Dynamics of an 89-kDa protein localizing at a specialized tip of the endonuclear symbiotic bacterium *Holospora* in infection

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The symbiotic bacterium *Holospora obtusa* infects the macronucleus of the ciliate *Paramecium caudatum*. After ingestion by its host, an infectious form of *Holospora* with an electron-translucent tip passes through the host digestive vacuole, and penetrates the macronuclear envelope with this tip. To investigate the underlying molecular mechanism of this process, we raised a monoclonal antibody against a tip-specific 89-kDa protein, partially sequenced this protein and identified the corresponding complete gene. The deduced protein sequence carries two actin-binding motifs. Indirect immunofluorescence microscopy shows that during escape from the host digestive vacuole, the 89-kDa protein translocates from the inside to the outside of the tip. When the bacterium invades the macronucleus, the 89-kDa protein is left behind at the entry point on the nuclear envelope. Transmission electron microscopy shows the formation of fine fibrous structures that co-localize with the antibody-labeled regions of the bacterium. Our findings suggest that the 89-kDa protein plays a role in *Holospora*’s escape from the host digestive vacuole, migration through the host cytoplasm, and invasion into the macronucleus.
Infection with *Holospora obtusa* changes digestive vacuole formation in the host, *Paramecium caudatum*

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The Gram-negative bacterium *Holospora obtusa* is an endonuclear symbiont of the ciliate *Paramecium caudatum*. *Holospora* cannot coexist in a host cell with other bacteria belonging to the same genus. For example, *H. obtusa*-bearing cells (symbiotic cells) eliminate newly introduced *H. elegans* from the host cell. However, it is unknown whether further bacteria of the same species are also eliminated from the symbiotic cells. Both non-*H. obtusa*-bearing (aposymbiotic) and symbiotic cells were mixed with infectious forms of *H. obtusa*, and were observed at 0.5–1.5 and 5–6 h after mixing. Compared with the aposymbiotic cells, the symbiotic cells formed fewer digestive vacuoles against the newly added *H. obtusa*. However, the aposymbiotic cells showed decreased digestive vacuole formation to *H. obtusa* at 6 h after mixing, although they did not decrease activity against the food bacterium *Klebsiella pneumoniae*, Chinese ink, the reproductive form of *H. obtusa*, or boiled or frozen infectious forms of *H. obtusa*. These results suggest that *P. caudatum* acquires the ability to distinguish the infectious form of *H. obtusa* from other objects within 5 h after infection with *H. obtusa*. 
Effects of protein synthesis inhibitors on infection by symbiotic Chlorella in the host Paramecium bursaria

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Chlorella-free cells of Paramecium bursaria can be reinfected with algae isolated from Chlorella-bearing cells by ingesting them into the digestive vacuole (DV). In a previous study, we showed that an alga can successfully escape from the host's DV and establish endosymbiosis after acidosomes and lysosomes have fused with the vacuole. When boiled or fixed algae were added to algae-free paramecia, all algae were digested. Therefore, algal resistance to the lysosomal enzymes is a property of living algae. To examine the effects of protein synthesis inhibitors on infection by symbiotic Chlorella of the host P. bursaria, isolated symbiotic algae were pretreated with cycloheximide, or algae-free hosts were pretreated with puromycin. The algae and the algae-free P. bursaria cells were then mixed, and the fate of the algae in the host DVs was observed. Infection of the host by Chlorella was not inhibited by host pretreatment with puromycin. However, algae treated with cycloheximide were not maintained in the host cytoplasm, although they could escape from the host's DV. These results suggest that algal protein synthesis plays an important role in establishing the P. bursaria–Chlorella endosymbiosis.
Aging control by the macronucleus, analyzed by macronuclear transplantation in *Paramecium tetraurelia*

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In *Paramecium tetraurelia*, the fission rate decreases with each cell cycle after conjugation or autogamy. Finally, it reaches zero and this results in clonal death. The lifespan after autogamy is generally about 200 fissions. Generally, clonal age is recorded by the length of the telomere. However, paramecium telomeres do not shorten in accordance with clonal age. So where is the clock for clonal age? It is known that the immaturity period for autogamy is measured by the number of rounds of DNA synthesis since autogamy. If this is also true for clonal aging, the clock must be integrated with chromatin replication. To analyze the clock for clonal age, macronuclear transplantation was used. When the macronucleus was transplanted from a young cell (20 fissions after autogamy) to an aged cell (170 fissions) from which the macronucleus had been removed, the fission rate of the aged cell recovered to that of the young cell by the 4th day after autogamy. The lifespan of the transplanted clones lengthened to 325 fissions. The total lifespan of the transplanted clone equals the sum of the recipient cell age (170 fissions) and the donor’s remaining lifespan (155 fissions). In the reverse experiment, when a young amacronucleate cell (20 fissions) was transplanted with the macronucleus of an aged cell (170 fissions), the young cell decreased its fission rate for about 4 cell cycles. These results suggest that the clock for fission number after autogamy must be in the macronucleus.
Histone magic in *Paramecium* mating pairs

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By using an expression vector harboring the *Paramecium* histone H2B gene conjugated with the codon-optimized yellow fluorescent protein (YFP) gene, PcVenus, we have found evidences relating to transport and incorporation of histone into the partner’s nuclei during the conjugation process in *Paramecium caudatum*. We have also demonstrated sharing of histones among all types of nuclei in a mating pair including both the old and new generations of nuclei. When histone H2B was produced as a fusion protein with PcVenus, significant fluorescent signals was detected in both the micro- and macronuclei of a transformed cell. The transformants showed normal growth and high sexual activity indicating the normal function of histone H2B-PcVenus. When the transformant expressing histone H2B-PcVenus was mated with an untransformed cell, clear fluorescent signal was observed in the micro- and macronuclei of the untransformed cell as early as 7 hours after pair formation. This indicates the transport of histone H2B-PcVenus and/or its mRNA from one member of a mating pair to its mating partner and incorporation into the partner’s nuclei. The discovery of exchange and shared use of histones among nuclei in mating pairs would be very important for the elucidation of epigenetic control mechanisms such as the histone code hypothesis in fertilization and early developmental processes.
Conjugation and encystment of the planktonic choreotrich ciliate

*Strombidium conicum*

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The planktonic choreotrich ciliate *Strombidium conicum* is known to be a cyst forming species. Its cysts were found in the sediment of Onagawa Bay and successfully excysted in laboratory. However, no attempt was made for encystment. In this study, we tried to induce its encystment in laboratory. Although environmental and physiological stresses usually induce the encystment in many species, stresses in temperature, light and feeding conditions did not induce encystment in this species when cultured in single clonal state. When cultured in mixed clonal state, many encystments were observed. During such mixed cultures, a pair of conjugating cells was observed after 36 days from original excystment. This suggests that *Strombidium conicum* encysts after conjugation, which occurs in matured populations.
Expression program of new zygote-specific (EZY) genes in *Chlamydomonas*

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The green algae *Chlamydomonas reinhardtii* differentiates into competent gametes under nitrogen starvation. When opposite gametes are mixed, they undergo a program of zygote formation: digestion of the minus chloroplast DNA, flagellar resorption, synthesis of zygotic cell wall, fusion of nuclei, digestion of mitochondrial DNA, and fusion of chloroplasts. We isolated the mRNAs from gametes and zygotes, and they were hybridized with a cDNA macroarray containing 10,368 ESTs. About 92 ESTs, whose average expression ratio of zygote:gamete exceeded 3-fold, were selected. Twenty-five non-redundant novel genes that were predominantly up-regulated in zygotes were identified by RNA blot analyses. Seven genes were transcribed specifically in the zygote, and these were designated as EZYs. EZY genes were classified into two categories based on profiles expressed in imp3, fus1 and normal mating. The mating signal alone was enough to induce two of the EZY genes, but cell fusion was required for expression of the other five EZY genes. When the competent gametes were pre-incubated with cycloheximide (CHX) for 30 min and mixed together, zygotes did not proceed with all the subsequent zygotic events. However, six EZY genes were transcribed in the presence of CHX.
Characteristics of intestinal flagellate faunae in the termite family Rhinotermitidae.

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The termites in family Rhinotermitidae, composed of 13 genera, usually possess symbiotic flagellate communities in their hindguts, which exhibit strong host specificity. The symbiont compositions of the rhinotermitid termites are, however, only partially investigated, and this prevents our understanding of the evolution of the symbiont community. In this study we collected termite species of three genera in the Rhinotermitidae (*Rhinotermes*, *Dolichorhinotermes*, *Stylotermes*) and of two genera in the closely related Serritermitidae (*Serritermes*, *Glossotermes*), to investigate their symbiont compositions. One to three flagellate genera (*Pseudotrichonympha*, *Spirotrichonympha*, *Hexamastix*) were found in the termites investigated in this study. The composition data from this study and published information about the composition in other host genera suggest that each species in the Rhinotermitidae and the Serritermitidae usually possess single *Pseudotrichonympha* species respectively, except in *Reticulitermes*. The symbiont fauna of the *Reticulitermes* host is unique in the family. Judging from the host phylogeny, host switchings might have taken place between some host lineages. The lack of symbionts in Termitidae, and one-to-one host–symbiont relationships found in some host genera could have evolved independently.
Establishment of monoclonal antibodies against the polar tube of *Encephalitozoon cuniculi* and identification of the corresponding antigenic polypeptide through proteome analysis

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We established three hybridomas able to produce monoclonal antibodies (MAbs) against the polar tube (PT) of *Encephalitozoon cuniculi*. The reactivity of two out of the three MAbs was examined in this study. Immunoreactive proteins specifically recognized by the MAbs were analyzed using immunoproteomics. Both MAbs exhibited positive reactions to similar multispots on 2-DE blots. Strongly positive signals were obtained from two Coomassie blue-stainable consecutive spots of identical Mr but different pI. These spots were identified as acidic protein PTP1.
Structure and function of general motor proteins systems for motility, including the spasmoneme in Vorticellidae stalk

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We present a concept for the contractile mechanism of the Ca\textsuperscript{2+}-driven motor protein (spasmin) and its receptor protein (spaconnectin) in the bundle of 3-nm filaments composing the Vorticellidae spasmoneme. The Ca\textsuperscript{2+}-binding protein, spasmin, belongs to the calmodulin superfamily and has an EF-hands structure of four or less. Any motor protein must have its own receptor protein for the transformation of ligand-binding or ligand-hydrolysis energy into work or force. It was recently revealed that the molecular weight of spaconnectin, tentatively the receptor protein of spasmin, is 190–200 kDa for the tetrameric form in the Carchesium spasmoneme, 90–100 kDa for the dimeric form in the Zoothamnium spasmoneme, and 50 kDa in the Vorticella spasmoneme. The large conformational change of the spasmoneme during its contraction and stretching is due to entropically elastic spaconnectin but not to spasmin. The concept of the Ca\textsuperscript{2+}-driven contractile mechanism of a Vorticellidae spasmoneme system as a bio-ratchet can be applied to the ATP-induced contractile mechanism of the myosin II and V systems, as well as the dynein-kinesin systems.

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Molecular characterization of a protein geranylgeranyltransferase type II and a Rab escort protein from *Entamoeba histolytica*

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Rab proteins function as molecular switches of signal transduction for intracellular vesicular transport. For Rab proteins to function, they must be post-translationally geranylgeranylated and attach to an intracellular membrane. Protein geranylgeranyltransferase type II (GGT-II) catalyzes this modification with the aid of a Rab escort protein (REP). We have been studying the prenyltransferases of *Entamoeba histolytica* (*Eh*), an enteric protozoan parasite of humans, in their biological similarity and differences as well as the feasibility of using them as a target for chemotherapy. We report here on their GGT-II and REP. The alpha (GGT-IIα) and beta (GGT-IIβ) subunits and REP from *Eh* consist of 317, 315 and 480 amino acid residues, respectively, and have characteristic conserved domains. These proteins are phylogenetically independent of those from other organisms. Recombinant GGT-II expressed in *Escherichia coli* was purified as a complex of both subunits. An anti-*Eh* GGT-IIα and an anti-*Eh* REP rabbit serum did not react with rat GGT-IIα and rat REP, respectively. REP-dependent geranylgeranylation of Rab by recombinant *Eh* GGT-II was confirmed using [³H]geranylgeranyl pyrophosphates. There was a difference in substrate specificity between amoebic GGT-II and rat GGT-II. In conclusion, GGT-II and REP from *Eh* are very different from those of mammals in phylogeny, antigenicity and substrate specificity.
Identification of adhesion molecule expressed at the insect stage of an African trypanosome

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Trypanosoma congolense, a causative agent of animal African trypanosomosis, is biologically transmitted by tsetse flies (Glossina spp.). The parasite undergoes cell differentiation during its lifecycle. Among three cell differentiation stages in the tsetse fly, only the epimastigote form (EMF) shows an adherent phenotype. It has been reported that cell adhesion of the EMF is a fundamental process for progression of its differentiation to the animal-infective metacyclic form. Recently, we found that the culture supernatant of the EMF contained a molecule involved in cell adhesion, and we tentatively named it trypanosome-derived cell adhesion molecule (TAM). Molecular mass of native TAM was 100 kDa. Adhesion activity of the native TAM was totally inhibited by heat (65 and 100°C), sodium periodate and proteinase K treatment. The full-length TAM gene (2,067 bp) was successfully cloned from an EMF cDNA library. TAM appeared to be localized on the cell surface of the EMF and the flagellum of the bloodstream form. According to the results of a BLAST search, TAM appears to be a new T. congolense protein. TAM appears to be a novel glycoprotein that is expressed on the EMF cell surface and is involved in cell adhesion processes in the EMF.
Electron microscopic observation of the invasion process of Cryptosporidium parvum in severe combined immunodeficiency mice

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Cryptosporidium parvum mainly invades the intestinal epithelium and causes watery diarrhea in humans and calves. However, the invasion process has not yet been clarified. In the present study, the invasion process of C. parvum in severe combined immunodeficiency (SCID) mice was examined. Infected mice were necropsied and the ilea were observed by scanning and transmission electron microscopy. In addition, ruthenium red staining was used to observe changes in the microvillus membrane that resulted from the invasion of C. parvum. Scanning electron micrographs showed elongation of the microvilli around the parasite. The microvilli were shown to be along the surface of the parasite. Transmission electron microscopy confirmed that the invading parasites were located among microvilli. Parasites were seen in a parasitophorous vacuole formed by the microvillus membrane. The parasite pellicle attached to the host cell membrane, and then the pellicle and host cell membrane became unclear. Subsequently, the pellicle structure became more complex and formed a feeder organelle. Invasion of the parasite was not observed in either a microvillus or the cytoplasm of the host cell. We have showed that C. parvum invades among the microvilli, is covered with membranes derived from numerous microvilli, and develops within the host cell.
Cloning and characterization of a gene encoding a protein disulfide isomerase from *Neospora caninum*

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A gene encoding a protein disulfide isomerase (PDI) was isolated from a *Neospora caninum* cDNA expression library. The nucleotide sequence of the cDNA clone revealed the presence of an ORF of 1,416 bp, which encoded 461 amino acids, showing a high degree of homology to *Toxoplasma gondii* PDI. The gene was cloned into a pGEX vector and expressed in *E. coli* as a GST fusion protein. The NcPDI was detected in *N. caninum* tachyzoite lysate and ES products with a molecular weight of 50 kDa. IgA antibody in 58.0% of individual cattle tear samples recognized both the recombinant and native NcPDI, which suggests that the PDI-specific antibody may be involved in defense against parasites. In addition, PDI-specific inhibitors showed significant inhibitory effect on the growth of *N. caninum* tachyzoites. The purified recombinant NcPDI demonstrated biological activity in vitro by catalysis and refolding of reduced RNase, and assisted in the recovery of native from denatured lysozyme. These findings indicate that the NcPDI possesses specific-PDI enzymatic activity and it offers a putative target for prevention and chemotherapy of neosporosis.
Loop-mediated isothermal amplification (LAMP) and its application for the detection of African trypanosomes

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While PCR is a method of choice for the detection of African trypanosomes in both man and animals, the expense of this method negates its use as a diagnostic method for the detection of African trypanosomosis in African countries where it is endemic. The loop-mediated isothermal amplification (LAMP) reaction is a method that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions using only simple incubators. An added advantage of LAMP over PCR-based methods is that DNA amplification can be monitored spectrophotometrically and/or with the naked eye, without the use of dyes. Here we present our conditions for a highly sensitive, specific and easy diagnostic assay based on LAMP technology for the detection of parasites of the Trypanosoma brucei group (including T. b. brucei, T. b. gambiense, T. b. rhodesiense, and T. evansi) and T. congolense. We show that the sensitivity of the LAMP-based method in detecting trypanosomes in vitro is up to 100 times higher than that of PCR-based methods in laboratory conditions. In vivo studies in mice infected with human-infective T. b. gambiense further highlight the potential clinical importance of LAMP as a diagnostic tool for identification of African trypanosomiasis.
Novel effects of D-glucose on the physiology of a Paramecium symbiont

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Glucose is a common nutrient, but sometimes it can work as an inducer or a signal. An endosymbiotic alga F36-ZK, isolated from Japanese Paramecium bursaria F36, grew faster in the presence of glucose, suggesting that glucose was a good nutrient. However, no glucose uptake was detectable in experiments using a radiotracer. On the other hand, it was found that glucose accelerated uptakes of several amino acids, such as L-Glu, L-Gln, L-Asp, L-Ser, L-Ala and L-Leu, approximately 2–5-fold in a mineral salt medium. A non-metabolizing glucose analogue, 3-OMG, also stimulated L-Ser uptake; this implies that glucose was not being used as an energy source. The effect was also observed in the presence of cycloheximide, indicating that the effect was not due to new synthesis of amino acid transporter. However, higher amino acid uptakes, but no stimulating effect of glucose were observed when Ca²⁺ and Mg²⁺ were absent, although amino acid uptake is generally increased in the presence of divalent cations in many organisms containing free-living Chlorella. These results suggest that divalent cations inhibit amino acid uptake, and glucose cancels the inhibiting effect of the cations. Glucose and divalent cations seems to have roles as regulators of the symbiont’s amino acid uptake in Paramecium symbiosis.
Effects of acrylamide on the symbiotic association of algae with the ciliate

*Paramecium bursaria*

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The monomeric, but not the polymeric, form of acrylamide induces carcinogenic or neurotoxic effects in both humans and laboratory animals. However, the mechanism of acrylamide toxicity to living organisms, including humans, is poorly understood. The present study aimed to investigate the mechanisms of acrylamide toxicity using a green paramecium, *Paramecium bursaria*. A previous report revealed that acrylamide induces a decrease in endosymbiotic algae in *P. bursaria* (Takahashi et al., *Toxicol. in Vitro*, 2005). In the present study, we monitored the proliferation of endosymbiotic algae throughout the host cell cycle in the presence of acrylamide to quantify the toxicity of acrylamide on the endosymbiotic algae. The results demonstrated that treatment with acrylamide (10 μg/ml) suppressed algal proliferation in the host cell. After treatment with acrylamide, several larger algal cells were observed in the host cells. This suggests that acrylamide induces multinuclear cells by interfering with algal cytokinesis.
Species composition of soil ciliates and their population size and biomass in upland soils treated with slurry

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SUMMARY

It has often been pointed out that protozoan ciliates might play important roles in the soil ecosystem. However, we have little information about the soil ciliate community. The main reason for this might be that there is no suitable method for detecting ciliates in soil samples or for estimating the number of individuals and/or their biomass. We previously proposed a modified MPN method, the MPN-SIPs method, to analyze ciliate communities in terrestrial habitats (Takahashi et al., 2003). The MPN-SIPs method estimates not only ciliate population size but also the faunal composition and the biomass of each ciliate species. However, whether this method can detect soil ciliates more efficiently than the MPN method has not been verified. We therefore estimated the number of ciliate individuals in each of four soil samples with different levels of slurry application (300 t/ha/0.5 y, 150 t/ha/0.5 y, 60 t/ha/0.5 y, and 0 t/ha/0.5 y) using both methods. In all samples, when the MPN-SIPs method was used we detected 7–10 times as many individuals as when the MPN method was used. These results indicate that the MPN-SIPs method is more suitable for analyzing the soil ciliate community than the MPN method.
Preliminary report

Establishment of faunal and taxonomic database of ciliated protozoa for infrastructure development in taxonomy and ecology in Japan

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Taxonomic and ecological studies on ciliated protozoa have been carried out in various areas in Japan since the end of the 19th century. However, the data from these studies have not been integrated into a database. The purpose of this study is establishment of faunal and taxonomic database using published information about free-living ciliates in Japan. We collected books, scientific journals and research bulletins, and made 1) a list of the literature, 2) a list of recorded species, and 3) a list of ciliate habitats. The total number of collected publications on ciliates was 405 (21 descriptions of new species; 384 records) as of October 1, 2005. The total number of ciliate species counted in this study is larger than that of species surveyed in a report of Japanese biota (Nakayama, 2003) based on our preliminary results. It was clarified that most of the literature was about ciliates in freshwater environments. In addition, about 70% of the reports were part of investigations of plankton. Our study will contribute to basic research, such as taxonomy and ecology, but also to applied sciences such as environmental assessment using ciliate species.
Photodynamic killing of *Blepharisma* (albino), *Climacostomum* and *Dileptus* by treatment with photosensitizer pigments

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*Blepharisma japonicum* produces a red pigment, blepharismin. Blepharismin is highly toxic to various ciliates in the dark. It is known that blepharismin has a defense function against predatory protists. Blepharismin is a photodynamic pigment. Even a dilute solution of blepharismin photosensitizes colorless cells. *Blepharisma* and *Climacostomum* are highly resistant to the toxicity of blepharismin in the dark. Are they also resistant to the toxicity of blepharismin in the light? We examined the photodynamic killing of *Blepharisma* (albino), *Climacostomum* and *Dileptus* by treatment with the photosensitizer pigments blepharismin, eosin, erythrosin, rose bengal and methylene blue. *Blepharisma* (albino) and *Climacostomum* were killed by the phototoxicity of blepharismin. *Climacostomum* was most resistant among the three ciliates to the phototoxicity of the acidic photosensitizer pigments and most sensitive to the phototoxicity of the basic photosensitizer pigment. *Blepharisma* (albino) was not resistant to the phototoxicity of any photosensitizer pigments tested in this work. These findings suggest that the mechanism of toxicity of blepharismin in the dark is different from that of its phototoxicity. The fact that *Blepharisma* is resistant to the toxicity of blepharismin in the dark but is sensitive to the phototoxicity of blepharismin suggests that *Blepharisma* developed the photophobic response to escape from harmful light.
A classification and functions of *Tetrahymena thermophila* myosins

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It is considered that actin and type-II myosin work together in assembly of the contractile ring during cytokinesis. Thirteen genes encoding myosin have been found in *T. thermophila*. However, type-II myosin is absent in this organism. We searched the functional domain in the tail regions of *Tetrahymena* myosins. They were finally classified into four groups which had FERM (lipid-binding), ATS1 (control of microtubules), Smc (dimeric coiled-coil structure), and other functions. To identify myosins involved in cytokinesis, we are analyzing the cellular distributions of Myo13 and Myo6, as both these myosins form dimers and could possibly function in cytokinesis instead of type-II myosin.
A comparative study on crystal vacuole formation of amoebae

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In order to compare the difference in crystal formation between *Amoeba proteus* and *Polychaos dubium* collected from a natural freshwater pond, a study was performed using a light microscope, transmission electron microscope (TEM), scanning electron microscope (SEM), and X-ray microanalyzer. The cytoplasm of *A. proteus* contained numerous small crystals 2–3 µm in length and bipyramidal in form, and that of *P. dubium* contained numerous large crystals 20 µm in length and of a plate-like rectangular form. Using the same organism as food did not change the shape, size or number of crystals in the two amoebae. For SEM observation and X-ray microanalysis, 50 individuals of each amoeba and white cells of *Paramecium bursaria* as a food organism were placed on the carbon specimen holder, and were naturally dried and then coated with carbon. We found that the crystals of *A. proteus* were composed of two types, one as described above, and another which was small, granular, and 0.1 µm in diameter. The elemental composition of both crystal types was the same and consisted mainly of carbon and oxygen, with small amounts of phosphorus, potassium, chlorine, and sulfur. The crystals of *P. dubium* were also of two types, but the plate-like rectangular crystals varied from 6–20 µm. In addition, many small granular crystals or clumps thereof adhered to the surface of these crystals. The plate-like rectangular crystals were composed of carbon and oxygen, and the small grains were mainly composed of carbon, oxygen, phosphorus, calcium and magnesium, with small amounts of sulfur, chlorine, and potassium. The elemental composition of small-grain crystals in *Paramecium bursaria* was the same as that of the small crystals of *P. dubium*. The variation in the plate-like crystals of *P. dubium* indicates that they gradually increase in size, while the crystals of *A. proteus* remain constant in size and shape. Because the elemental composition of the crystals of the amoebae and the paramecium were different, it is probable that the crystals and small grains were synthesized within each cell.
Lateral gene transfer occurs from the micronucleus-specific bacterium *Holospora elegans* to the host *Paramecium caudatum*

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The bacterium *Holospora elegans* is a micronucleus-specific symbiont of the ciliate *Paramecium caudatum*. *Holospora*-bearing paramecia survive well compared with *Holospora*-free paramecia under heat-shock conditions. Furthermore, aposymbiotic cells (paramecia treated with penicillin to remove *H. elegans*) retain this tolerance. We therefore looked for a substance originating from *H. elegans* in aposymbiotic cells. We found that a 16S rDNA of *Holospora* remained in the macronucleus of the aposymbiotic cells. This suggests that the stress response of the host would be enhanced irreversibly, because of lateral transfer of bacterial genes to the macronucleus. The result further suggests that infection with *Holospora* enables the host to adapt to a hot environment.
Comparison of protozoan communities between winter-flooded and conventional rice fields

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Under ordinary management in Japan, rice fields dry up from August to April. In non-tilled, winter-flooded rice fields, however, the rice field is flooded with water even in winter (January and February). Rice stubble slowly decomposes in the water. In the present work, we compared ciliates of protozoan communities in a non-tilled, winter-flooded rice field with those in a conventional rice field. Ciliates were observed by phase contrast microscopy and by protargol staining. In July and August, 12 species were found in the winter-flooded rice field, and 11 species in the conventional rice field. There was no significant difference in species. In May and June, 14 species were found in the winter-flooded rice field, while 9 species were found in the conventional rice field. It is probable that the ciliates ate bacteria and organic matter produced by decomposition of the stubble. More ciliates were also found in the winter-flooded rice field in January and February. In particular, genus Halteria, class Colpodea and subclass Stichotrichia were common.
A new gregarine parasite of *Plodia interpunctella* (Insecta: Lepidoptera)

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Gregarines are a group of apicomplexans that are closely related to the human pathogen *Cryptosporidium*. Although this group of protozoans does not have any close impact on human life, it has been suggested that some species are potentially useful for the biological control of insects. Gregarines are also of interest from an evolutionary point of view, because they are thought to be deep-branching apicomplexans. In the present study, a species of gregarine parasite was found in the midgut of a lepidopteran insect, *Plodia interpunctella*. It was characterized by a septum between protomerite and deutomerite, solitary sporadines and simple knob-like epimerites. Although morphological features of gametocysts and spores were not examined, the above morphological features and its host specificity indicate that the species can be assigned to the family Leidyanidae, which contains a single genus *Leidyana*. The genus *Leidyana* is very similar in many respects to *Gregarina*, the only difference being that *Leidyana* is always solitary before syzygy, while *Gregarina* gamonts form associations. This is the second record of *Leidyana* in a lepidopteran insect in Japan, following that of *L. latiformis* in a tineid moth (Hoshide, 1958).
Tubular filaments in the cytoplasm of *Paramecium bursaria*

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By transmission electron microscopic observation, tubular filaments of 50–60 nm in diameter were found in the cytoplasm of *Paramecium bursaria*, extending from the surface of symbiotic *Chlorella* toward the inside of the cell. The tubular filaments appeared to be membranous structures, as they disappeared after treatment with Triton X-100, while subcortical microtubules remained intact after the detergent treatment. SDS–PAGE and Western blotting showed that *P. bursaria* possesses two proteins, of 35 kDa and 50 kDa, that react with α-tubulin antibodies. The 50-kDa protein is most likely α-tubulin, but the 35-kDa protein is unique to the cytoplasm of *P. bursaria*, and is neither detected in cilia nor originates from the symbiotic *Chlorella*. The origin of the protein and its relationship to the tubular filaments are obscure.
Isolation and characterization of mastigoneme proteins of *Ochromonas* sp.

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The phytoflagellate, *Ochromonas* sp., has two flagella of different lengths projecting from the anterior end of the cell. The fine, threadlike projections, called tubular mastigonemes, are connected to the surface of the longer flagellum, which is considered to control the direction of flagellar swimming. In this study, to understand the molecular architecture of the tubular mastigonemes and their role in flagellar motility, we isolated mastigonemes from *Ochromonas* sp. and characterized constituent proteins. SDS–PAGE analysis of the mastigonemes showed four major protein bands, and one of these bound specifically to concanavalin A.
Axopodial degradation by arsenic ions and pH in the heliozoon
*Raphidiophrys contractilis*

S. M. Mostafa Kamal Khan, Toshinobu Suzuki


In this study, we have observed the effect of pH and arsenic ions on the heliozoon *Raphidiophrys contractilis*. In the presence of arsenic ions and pH variations, this heliozoon shows different sensitivities; axopodial degradation is one of these. Most organisms have adapted to life in water of a specific pH, and may die if the pH changes even slightly. At extremely high or low pH values the water becomes unsuitable for most organisms. The accumulation of trace elements, such as arsenic, in the environment is a potential risk to human health due to their transfer in aquatic systems. It is important to monitor and ensure the quality of our aquatic environments. The heliozoon *R. contractilis* might be used as a bio-indicator to monitor and assess water quality.
Analysis of a glycoprotein and extrusomes in the feeding behavior of the heliozoon

*Actinophrys sol*

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The heliozoon *Actinophrys sol* captures prey organisms with its axopodia. Extrusomes are known to be located beneath the cell membrane of axopodia and to discharge their contents when prey organisms make contact with the axopodia. It has been suggested that a 40-kDa glycoprotein (gp40) that is secreted from the extrusomes is involved in prey capture, especially in prey adhesion (Sakaguchi et al, 2001). In this study, we investigated the effects of inhibitors on prey capture by *A. sol*. It was found that concanavalin A, which binds to gp40, inhibited prey adhesion. This inhibition was relieved by the addition of mannoside. In addition, prey adhesion was inhibited by removing calcium ions from the extracellular medium. This inhibition was relieved by the addition of calcium ions. Consistent with this observation, calcium ion channel blockers (nifedipine, verapamil and lanthanum) and a calmodulin antagonist (W-7) inhibited prey adhesion. These results indicate that the presence of some glycoconjugates and calcium influx into the cell are necessary for prey capture.
Bio-monitoring system for aquatic hazards using heliozoons

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A biological monitoring system for toxicants in water has been developed by using the centrohelid heliozoon Raphidiophrys contractilis as an indicator organism for water quality. A flow-through type chamber was developed for toxicity testing on R. contractilis. It was placed on a light microscope stage, and changes in the heliozoon’s axopodial length were continuously monitored with a CCD camera. The image was digitized, and analyzed to quantify the length of the axopodia. The test results revealed that this monitoring system has a high sensitivity and durability, enabling us to quickly and easily detect toxic substances in water.
Amino acid sequence analysis and molecular cloning of *Naegleria fowleri* proteins

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*Naegleria fowleri* is a thermophilic free-living amoeba that causes primary amoebic encephalitis (PAM) in humans, while *N. lovaniensis*, the morphologically identical species, is not. A two-dimensional (2-D) gel analysis was employed to compare total proteins in *N. fowleri* to those of *N. lovaniensis* in order to identify proteins that may link to its pathogenesis. Until now we have detected 63 protein spots in *N. fowleri* by means of N-terminal and/or internal amino acid sequences analyses. Due to lacking in the available genetic data of DNA of *N. fowleri*, it is sometime difficult to link information from the proteome analyses with information on DNA. In the present experiment, we partially cloned the genes of 2 protein spots, tentatively designated as #15 (24.1 kDa, pI 6.5) and #35 (50.9 kDa, pI 16.7), that are specific to *N. fowleri* by means of degenerate PCR. The respective PCR products had approximately 250 bp and 400 bp in sizes. From the amino acid sequence predicted from the amplified DNA, #15 was identified with high confidence as thioredoxin peroxidase (22.3 kDa, Q6DV14) with 80% (54/67) homology. Similarly the predicted amino acid sequence of #35 showed 55% (67/120) homology to that of glutamate dehydrogenase (55.0 kDa, Q54KB7).
Identification of a set of genes expressed during induction of conjugation in the ciliate *Blepharisma japonicum*

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Conjugation in *Blepharisma japonicum* is induced by interaction between cells of complementary mating-types I and II, when they are moderately starved. Specific genes activated during conjugation, and the way they regulate these processes, are not known. To identify genes activated during conjugation, we isolated genes expressed in conjugation-induced cells by using a cDNA subtraction method. So far we have identified such genes, including CDK family genes (*cdc2, Cdk2*), a 4-hydroxyphenylpyruvate dioxygenase (*4-HPPD*) homolog, and a cyclin dependent kinase regulatory subunit (*Cks*) homolog. In this study, we newly isolated two genes that had no significant homology from conjugation-induced type-II cells. We also isolated two genes from conjugation-induced type-I cells and showed that one of them had homology to the hsp-90 family. We examined the level of expression in some of these conjugation-specific genes (*cdc2, Cks, 4-HPPD, hsp-90*) in both mating types in the logarithmic growth phase, early stationary phase and during induction of conjugation. We found that *cdc2, Cks* and the *4-HPPD* homolog were specifically expressed in starved mating type-II cells, and their expression remarkably increased during induction of conjugation. Although the *hsp-90* homolog was expressed in both mating types, under all conditions, the expression level increased in conjugation-induced cells.
The interaction between protozoa and bacteria in the soil is very important in agricultural systems. The beneficial effects of protozoa on plant growth have been attributed to nutrients released from the consumed bacterial biomass. However, there are few reports analyzing protozoan diversity in soils. Although culturing techniques and microscopy are commonly used, procedures for isolation of protozoa from soil have not been fully exploited. Molecular techniques offer a powerful tool, which is rapid and high throughput, for analyzing the diversity of the microbial community. We designed a ciliate-specific PCR primer, and performed PCR amplification of 18S rDNA of five ciliates, three flagellates, two amoebae, four nematodes, three fungi and one yeast species. Eukaryote 18S rDNAs of all organisms were amplified using the universal primer set EU347F & EU929R (ca 582 bp in length). The PCR bands for ciliates were observed using the ciliate-specific primer set CS322F & EU929R. Unexpectedly, 18S rDNA of one of the tested ciliates (Dileptus anser) was not amplified, while that of Aphelencus avenae (a nematode) was amplified. The DNA sequence of A. avenae is identical to that of the CS322F primer except for one position. The designed ciliate-specific PCR primer is therefore not completely specific for ciliates, but may nevertheless be useful to study the diversity of ciliates. To analyze the communities and diversity of ciliates, and their interaction with bacteria in the soil, we used the ciliate-specific primer and PCR-denaturing gradient gel electrophoresis (DGGE) analysis based on 18S rDNA.
Sequencing and bioinformatics analyses of ciliate eRF1s toward understanding stop codon recognition

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Eukaryotic release factor 1 (eRF1) is believed to play an important role in the stop codon reassignment in ciliates. To clarify the specificity of stop codon recognition in ciliate eRF1s, we have sequenced eRF1 genes from four ciliate species. Phylogenetic and structure-based analyses of eRF1s have been carried out. We have statistically analyzed protein–RNA complexes available in a structure database (Protein Data Bank) and obtained a propensity for each amino acid residue being located in the RNA-binding sites. Using the RNA-binding propensity, we have suggested areas on the protein surface that are important for stop codon recognition in ciliate eRF1s.