became possible to switch its light path from the still camera to the CCD video camera instantly. The primary movie sessions, recorded as DV format, have been fragmented as short time video clips (10 - 60 sec). Then those clips have been trimmed and/or reduced its size, compressed using the photo-JPEG codec of QuickTime, then stored as QuickTime movies. For various speeds of network connections, each movie is provided as 4 grades of compress ratios from several ten kilobytes to a few mega bytes in data size. By using those equipments and methods, we have provided up to 1200 movie clips at http://protist.i.hosei.ac.jp/Movies/htmls/indexE.html.

ANALYSIS OF THE FEEDING SYSTEM IN THE HELIOZOON *ACTINOPHRYS SOL*

Souichiro Kakuta, Toshinobu Suzaki
Department of Biology, Faculty of science, Kobe University Nada-ku, Hyogo 657-8501, Japan

Heliozoon *Actinophrys sol* is a predatory protozoan which has a number of needle-like axopodia radiating from a cell body. Beneath the plasma membrane, there are a lot of small vesicular organelles called extrusomes. *A. sol* captures food organisms by using axopodia, from which contents of extrusomes are discharged toward the prey. A 40-kDa glycoprotein (gp40) is included in the extrusome, and is considered to play an important role in prey recognition and ingestion. We sequenced the cDNA that was amplified using degenerate primers designed from the N-terminal amino acid sequence of gp40. The sequence was not identical to the amino acid sequence of gp40, but had a high similarity with the sequence of peptidases of the members of sedolisin family. Antibody against N-terminal amino acid sequence of gp40 and putative peptidase of *A. sol* were raised followed by immunofluorescence and immunoelectron microscopic observation. As a result, we could confirm that gp40 existed in the extrusome, and that the putative peptidase was localized in late lysosomes.

CELL SIZE AND DNA CONTENT IN *PARAMECIUM*: SIZE CHANGES DURING LOG AND STATIONARY PHASES

Shiori Echigoya, Yoshiomi Takagi
Department of Biology, Faculty of Science, Nara Women's University, Nara 630-8506

We studied the cell size and macronuclear DNA content in 7 species of Paramecium, and found that they were positively correlated. Wd studied changes in the cell size during a cell cycle, and found that the cell size increased in two steps with a DNA independent manner in G1 stage and a DNA dependent manner in S stage. We studied changes in the cell size through the log and stationary phases of growth, and found that the cell size increased tentatively during the log phase and decreased by half during the stationary phase.

DETERMINATION OF A GERMINAL MICRONUCLEUS IN EXCONJUGANTS OF *PARAMECIUM CAUDATUM*

Noriko Taka, Kazuyuki Mikami
EEC Miyagi University of Education, 149, Aza-Aoba, Aramaki, Aoba-ku, Sendai 980-0845, Japan

In *Paramecium caudatum*, each cell has a germinal micronucleus. However, there are four presumptive micronuclei in the exconjugant. At the first fission after conjugation, only one micronucleus divided. This is an exconjugant specific phenomenon, because two micronuclei after nuclear transplantation were able to divide during vegetative phase. The results obtained with DAPI staining and immunofluorescence of α tubulin antibody showed that four micronuclei remained in most of cell at the first postconjugational fission regardless of nutritional condition. When the cells were reacted with FITC labelled α tubulin antibody, one of the four presumptive micronuclei changed from round to spindle form prior to the first postconjugational division. The evidence means that the micronucleus must be selected to divide just before the cell division. The selection mechanism of the micronuclei will be discussed.

AFFECT OF HEAVY METALS ON THE HELIOZOON *RAPHIDIOPHRYS CONTRACTILIS*

Khan Mostafa Kamal[1], Mikihiko Arikawa[1,2], Toshinobu Suzaki[1]
[1]Department of Biology, Faculty of Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan and [2]Graduate School of Human Culture, Nara Women's University, Kitauoyanishi-machi, Nara 630-8506, Japan

The affect of heavy metals on natural microbial communities have attracted increased attention. Heavy metal pollution resulting from human, industrial and agricultural activities affects biological systems. This pollution affect the growth, development, morphology, metabolism and many other cellular and molecular biological activities of all eukaryotic organisms including unicellular microorganisms. The effect of copper, lead, mercury and zinc on the axopodia of the centrohelid heliozoon *Raphidiophrys contractilis* was studied. In the presence of these heavy metals, axopodial length of the heliozoon *R. contractilis* became shorter significantly than its normal length. In the same concentration of these four heavy metals, the effect of mercury was higher than the others, and mercury produced a pronounced effect on the axopodial length. In the presence of high concentration of these heavy metals, it was observed that axopodia disappeared and the cells were disrupted very quickly. From these observations, the heliozoon *R. contractilis* was found to be useful as a monitor organism for detecting toxic chemicals in water.

DEVELOPMENTALLY AND ENVIRONMENTALLY REGULATED EXPRESSION OF MATING PHEROMONE IN THE CILIATE *BLEPHARISMA JAPONICUM*

Mayumi Sugiura, Seiko Kawahara, Terue Harumoto
Department of Biological Science and Environment, Graduate School of Human Culture, Nara Women's University, Nara 630-8506, Japan

The protozoan ciliate *Blepharisma japonicum* switches the reproductive process from asexual (binary fission) to sexual (conjugation) when they are exposed to food-deprived condition. However, this switching mechanism has not been elucidated yet. Conjugation in *B. japonicum* is induced by interaction between complementary mating-type cells, I and II, which excrete mating pheromones, gamone 1 and 2, respectively. In this study, we examined the pattern of the expression of gamone 1, which was a key factor to initiate conjugation, in the cells under different developmental stages or nutritional conditions. We also examined the influence of gamone 2 on the expression of gamone 1. We found that the transcript of gamone 1 was specifically expressed when sexually mature mating-type I cells were starved. It was not detected in immature cells, mating-type II cells and growing cells. The transcriptional level was remarkably increased when the starved mature mating-type I cells were stimulated with gamone 2. These results indicated that the expression of gamone 1-activity was strictly regulated by the developmental and environmental factors at the transcriptional level.

IDENTIFICATION OF THE UPSTREAM SEQUENCES OF GAMONE 1 GENE IN THE CILIATE *BLEPHARISMA JAPONICUM*

Risa Takami, Mayumi Sugiura, Terue Harumoto
The Division of Biological Science, Graduate School of Human Culture, Nara Women's University, Nara 630-8506, Japan

In the conjugation of *Blepharisma japonicum*, cells of complementary mating-types I and II are participated in the interaction for the formation of mating pairs. The pair-formation is induced by the mating pheromones, called gamones, which are secreted by each mating-type cells. Gamone 1 is not constitutively secreted, and is secreted exclusively by the moderately starved, sexually matured, type I cells. Moreover, synthesis of gamone 1 is further promoted by gamone 2 which is secreted by the cell of mating-type II. Therefore, the expression of gamone 1 is regulated by various conditions. First, we determined the sequence of gamone 1 gene in the genome, and it was clearly shown that the intron was not included. Second, we tried to identify the transcriptional regulatory region of the gamone 1 gene to elucidate the expression mechanism of gamone 1. 5'-flanking region of gamone 1 gene was amplified using the inverse PCR, and the upstream sequences of 112 bp were determined. The sequences contained proximal sequence element (PSE)-like sequence which is considered to be the transcriptional regulatory sequence for RNA polymerase III, and TATA box-like sequence.

CLONING OF THE GENES WHICH SPECIFICALLY EXPRESSED DURING THE INDUCTION OF CONJUGATION IN TYPE II CELLS OF *BLEPHARISMA JAPONICUM*

Yuri Tanaka, Mayumi Sugiura, Terue Harumoto
The Division of Biological Science, Graduate School of Human Culture, Nara Women's University 630-8506, Japan

Conjugation of *Blepharisma japonicum* is induced by the interaction between complementary mating-type cells I and II. The both mating-type cells which received complementary mating pheromones (gamones) underwent morphological changes and started to unite. It was reported that protein synthesis drastically increased during this time, and this protein synthesis was indispensable for the formation of conjugating pairs. However, it is still unknown whether the new genes specific to conjugation were transcribed. In order to identify genes involved in these processes, we used the cDNA subtraction method. We treated type II cells with the cell-free fluid of type I cells containing gamone 1 (10^3 U/ml) for 4 hours, then purified polyA$^+$RNA. PolyA$^+$RNA was subjected to cDNA synthesis, and the cDNA was subtracted between such treated cells and untreated cells. We cloned several cDNAs specific to the treated cells. Homology search revealed that one of these gene showed significant homology to cdc2 (cell division control protein 2 gene). We performed Northern hybridization and confirmed that the transcript appeared specifically during the induction of conjugation.

AXENIC CULTIVATION OF THE CILIATE *TETRAHYMENA* SP. FOUND IN A DEAD MOSQUITO'S LARVA

Yuko Hisatomi, Yumi Akagawa, Masako Uchida, Tadao Takahashi
Nishikyushu University, Biology laboratory, Kanzaki-cho, Saga 842-8585, Japan

The ciliate *Tetrahymena* sp., which was found in a dead mosquito's larva, *Ades albopictus*, was allowed to grow well axenically by performing the following

procedure. First, the exponentially growing cells were washed with an inorganic salt solution SMB II (Miyake & Beyer, 1973). These cells were, then, seeded onto a PY agar plate medium (0.9% agar) containing antibiotics and were incubated at 23C. At 7 days after the start of incubation, pure colonies of the ciliate were yielded on the agar plate. Finally, each of these colonies was transferred to different containers that held 2ml of PY liquid medium containing antibiotics. However, almost of cells broke down at the late stationary phase of growth in the axenic culture. In order to determine whether the cause of this phenomenon was due to the unsuitable concentration of the PY medium, we examined the growth of the ciliate in various concentrations of media. However, the cells also broke down in any PY concentration at the late stationary phase of growth. These phenomena, therefore, imply that such a cytolysis might be induced by some accumulated substances in the medium during cultivation.

EXISTENCE OF SIALOMUCIN IN THE EXTRACELLULAR CYST IN THE THYMUS OF JUVENILE MICE

Kiyoshi Shimizu, Jinzo Yamada
Department of Anatomy, Tokyo Medical University, Shinjuku-ku, Tokyo 160-8402, Japan

The extracellular cyst which exists at the cortico-medullar region in the thymus of many vertebrates might be a graveyard for T cells undergoing death.With a lectin, we observed whether or not mucous cells in the epithelium of the extracellular cyst secreted the sialomucin.Animals used were, 2 days of age, male mice of the IVCS strain.The thymus was fixed with glutaraldehyde, embedded in LRwhite, stained with 10nm gold colloid conjugated lectin (LPA), re-stained with uranyl acetate and lead citrate and viewed with an electron microscopy.Sialic acid existed in the secretory granules in the mucous cells and also in the content of the cyst. These findings indicated that the mucin secreted by the mucous cells had a high viscocity and might play a role for catching negative selected T cells which might move ino the cyst.

SUBCELLUAR LOCALIZATION OF NEUROTROPHIN RECEPTOR IN CULTURED SLICES OF MOUSE CEREBELLUM

Juan Luo, Ryoichi Yoshimura, Yasuhisa Endo
Department of Applied Biology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606-8585, Japan

It has been well demonstrated that the binding of neurotrophins to their relevant Trk receptors plays a siginificant role in regulation of survival, proliferation and differentiation of neurons.To date, however, there is little information on the subcelluar localization of these neurotrophin receptors in the brain. The expression and localization of p75, TrkA, TrkB in cultured slices of mouse cerebellum were examined by Western blot and immunohistochemistry. In the organotypic slices, the immunoreactivity of p75 was found mainly in Purkinje cells, and the immunostainig of TrkA and TrkB was intense in Purkinje cells and granule cells. At the electron microscopic level, we found that TrkB localized in the plasma membrane, small vesicles in the cytoplasm and dendritic processes of Pukinje cells.

THE MUCOUS CELLS OF THE ADHESIVE ORGAN OF THE TERRESTRIAL PLANARIAN *BIPALIUM SP.*

Yasuko Shirasawa[1], Isao Yoshihama[2], Naomi Seo[1], Emiko Furuta[3]
[1]Department of Biology, TokyoMedical University Shinjuku-ku, Tokyo 160-8402, Japan, [2]Electoron microscopy laboratory, Tokyo medical University, Sinjuku-ku, Tokyo 160-8402, Japan and [3]Institute of Comparative Immunology, Saitama-city, Saitama 337-0015, Japan

Histological observations were examined on the adhesive organ of the terrestrial planarian, *Bipalium sp.* We already reported that the epidermal mucous cells of this animal were classified into six types with electron microscopy. In present study, we investigated the mucous cells with scanning and transmission electron microscopy. The organ was composed of the tile shaped papillae and observed comb like arrangement on the margin of the head. The component cells of the papillae-surface were observed eosinophilic. Small pore, pit which was covered with cilia, existed among papillae. Two types of mucous cells were observed on the surface of the papillae. One was lattice granular cells and the other was electron dens granular cells. The result suggests that the two types of mucous granules play an important role of taking up the live baits.

CHANGE OF BROWN ADIPOSE CELLS OF LAND LEECHES, *HAEMADIPSA ZEYLANICA JAPONICA* WITH TEMPERATURE IN SUMMER

Hiroko Inamura[1], Ikuo Yamanaka[2], Chieko Yamanaka[2]
[1]Department of Biology, Tokyo Medical University, Shinjuku, Tokyo 160-8402, Japan and [2]University Forest, The University of Tokyo University, Awa-gun Chiba 299-55

Brown adipose cells were swollen eggs shaped in the winter and summer. They included large oil droplets and mitochondria. We studied again how its form appears in summer during 1999 to 2003 a University Forest in Chiba. The monthly average temperature in late May was 18.1, at which time the form was spindle shaped. The temperature in late June was 21.3, at which time the form showed the shape of swollen eggs. Also, in late July it averaged 23.9, at which time the shape of swollen eggs spread throughout the internal area. The results indicate that these cells adapt to change in temperature.

FIBROMUSCULAR LAYER IN THE RESPIRATORY TRACT OF RED-BELLIED NEWT (*CYNOPS PYRRHOGASTER*)

Hideo Naruse[1], Akihiko Kimura[1], Ayano Ogawa[1], Keiko Kondoh[1], Hiroyuki Hayashi[2], Sachiko Kakuta[1], Toshiaki Gomi[3], Kiyoshi Kishi[1]
[1]Department of Anatomy, School of Medicine, Toho University Ota-ku, Tokyo 143-8540, Japan, [2]Department of Oral Histology, Kanagawa Dental College Yokosuka city, Kanagawa 238-8580, Japan and [3]School of Health and Social Services, Saitama Prefectual University Koshigaya city, Saitama 343-8540, Japan

Serial sections of the respiratory tract of red-bellied newt (*Cynops pyrrhogaster*) were made, and the relationship between the density (cells/$10^6\mu m^3$) of serotonin-immunoreactive neuroepithelial endocrine (NEE) cells and fibromuscular layer was studied. The respiratory tract was divided into five laryngotracheal portions (LT1-LT5) and one pulmonary portion (P). Then, each portion was further subdivided into ventral (V), dorsal (D) and lateral (L) surfaces. The density of serotonin-immunoreactive NEE cells was remarkably high in LT4-L and LT3-L subdivisions. In those portions, smooth muscle and many capillaries were observed in fibromuscular layer between the lateral cartilages and epithelium.

CULTURE OF LANCELET CELLS DERIVED FROM GONADS

Kinya Yasui[1], Kaoru Ikenaga[2], Yasuhisa Henmi[3]
[1]Marine Biological Laboratory, Graduate School of Science, Hiroshima University Mukaishima, Hiroshima 722-0073, Japan, [2]Institute of Molecular Embryology and Genetics, Kumamoto University Kumamoto 860-0811, Japan and [3]Center for Marine Environment Studies, Kumamoto University Aitsu, Kumamoto 861- 6102, Japan

To establish lancelet (amphioxus) assay systems for analyzing gene function and control of gene activation under background of this animal, we are culturing cells from lancelet branchial epithelium, gut epithelium, nerve cord, and gonads. Of these, cells from gonads spread well and proliferated in a medium consisting of Millipore filtered seawater 77%, HEPES 10 mM, D-MEM 16%, calf serum 5%, and L-glutamine supplemented with 4% (v/v) B27. GFP-expressing vector was successfully introduced into cells from gonads with an electroporator changing cell density or DNA concentration, and/or voltage/duration of pulse, but at very low rate.

EFFECTS OF LYSENIN ON CULTURED CHROMATOPHORES OF TELEOST FISH

Noriko Oshima[1], Masaki Honma[1], Akihiro Kanaya[1], Yoichi Inada[1], Naoshi Ohta[2], Hideshi Kobayashi[2]
[1]Department of Biomolecular Science, Faculty of Science, Toho University, Funabashi, Chiba 274-8510, Japan and [2]Research Laboratory, Zenyaku Kogyo Co., Nerima-ku, Tokyo 178-0062, Japan

Lysenin binds sphingomyelin (SM) specifically. It has lethal effects on various cultured cells of mammals excepting mouse melanoma cells (B16F10). In the present study, we examined the effects of lysenin on normal pigment cells cultured from swordtail, Nile tilapia, zebrafish, sumatra and topmouth minnow. Several min after the beginning of the treatment with lysenin (100 ng/ml), saltatory movements of pigment granules began at the tips of dendritic processes and the peripheral regions of the cell. The saltatory movements then spresded all over the cytoplasm of pigment cells. Tilapia melanophores, and melanophores, xanthophores and erythrophores of swordtail changed to a sphere in form or were cut into some small spheres, although the outlines of cultured pigment cells of other species were almost maintained. Immunofluorescence study using swordtail melanophores showed that radial arrays of microtubules within cells disappeared by the treatment with lysenin. Any lysenin-treated cells did not respond to norepinephrine, suggesting lethal effects of lysenin. Lysenin (100 ng/ml) that had been incubated beforehand with SM-liposomes (0.03 mM) for 10 min had no effects on pigment cells.

SUBCELLULAR LOCALIZATION OF INNEXIN, GAP JUNCTION RELATED PROTEIN IN INSECT CELLS

Tsutomu Ono[1], Daisuke Fushiki[1], Ryoichi Yoshimura[1], Yasuhisa Endo[1], Shigeo Imanishi[2], Kikuo Iwabuchi[3]
[1]Department of Applied Biology, Kyoto Institute of Technology, Sakyo-ku, kyoto 606-8585, Japan, [2]National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan and [3]Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Futyu-shi, Tokyo 183-8509, Japan

Innexin, gap junction related protein in insect, is thought that it isconcerned with intercellular direct communication. But, the cell biologicalanalysis has not been performed so far. To date, we have showed that gapjunction is formed and innexin is expressed in cultured insect cells, usingby microinjection of Lucifer yellow and immunocytochemistry of anti-innexinantibody. In this study, we will report localization of innexin in detail byimmunoelectron microscopy, as well as the changes of amount of innexinexpression under the various culture conditions by western blotting.