ESTABLISHMENT OF INBRED STRAINS OF *ARTEMIA FRANCISCANA*

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The brine shrimp *Artemia* is a phylogenetically important organism because of its progenitorship in Crustacea. All of *Artemia* is wild and genetically polymorphous. It is small and easily reared. Its generation time is about one month. Bisexually reproductive *Artemia* could breed in and in. *Artemia* lays dormant cysts. This is good for keeping and transporting the strains. In consideration of the above mentioned, inbred strains of *Artemia franciscana* from the Great Salt Lake in Utah, USA, have been established and kept, being over F_{30}.

MACRONUCLEAR GENOME WAS GENERATED BY TWO DISTINCT DNA-SPLICING MECHANISMS IN *PARAMECIUM CAUDATUM*

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Heterogeneity of DNA fragments containing globin (hb) gene was found in *Paramecium caudatum* macronuclear genome. To clarify its nature, we cloned and sequenced five DNA fragments with different sizes. These five fragments had the same sequence except for an internal eliminated sequence-like (IES-like) structure at the middle portion. The putative IESs, which were characterized by the presence of direct repeats of 2–11 nt at the both boundaries, leaving one copy of the repeat sequence at the junction. Inverted repeats were also found at the boundaries, and which agreed partially with the consensus sequence, TAYAGYNR, found in other species of *Paramecium*. We also found that a DNA fragment in which hb gene was fused head to head with nucleosome assembly protein 1 (nap1) gene was amplified by PCR with total genomic DNA. The sequence of the fused site indicates that this fusion gene was produced by a novel mechanism, different from usual IES removal. These results suggest that the heterogeneity found in the Paramecium macronucler genome produce not only compact assignment of the genes to remove intergenic regions but also non-functional fusion gene, by two distinct mechanisms.

ENDONUCLEAR SYMBIOTIC BACTERIUM *HOLOSPORA* SPECIES DISTINGUISHES THEIR TARGET NUCLEAR ENVELOPES

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The Gram-negative bacterium *Holospora* species distinguishes two kinds of nuclei, a macro- and a micronucleus, of the ciliate *Paramecium* species. For example, *H. obtusa* infects the macronucleus and *H. undulata* infects the micronucleus of *P. caudatum*. *Holospora* species are the only bacteria known so far that can distinguish two kinds of host nuclei. We developed monoclonal antibodies specific for outer membranes of *H. obtusa* and *H. undulata*. Indirect immunofluorescence microscopy showed that the antigens extracted from SDS-PAGE gels of each *Holospora* species bound to their target nuclear envelopes when the antigens were mixed with isolated macro- and micronuclei. Immunoblots showed that relative molecular masses of the antigens were 16 kDa in *H. obtusa* and 13 kDa in *H. undulata*. These antigens were unstained with ordinary silver staining, but stained with silver for bacterial lipopolysaccharide. Thus, our results showed that nucleus-specific infection of *Holospora* was controlled by an affinity between lipopolysaccharides of the outer membranes of the infectious forms of *Holospora* and unknown receptor substances exposed on the nuclear envelopes of the target nuclei.

COMPARATIVE STUDY OF AXOPODIAL CONTRACTION IN HELIOZOA

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Mechanism of axopodial contraction in heliozoa is still poorly understood. In this study, we investigated the axopodial contraction induced by artificial stimuli in two heliozoon species, *Raphidiophrys contractilis* and *Actinophrys sol*. Axopodial contraction in *R. contractilis* was found to be induced by both electric and physical shock. However, in case of *A. sol*, the axopodial contraction was not induced by physical stimulation. In both species, axopodial contraction was not observed in Ca^{2+}-free media. To identify cytoskeletal components required for axopodial contraction, electron microscopy was curried out. In *A. sol*, a bundle of contractile tubules was observed inside the axopodial cytoplasm, while only the axonemal microtubules were observed inside the axopodia of *R. contractilis*. These results indicate a possibility that the mechanism of axopodial contraction in *R. contractilis* is different from that in *A. sol*.

CA^{2+}-DEPENDENT NUCLEAR CONTRACTION IN THE HELIOZOON *ACTINOPHRYS SOL*

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Ca^{2+}-dependent contractility was found to exist inside isolated and detergent-extracted nucleus of the heliozoon *Actinophrys sol*. Nuclei were isolated in a Ca^{2+}-free solution. Diameter of the isolated nucleus (16.5 ± 1.7 μm) was slightly larger than that in living cells (15.1 ± 1.7 μm). Upon addition of Ca^{2+} ($[Ca^{2+}] = 2.0 \times 10^{-3}$ M), diameter of the isolated nucleus became reduced to 11.0 ± 1.3 μm. The threshold level of $[Ca^{2+}]$ for contraction was 2.9×10^{-7}M. Contracted nuclei became re-expanded when Ca^{2+} was removed by EGTA, thus cycles of contraction and expansion could be repeated many times by alternate addition of Ca^{2+} and EGTA. Ca^{2+}-dependent nuclear contractility remained after treatment with 2 M NaCl for 30 min, which suggests a possible involvement of the nucleoskeletal components in the nuclear contraction. Electron microscopy showed that, in the expanded state, filamentous structures are spread to form a network in the nucleus. After addition of Ca^{2+}, they became aggregated and constructed a mass of thicker filaments. These results suggest that the nuclear contraction may be induced by transformation of the filamentous structures in the nucleus.

ANALYSIS OF A PIWI-RELATED GENE IMPLICATES SMALL RNAs IN GENOME REARRANGEMENT IN *TETRAHYMENA*

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During development of the somatic macronucleus from the germline micronucleus in ciliates, chromosome rearrangements occur in which specific regions of DNA are eliminated and flanking regions are healed, either by re-ligation or construction of telomeres. We identified, a gene, *TWI1*, in *Tetrahymena thermophila* that is homologous to *piwi* (a gene required for stem cell maintenance in *Drosophila*) and is required for DNA elimination. We also found that siRNA-like small RNA molecules were specifically expressed prior to chromosome rearrangement during conjugation. These RNAs were not observed in *TWI1* knockout cells and required *PDD1* (another gene required for rearrangement) for expression. We propose that these small RNAs function to specify sequences to be eliminated by a mechanism similar to RNA-mediated gene silencing.

STRUCTURE OF MICROTUBULAR BUNDLE EXTENDED FROM BASAL BODY OF THE CILIATE *SPIROSTOMUM*

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At the cell surface of ciliate *Spirostomum*, it is known that the microtubular bundle extend toward the axis of the cell body. In this study, the structure of microtubular bundle extended from basal body were analyzed in three dimensions, using the transmission and scanning electron microscope. These bundles of microtubule were ribbon-like structure, and each ribbon was consisted of 6-7 or 3 microtubules. The number of microtubules in each microtubular ribbon was decreasing as it closed to the axis of a cell body. These microtubular ribbons which were extended from each group of the ciliary line of membranella became single bundle. Moreover, it became clear that these bundles were re-arranged and become the shape of large sheet. This sheet changed the run direction.and was not runing in the direction of an axis of a cell body, and running to parallel with the cell membrane. When microtubular bundle changes to sheet-like arrangement, it is thought that the resistance force become of opposing a twist force of a cell. Furthermore, it may be related to cooperation movement of membranella that ribbons became single bundle.

PROLIFERATION AND AGING OF *PARAMECIUM TETRAURELIA* UNDER HYPERGRAVITY

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Several papers reported that proliferation of *Paramecium* was sensitive to altered gravity condition. In order to investigate the direct effect of gravity solely on *Paramecium*, cells were axenically grown under hypergravity. The cell density was monitored in the course of the culture by a method consisting of optical slice and computer-aided image analysis. *P. tetraurelia* grown under 20xg had significantly slower proliferation rate, and had lower density at stationary phase. The reduced proliferation rate was maintained as long as cells were exposed to hypergravity (> one month). Hypergravity also changed the morphology of *Paramecium*. However it did not change the length of autogamy immaturity measured by mean fission age. The reduced proliferation rate under hypergravity, as a result, prolonged the immaturity period measured by clock time. Previous space experiments denoted that the proliferation of *P. tetraurelia* became faster under microgravity. If we assume the symmetrical effect of gravity on the maturation process of *Paramecium*, microgravity in space might reduce the length of immaturity period of *Paramecium* in clock time while maintaining the fission age unchanged.