CHICKEN DIAPHANOUS HOMOLOG ISOLATED FROM A cDNA LIBRARY OF CHICKEN GIZZARD.

M. Ito, H. Iseki, K. Yuza, S. Kohei, A. G. Terasaki and K. Ohashi. Dept. of Biol., Fac. of Sci., Chiba Univ., Chiba.

We isolated a clone of 6,222 bp from a cDNA library of chicken gizzard. The nucleotide sequence of the insert was determined. Analysis of the cDNA identified an open reading frame predicted to encode a 1,253 amino acid protein similar to *Drosophila* diaphanous, which we termed chicken Dia. Its calculated mol. wt was 139,257. Chicken Dia possesses the two conserved domains FH1 and FH2 (formin homology 1 and 2) and a RhoA-binding domain. Chicken Dia showed very high similarity with human Dia-156 besides the polyproline region (FH1). The FH1 domain of mouse p140mDia showed relatively high similarity to that of chicken Dia. The polypeptide encoded the N-terminal 176 amino acids was expressed in *E. coli*. An antiserum raised against this polypeptide. Affinity-purified antibody was used for immunoblotting and immunofluorescence microscopy. The antibody did not clearly detect any peptide bands of whole extracts of chicken breast muscle and gizzard smooth muscle. However, by immunofluorescence, striated staining patterns were observed in chicken breast muscle.

ANALYSIS OF MICROTUBULAR STRUCTURES OF *XENOPUS* EGGS DURING CYTOKINESIS

Makiko Takayama[1], Tatsuhiko Noguchi[1], Issei Mabuchi[1,2]. [1]Division of Biology, School of Arts and Sciences, University of Tokyo, Tokyo 153-8902, and [2]Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585.

It has been known that MTs play an important role in cytokinesis. In amphibian eggs, microinjection of microtubular poisons inhibits cytokinesis (Sawai et al., 1992). However, organization of MT during cytokinesis and their role have not been known yet.Therefore, we examined the MTs in *Xenopus* eggs during cytokinesis by immunofluorescence microscopy. The MTs extended from each centrosome seemed to merge and connect with each other in the cortex just beneath the furrow. This connection was continuously formed at the growing end of the cleavage furrow.

A STUDY ON VARIETY OF THICK FILAMENT DIAMETERS IN ADDUCTOR SMOOTH MUSCLE CELLS OF BIVALVES

K.Hamanaka and A. Matsuno[1]. Dept. of Biol., Fuc. of Sci. Shimane Univ., and [1]Dept. of Biol. Sci., Fuc. of Life and Environ. Sci., Shimane Univ., Matsue.

Adductor smooth muscle cells of bivalves bear paramyosin cored ultra-thick myofilaments measuring 50-100 nm in diameter. These myofilaments had been discussed whether they might have some parts of "catch contraction". However, it is generally believed that bivalves prepare those thick myofilaments to polymerize myosin molecules on the paramyosin cores under high-ionic conditions at habitats of the bivalves. Under these circumstances, we try to conform whether diameters of those ultra-thick myofilaments show varieties among the specimens living in fresh water, brackish water and sea water. Muscle cells of the three specimens were observed by an electron microscope and photographed. Diameters were measured from photographs and the measurements were analyzed statistically. As the results, muscle cell of *Anodonta* (fresh-water) had three kinds of thick myofilaments;44,54 and 68nm in diameter. Cells of *Corbicula* (brackish water) and *Tapes* (sea water) bear also three kinds of thick myofilaments measuring about 42,62 and 74 nm, and 48,62 and 72nm respectively. The muscle cell of *Corbicula* (brackish water) has the largest numbers of thick myofilaments among them, and *Anodonta* (fresh-water) has smallest numbers.

THE POLARIZED ORGANIZATION OF CELLS OBSERVED WITH A CENTRIFUGE POLARIZING MICROSCOPE

M. Goda[1], S. Inoué[2], G. Gundersen[3] and R.A. Knudson[2].
[1]Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto. [2]Marine Biological Laboratory, Woods Hole., USA [3]Department of Anatomy and Cell Biology, Columbia University, New York, USA

Using a centrifuge polarizing microscope (CPM), we explored the polarized organization of cells induced by high gravitational forces. The CPM [developed jointly by MBL, Olympus Optical, and Hamamatsu Photonics] displays birefringence and DIC images resolved to better than 1 μm at up to 10,000 times gravity (x g). Oocytes of *Cheatopterus pergamentaceous* in isopycnic seawater showed strikingly different patterns of stratification and fine structural organization before and after nuclear envelope breakdown. These differences suggest changes in cytosolic calcium pumping activity associated with maturation of the oocytes. Mouse fibroblasts, growing directly on glass coverslips (surface oriented parallel to the g-force) and bathed in standard culture media, maintained their shape and were not detached except in metaphase even at 10,000 x g. Also unexpectedly, organelles were barely redistributed within the fibroblasts whether or not they contained vimentin fibers. However, we did observe accentuated ruffling, growth, and pinocytotic activity that seemed to occur preferentially at the centrifugal side of cells involved in tissue wound healing. These preliminary observations show the CPM's ability to unveil unsuspected physiological dynamics and mechanical attributes of fine structure within living cells.

PROCESS OF NUCLEAR PORE COMPLEX FORMATION IN THE NUCLEAR MEMBRANE OF SPERM HEAD CHROMATIN UNDERGOING NUCLEAR RECONSTITUTION IN THE *XENOPUS* INTERPHASE EGG EXTRACTS

A.Shinozaki, S.Tsuchida and T.Yagura
Laboratory of Life Science, Department of Chemistry, Faculty of Science, Kwansei Gakuin University, Nishinomiya-shi, Hyogo

The sperm head chromatins undergo the reconstitution process of nuclear membrane with nuclear pore complexes in the Xenopus interphase egg extract. The detailed process of formation of nuclear pore complex was examined using this reconstitution system. Addition of hemin, a strong hydrophobic compound, to the nuclear reconstitution system resulted in abnormal nuclei exhibiting flattened membrane patches randomly distributed both on the surface and inside the nuclei. We have found several structures in these nuclear membrane patches that appeared to be intermediate forms of nuclear pore complex in addition to the normal nuclear pore complexes. By the experiments using the antibody specific to the nuclear pore complex protein, we revealed a part of the process of nuclear pore complex formation in the nuclear membrane. At first, the nuclear pore proteins assembled to the inner surface area of nuclear vesicle on side attached to the chromatin. Then, when the membrane vesicles were flattened to make nuclear double-layered membrane, the pores were formed as the cluster of nuclear pore proteins was in contact with outer side of membrane.

ABNORMAL EXPRESSION OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN CEREBELLAR CORTEX PRECEDEDS THE ONSET OF ATAXIA IN *DILUTE-LETHAL* MICE.

K.Sawada, S.Komatsu, H.Haga, Y.Fukui, Dep of Anat, Univ of Tokushima Sch of Med, Tokushima

Expression of tyrosine hydroxylase (TH) immunoreactivity in the cerebellum was examined in *dilute-lethal* mice (DL) prior to and following the onset of ataxia. Walking of DL were daily monitored from postnatal days 7 to 21. Mice falling over within 30 steps were regarded as ataxic. After behavioral testing, some mice were perfused at 7, 8, 9, 10 and 12 days of age with Bouin's solution without acetic acid. Cerebella were immersed in the same fixative, embedded in paraffin, and sectioned serially in the frontal plane at 3 μm. Sections were reacted with anti-TH mouse monoclonal antibody at 4°C overnight. The immunoreactive products were visualized by Vectastain ABC elite kit

DL walked normally on days 7 and 8. Falling over when walking was exhibited by about 20% of DL on day 9 and by all DL by day 10. TH-positive Purkinje cells in lobules IX and X of the vermis of either ataxic or non-ataxic DL were clearly observed on day 9 when compared to control mice, and had drastically increased by day 10. On day 12, parasagittal organization of TH-positive Purkinje cells became evident. These results revealed that abnormal TH expression occurred in some Purkinje cells of DL cerebella, preceding the onset of ataxia.