# 1430

# Cell Biology and Morphology

the circulatory system in a pycnogonid, Ammothella biunguiculata by LM with serial semithin sections and TEM reveals that the resemblance to mandibulates would be superficial. In addition, some possible synapomorphies with euchelicerates are found. The musculation of dorsal vessel wall and horizontal septum is very weak. Observation on the living specimens suggests that the peristalsis of gut is more effective in hemolymph propulsion than the contraction of dorsal vessel and horizontal septum

#### **IMMUNOHISTOCHEMISTRY OF INSECT INNEXIN (GAP JUNCTION-RELATED PROTEIN)**

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Gap junction in invertebrates consists of innexins, which have no homology in amino acid sequence to connexins in vertebrates. Up to date, it remains to clarify the distribution of innexins in cellular and tissue level of insects. We prepared the peptides containing carboxyl terminals of Bombyx innexine 2 and immunized mice. Using the specific antisera against Bombyx innexine 2, we tested the various tissues of cockroaches and silkworms by immunohistochemistry and Western blot.

#### BROWN ADIPOSE CELLS OF THE LAND LEECH, HAEMADIPSA ZEYLANICA JAPONICA IN SUMMER

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In dry weather, no land leeches appear, but immediately the rainy season bigins, they are active. We have reported that the brown adipose cells of land leeches in the cold season change to the shape of swollen eggs. They included very large oil droplets and also mitochondria which were located around the nucleus. In this study, we observed how the form of the brown adipose cells reacted to summer. We collected them at the University Forest in Chiba of the University of Tokyo during summer 2001. We also made use of weather data obtained on the University Forest in Kiyosumi. The monthly average temperature in the height of summer was 25°C, at which time the form of the adipose cells and oil droplets resembled their winter appearance. The cytoplasm increased in volume and became lighter in color than in June at which the monthly temperature was 20°C. It was of interest that mitochondria in midsummer had something in common with those at low temperatures. These results suggest that the brown adipose cells may have an important role in thermolysis and metabolism to enable adaptability to the environment temperature in midsummer.

# MELATONIN INVOLVED IN THE MECHANISM OF ANDROGEN ACTION IN LNCaP CELLS

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Although it has been reported that melatonin exhibits an anti-proliferative effect on some cancer cells, the mechanism of this action is not yet fully understood. The present experiments were performed to verify whether melatonin might modulate the production of PSA in LNCaP cells, and to obtain the information on its possible mechanism of melatonin action. LNCaP cells were used throughout the experiments. The cells were maintained in RPMI-1640 medium supplemented with 0.1% FBS in the presence or absence of melatonin and DHT for 4 days. DHT significantly enhanced PSA production, and DHT-induced PSA increase was completely abolished by melatonin. However, melatonin at doses used did not influence on cell proliferation. The effect of melatonin on the expression of AR was less pronounced, though the effect on IGF-1R was significant. Present results seem to show that melatonin inhibits a mechanism involving in PSA production rather than in AR expression and cell proliferation. proliferation.

### ASCORBIC ACID INDUCES NECROSIS

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Ascorbic acid is coenzyme of collagen generation, therefore ascorbic acid is essential for vein maintenance. However, high concentration of ascorbic acid induced cell death had been observed in several cell lines in vitro. We previously reported that high concentration of ascorbic acid losserved by phase-death. In the present research, high concentration of ascorbic acid (< 3 mM) induced vacuolation, vacuole swelling, and finally membrane burst, as observed by phasecontrast microscope. The cell death was not inhibited by caspase inhibitor, Z-VAD-fmk. As analyzed by agarose gel electrophoresis, ascorbic acid did not induce internucleosomal DNA fragmentation. These results suggest that ascorbic acid induces necrosis characterized by autophagocytosis in bovine aortic vascular endothelial cell line

# INITIAL ANALYSIS OF FACTORS THAT REGULATE THE DIRECTION OF SEROTONERGIC NERVE FIBER ELONGATION IN ECTODERM CELL REAGGREGATES IN SEA URCHIN EMBRYO

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During early period of serotonergic apical ganglion formation in plutei of the sea urchin, *Hemicentrotus pulcherrimus*, neve fibers are extended from the basal side of serotonin cells toward the embryonic middle line to form a neuro plexus (Yaguchi *et al.* 2000). The serotonergic cell neuro plexus, thus is formed by nerve fibers that are extended toward homophilic cells. Serotonergic cells are derived from ectodermal cells at the apical tuft region. To elucidate the guidance cue that regulates such directed nerve fiber elongation *in vitro*, we have used ectodermal cell reaggregates prepared from midgastrulae. The dissociated ectodermal cells reaggregates. They serve fibers in the reaggregates. They have not been the reaggregates that they directed nerve fibers in the reaggregates. They never extended the fiber outwardly from the reaggregate. We will report how the direction of nerve fiber elongation is regulated by microenvironment in ectodermal cell reaggregates.

# PHENOTYPE OF CULTURED NEURONAL CELL LINE (NG108) CHANGED UNDER THE CO-CULTURE WITH SMOOTH-MUSCLE CELL LINE (SM3)

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NG108 cells, one of typical cultured neuronal cells, are differentiated into multipolar type by the addition of dbcAMP to the culture medium. We tried the construction of in vitro co-culture system in order to resolve the contribution of neurons on the vascular regeneration. Under the co-culture with vascular smooth muscle cell line (SM3), a half of NG108 cells was unipolar in type. The rest quarters were bipolar and multipolar, respectively. Using filter-insert culture system, our results indicated that the secretory factors fromSM3 cells caused these changes.

# **GROWTH FACTOR ARRAY FABRICATION USING AN INK JET PRINTER**

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In recent years, ink jet technology has achieved remarkable progress, which now makes it possible to manipulate tiny droplets in desired patterns and quantities. In this study, we used a commercial color ink jet printer to print growth factors on cell culture substrates. We fabricated an array of growth factors consisting of multiple small squares with different densities of insulin-like growth factor-I (IGF-I), basic fibroblast growth factor (bFGF) and bone morphogenic protein-2 (BMP-2). C2C12 myoblast cells were cultured on this substrate and the onset of myogenic and osteogenic differentiation was monitored for the expression of myogenin and alkaline phosphatase. The ratio of the cells expressing myogenin varied with the doses of IGF-I, bFGF and BMP-2 in each square. Since the ink jet printer manipulates several colors, this method can easily be applied to multivariate analysis of growth and attachment factors affecting cell growth and differentiation. This method may provide a powerful col for tissue engineering and cell biology. powerful tool for tissue engineering and cell biology.

# COMPARATIVE STUDIES ON THE ROLE OF COFILIN/ADF PROTEINS IN MYOFIBRILLOGENESIS DURING AVIAN MUSCLE DEVELOPMENT

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Recently increasing numbers of reports have accumulated that indicate that cofilin/ADF family proteins play important roles in muscle tissues. In this study, we compared how cofilin and ADF are involved in muscle morphogenesis as follows. First, relative proportion of the dephosphorylated form of each protein, an active form, was analyzed in developing muscle as well as the whole amount of each protein. The results indicated that the proportions change as muscle develops, but the changing patterns are somewhat different between the two proteins. Secondly, the ability of the proteins to bind to F-actin and their actin-depolymerizing ability were estimated by co-precipitation assay, and some difference was observed between the two proteins. Thirdly, the recombinant proteins were introduced into cultured muscle cells to test their effects on actin in filament in the cytoplasm. The results indicated that cofilin generates actin rod more efficiently than ADF. These results suggest that in developing musce alls cofflin plays more important roles in durantic organization of acting that a DF. developing muscle cells, cofilin plays more important roles in dynamic organization of actin than ADF.