ROLE OF LIGHT CHAINS IN THE MOTOR FUNCTION OF RAT CARDIAC MYOSIN

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We studied the role of light chain in the motor function of cardiac myosin, by comparing two different rat cardiac myosins with the same heavy chain; one contained atrial type light chains (A-myosin), while the other contained ventricular type light chains (V-myosin). Both the actin-activated ATPase activity and the actin sliding velocity did not differ significantly between the two myosins. The time-averaged force generated by a single myosin molecule was appreciably higher in V-myosin than in A-myosin, and the optical trap experiments showed that the duty ratio was longer in V-myosin than in A-myosin.

X-RAY DIFFRACTION FROM QUICK-FROZEN MYOFIBRILS OF STRIATED MUSCLES FROM VARIOUS ANIMALS

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The myofilaments of striated muscle are arranged in a hexagonal lattice within a sarcomere. We have shown that, by the use the microdiffraction technique, the lattice planes from the flight muscle of higher insects (including Hymenoptera and Diptera) were almost exactly in register over a distance of several millimeters, or ~1,000 sarcomeres in series. To examine to what extent the lattice planes are in register in vertebrate striated muscles, we quick-froze skinned rabbit skeletal muscle fibers, cutting them into short (~0.3 or ~100 sarcomeres) segments and irradiated them end-on with 2-μm X-ray microbeams while they were kept frozen. The diffraction patterns recorded in this way did not show a slightest sign of hexagonal myofilament arrangement, but simply consisted of concentric circles with uniform intensities along their circumferences. This result suggests that, in vertebrate skeletal muscle, the register of filament lattices is lost within a very short stretch of the myofibril (much less than 100 sarcomeres).

GROWTH FACTOR ARRAY FABRICATION USING AN INK JET PRINTER II

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In our previous paper (Zool. Sci. 20; 429-434, 2003), we used 4-azidbenzoic acid N-hydroxysuccinimide ester to immobilize peptide growth factors and to fabricate multi factor array. In this study, we improved the method of immobilization of growth factors. Growth factor was immobilized on dishes via covalent bonds to an activated dextran. When we cultured the mouse C2C12 cells on FGF-2-immobilized substrata, cell proliferation was promoted. Immobilized BMP-2 induced osteogeninc differentiation. On IGF-I-immobilized substrata, myogenic differentiation was enhanced. By this method, the multiple actions of these three factors and the comparison of the liquid and the solid systems demonstrated same tendency. Furthermore we developed new analysis methods. These methods may provide a novel tool for cell biology and tissue engineering.

CELL-BEHAVIOR DATABASE

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Has modern biology devoted itself to understand 'living cells' to the same extent as it has devoted it to understand 'molecules'? On this introspection we have started an attempt to recognize 'cells' as active subjects or entities and to register their behavior in a database.

When we see a chain of persons hand-in-hand forming a circle we immediately think of the meaning of their action. Are they dancing? Are they peace demonstrators? Are they measuring the diameter of a tree trunk? We only say 'people are connected to one another in a closed circle by their hands' when we don't understand the meaning of the situation. Likewise, evaluation of the 'aim' of a cell's action is expected to bring a deeper insight into the understanding of the functional aspect of the cell. For example, epithelial cells are not just 'adhering' to one another by cell junctions, but are 'forming' an epithelium. The latter expression implies all the functional aspects of epithelium, even those unknown to us, instead of just describing the actual state of cells. Regarding cells as entities pursuing their aims will lead us much closer to understanding what is going on in living phenomena.

MYOFIBRILLOGENESIS OF CULTURED SKELETAL MUSCLE ON POLYACRYLAMIDE GEL: DESTRUCTION BY STATIC-STRETCH AND ITS RECOVERY

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Myofibrillogenesis of chick skeletal muscle proceeded normally on collagen-crosslinked polyacrylamide gel equipped on a extensible device. When judged by the location of desmin, matured myofibrils were formed by day 7 of culture. However, this myofibrils decomposed by stretching the acrylamide-gel substrate for 18h by 5 % (static stretch) without affecting the overall shape of myotubes. Immunofluoresence microscopy indicated that sarcomeric striation disappeared and actin and myosin distributed diffusely throughout cytoplasm. SDS-polyacrylamide gel electrophoresis of the myotubes showed that about 70% of total proteins were in Triton X-soluble fraction. The ratio of actin detected in the soluble fraction to that remained in cytoskeletal (attached) fraction was 4:6 while the ratio was 2:8 in control un-stretched myoblasts. On the other hand, almost all myosin was found in the cytoskeletal fraction. We found actin filaments with 1 um-length by negative staining of the soluble fraction. These observation suggested that decomposition of myofibrils by stretch occurred at A- and I-filament level and further depolymerization of these filaments would be small.

SUPPRESSION OF COFILIN EXPRESSION BY ANTI-SENSE MORPHOLINO OLIGO NUCLEOTIDES AND MYOFIBRILLOGENESIS IN CULTURED SKELETAL MUSCLE CELLS

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Cofilin is an actin regulatory protein that plays a critical role in actin filament dynamics in a variety of cells. We have previously demonstrated that excess of cofilin in myotubes leads to disruption of actin filaments followed by actin-cofilin rod formation in the cytoplasm. In this study, to further clarify how cofilin is involved in regulation of actin assembly in the process of myofibrillogenesis, we attempted to suppress cofilin expression in cultured myotubes by applying an anti-sense method with Morpholino oligonucleotides. Anti-sense or sense probe was directly injected into chicken skeletal myotubes in culture and the cofilin level was examined by immunofluorescence staining with anti-cofilin antibody (MAB-22). Fluorescence intensity was obviously decreased in the myotubes where the anti-sense oligonucleotide were introduced and in these myotubes, ordered assembly of actin into sarcomeric structures was significantly suppressed. Myosin assembly into myofibrils was scarcely affected. These results indicate that cofilin has a critical role in the regulation of actin assembly at the early process of myofibrillogenesis.

DISORGANIZATION OF MYOFIBRILS IN COFILIN-DEFICIENT SKELETAL MUSCLE CELLS

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Cofilin (CF) is an actin-binding protein that is important for actin dynamics in a variety of cells. Here, we report the effect of CF deficiency on mouse muscle cells in vivo . Although two CF isoforms exist in mammals, muscle-type cofilin (MCF) is solely expressed in differentiated skeletal muscle cells. We prepared MCF-deficient muscle cells in chimeric mice that were generated with MCF-/- ES cells. MCF-deficient cells were detected using b-gal expression as a marker, since b-gal gene was put in MCF gene locus in the MCF-/- ES cells. Abnormal morphology, such as variation in fiber size, accumulation of mono-nucleated cells and fiber degradation, was observed in the MCF-deficient skeletal muscle regions. When transverse and longitudinal cryo-sections of the muscle were stained with anti-b-gal antibody plus anti-myosin antibody or rhodamine-phalloidin, actin and mysoin were detected in a disorganized pattern in the muscle cells that contained b-gal. These results indicate that CF is required not only for myofibrillogenesis during development, but also for the maintenance of myofibril structure in differentiated muscle cells.

A UBIQUITOUSLY DISTRIBUTED MICROTUBULE-ASSOCIATED PROTEIN 4 (MAP4) EXPRESSES A NEURON-SPECIFIC ISOFORM

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The microtubule-binding domain of MAP4 contains a region rich in proline and basic residues (proline-rich region). We searched the bovine adrenal gland for

MAP4 isoforms, and identified a novel variant lacking 72 consecutive amino acid residues within the proline-rich region, as compared with the full-length MAP4. The amino acid sequence of the missing region was highly conserved among the corresponding regions of bovine, human, and mouse MAP4, which suggested the functional significance of this region. A comparison of the genomic sequence with the cDNA sequence revealed that the missing region is encoded by a single exon. It is possible that the new isoform can be generated by alternative splicing, not only in bovine but also in other mammalian species. The mRNA expression of the novel isoform was restricted to the brain and adrenal medulla, suggesting that this is a neuron-specific variant.

ELECTRON MICROSCOPIC STUDIES ON EYE-REGION OF "MENASHI MUTANT" IN THE PLANARIAN *DUGESIA RYUKUENSIS*

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Worms of an asexual clone (the OH strain) in the planarian *Dugesia ryukuensis* are sexualized by being fed with sexually mature planarians containing the sexualizing substance. Inbreeding of the sexualized worms produces "Menashi mutants" which represent eye-less like phenotype and emerge at a rate of 2.5% in the F1 population. In order to confirm the abnormality of eyes, we examined a fine structure of cells in the eye-region by TEM. In the wild type, the eye consisted of both pigment cells containing electron-dense pigment granules and visual cells with well-developed endoplasmic reticula (ER). The pigment cells contacted with rhabdomes of the visual cells. Although the Menashi mutants possessed both pigment cells and visual cells, their morphology was extremely irregular. In the pigment cells, multivesicular bodies containing small electron-dense vesicles, namely pigment granules like so-called premelanosome granules were abundantly observed. In the visual cells, ER were seldom observed and immature rhabdomes were surrounded by stalks of the visual cells, resulting that the rhabdomes were not adjacent to pigment cells.

ULTRASTRUCTURE OF THE VISUAL ORGAN OF THE TERRESTRIAL PLANARIAN, *BIPALIUM NOBILE*

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In the animal kingdom, planaria is the first animal that has obtained the brain and the specialized sense organs in primitive forms. We already reported that photoreceptor organs, eyes, in *Bipalium nobile* consisted of four optic cells and a pigment cell. Each distal portion of four optic nerve cells became sensory hair-like microvilli(dendrites), encircled with a goblet-shaped pigment cell(pigment cup) and the opposite portion of the microvilli lined up in the shape of a fan was named cones. In the upper side of the cones, a great number of mitochondria was observed. At the top of the pigment cup, four cones were gathered, and then the cytoplasm of each optic nerve cell became attenuated. Each narrow cytoplasmic band of the cells ran back to the pigment cup. The axon proceeded from the base of the cell(opposite to the apical dendrite). In the pigment cell(pigment cup), its granules accumulated perinuclear area and the cytoplasm in both ends of the cell did not possess the granules. These observations indicated that terrestrial planaria could only recognize the direction of light.

STRUCTURE OF BROWN FAT CELLS OF THE LAND LEECHES, *HAEMADIPSA ZEYLANICA* VAR. *JAPONICA* IN SPRING

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In winter, the brown fat cells of land leeches became swollen and contain very large oil droplets and many mitochondria. In summer, brown fat cells showed similar characteristics. In this study, we observed the structure of cells in land leeches collected at the University of Tokyo, Forest in Chiba which displayed activity in spring. There were some differences in cells of feeding leeches and leeches which were not feeding. Although in both subjects the shape of the cells were long spindle including many small oil droplets, the cells of the feeding leeches showed bright colors. However, the cells of non feeding leeches were darken because they included many mitochondria. These results suggest that the influence of temperature rise and feeding are related to the role of the structural changes of the brown fat cells in spring.

ANATOMY OF THE CRANIAL NERVES OF MEDAKA, *ORYZIAS LATIPES*

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In recent years, large-scale production of the mutants has been carried out using Medaka, identifying various mutations affecting development and organization of a nervous system. To understand the functional organization of the nervous system, it is essential to have a detailed anatomical description the brain and cranial nerves. This work was undertaken for this purpose, using adult fish of a Cab strain of Medaka. Besides Biocytin and DiI labeling, Medaka was utilized which expresses GFP in the cranial nerves. Positions and morphological characteristics of the nuclei in the brain, routes of the neurite bundles, and their projections are documented for each cranial nerve.

DISTRIBUTION OF NEE CELLS AND NEBS IN THE RESPIRATORY TRACT OF RED-BELLIED NEWT (*CYNOPS PYRRHOGASTER*)

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Serial sections of the respiratory tract of red-bellied newt (*Cynops pyrrhogaster*) were made, and the density of NEE cells and NEBs in different regions of the respiratory tract was studied. The respiratory tract was divided into five laryngotracheal portions (LT1-LT5) and one pulmonary portion (P). Then, each laryngotracheal portion was further subdivided into ventral(V), dorsal(D) and lateral(L) surfaces. The density of NEE cells was highest in LT4-L, and lower in LT3-L, LT4-D, LT5-D and LT2-L in descending order. The density of NEE cells was lowest in P-L, and higher in P-V and P-D in ascending order. In addition, a small number of NEBs, which are organoid groups of NEE cells, was detected in the caudal lining of the laryngotrachea.

VASOACTIVE INTESTINAL POLYPEPTIDE AND CALBINDIN-D28K IMMUNOREACIVE NEURONS IN THE OLFACTORY BULB OF THE MUSK SHREW, *SUNCUS MURINUS*

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The distribution of vasoactive intestinal polypeptide (VIP)-immunoreactive elements in the main olfactory bulb of the musk shrew, *Suncus murinus*, were studied by immunostaining, and determined the degree of colocalization of VIP and calbindin-D28k (CB). VIP-positive (+) neurons were identified as subpopulations of periglomerular and perinidal cells in the periglomerular region, as the superficial short-axon, Van Gehuchten and satellite cells in the external plexiform layer (EPL). In the periglomerular region, no cells colocalized VIP and CB. The EPL neurons were divided into three groups, which were VIP+ and CB+, VIP+ and CB-negative (-), and VIP- and CB+ neurons. VIP+ superficial short-axon cells extended some neuronal processes toward the nidi, and others with VIP+ varicosities surrounding the tufted cells. VIP+ Van Gehuchten and satellite cells extended many branches of neuronal processes with varicosities surrounding the mitral and tufted cell. Some varieties and numerous VIP+ neurons in the EPL, as compared with a few VIP+ periglomerualr and perinidal cells, suggests that the neuron circuits in the EPL of this species much more complicated than originally thought.

THE APPEARANCE OF UNUSUAL LEUCOPHORE-LIKE CELLS IN THE PERIODIC ALBINO MUTANT OF *XENOPUS LAEVIS*

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The periodic albino mutant of *Xenopus laevis* is characterized by the delayed melanization and subsequent depigmentation in both retinal pigment epithelium and melanophores. Previous study demonstrated that dermal melanophores with abnormal melanosomes disappear after metamorphosis, and epidermal melanophores do not appear in this mutant. However, it is still not known how a^p gene acts in pigment cells. In the present experiment, three types of pigment cells (melanophores, xanthophores, and iridophores) were thoroughly investigated both in vivo and in vitro. It was revealed that unusual pigment cells, which reflect light and look white, appear in the lineage of melanophores in the periodic albino mutant. These leucophore-like cells also fluoresce under blue light, but are different from xanthophores or iridophores. The leucophore-like cells that appear specifically in the mutant do not exist in the wild-type.

DETECTION OF NUCLEAR/CYTOPLASMIC PROTEINS RELATED TO VORTICELLID SPASMIN IN HeLa CELL

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Spasmin is a calcium-binding protein that is the major component of the calcium-induced contractile filaments, called spasmoneme found in vorticellid ciliates. Such filaments have not been observed in any organisms other than green algae. To determine whether calcium-induced contractile filaments like spasmoneme are