Morphological observation of single myofiber

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It is well known that myofiber changes its morphological features in regeneration process followed by degeneration. However, it still remains unclear how the morphological changes would occur through overall of the myofiber because a huge cytoplasm of the cell makes us difficult to evaluate whole cellar morphology by microscopic observation on tissue section samples. To address this problem, we first established a new method to isolate a large number of myofibers by alkali maceration. Isolated myofibers were suitable for morphological analysis, since they had neither non-myogenic cells nor artificially induced structural damages. Then, we should do morphological analysis on isolated myofibers obtained from the mdx mouse, a mutant animal with congenital membrane disorder and its wild type. As the results, we found followings. 1) Unique myonuclear clusters and cytoplasmic bifurcation were found through overall of the myofiber in the damaged muscle. 2) The appearance rate of the abnormal myofiber would reflect the degree of the muscular damage.

Structural changes in the smooth muscle fibers of digestive tracts in tadpoles (Rhacophorus owstoni) during metamorphosis

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The tadpole intestine is remodeled during metamorphosis, the long larval thin intestine begins to shorten, and is modified the adult thick intestine. We studied the musculature of the intestine in tadpoles of the treefrog by both scanning electron microscopy and immunohistochemistry with a-smooth muscle actin antibody. Before of metamorphosis, the smooth muscle fibers (SMFs) are rearranged in place two layers that are longitudinal and circular. End of metamorphosis, the density of SMFs is high in both layers, and it consists of several layers that superposed of SMFs. In longitudinal muscle layers of large intestine, the density of SMFs in lateral bend is concentrated greater than in the median bend. The results suggest aggregation in the SMFs of muscle layers of the intestine, as well as rearrangement of SMFs, with the metamorphosis.

BIOLUMINESCENCE IN THE TUNIC OF THE COLONIAL ASCIDIAN, Clavelina miniata.

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When the tunic of the colonial ascidian, *Clavelina miniata*, is stimulated mechanically, it emits green light(λ max=535nm). Three types of tunic cells can be distinguished morphologically. Previous work demonstrated that phagocytes exhibit luminescence by the physical stimulation. However, the luminescent system of *C. miniata* is unclear.

To study molecular mechanism of bioluminescence, the tunic cells of *C. miniata* were lysed by freeze-thaw in the presence of 1.0% cholic acid, CHAPS and *n*-Octyl- β -D-glucoside. Extract of tunic cells was resolved by 2-dimensional gel electrophoresis (native PAGE / SDS-PAGE). When the gel was scanned on the fluorescent image analyzer, a green fluorescent band was clearly detected, suggesting that the fluorescent protein may be involved in the bioluminescent system of *C. miniata*.

It is known that round granules and clear vacuoles are found in the tunic phagocytes in the electron microscopic observation. When phagocyte was observed with fluorescent microscope connected with SIT camera (C2400-08, Hamamatsu Photonics), green fluorescence was detected in the round granules. Thus, the fluorescent protein is likely to be distributed in the round granules.

COMPARATIVE STUDIES ON THE EPIDERMAL MUCOUS CELLS IN SEVERAL TERRESTRIAL ANIMALS

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The land planarian, *Bipalium* secretes rich mucus when moving, but there have been few histological reports on them. A land slug, *Incillaria* and an earth worm, *Pheretima*, which are similar to them in living conditions and behavior, were previously studied (Furuta et al). We compared *Bipalium* with the above animals of the epidermal mucus cells. In *Bipalium*, several mucus cells can be distinguished by staining and seem to contain granules on light microscopy. In this study we observed them morphologically by TEM. Basement membranes of the epidermal cells were clearly observed *Bipalium* and *Pheretima*, but not in *Incillaria*. And in *Incillaria* and *Bipalium*, microvilli and cilia of epidermal cells were also observed remarkably. There were various kinds of epidermal mucous cells: at least 3–4 types *Bipalium* with regard to excretion and differences of the electron density of the granules. These observations suggest that various secretory cells not only adhire strongly during periods of movement are also able to recognize each other.

THREE-DIMENSIONAL ULTRASTRUCTURES OF EPITHELIALL CELLS IN THE MOUSE EPIDIDYMAL DUCT

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The epididymal duct is a long, extremely tortuous tube, whose morphology and function in the epididymis differ slightly in the ductal segments and according to anima species. In mouse, this duct is comprised of five segments. Sperm following discharge from the testis undergoes functional and morphological differentiation during passage through the epididymal duct. Morphological and stereostructural differences in the epithelial cells in all five segments of mouse epididymal duct were examined by SEM, with the O-D-O method which permits 3-dimensional observation of cell organella.

Comparative histochemical studies of the liver of fishes in relation to their behaviour

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Teleost livers are classified (roughly) into two groups by histochemical properties in hepatocytes. One group is glycogen-rich hepatocytes, and the other is a lipidrich. The hepatic metabolism is in intimate connection of hepatic blood circulation and biliary pathway. We discussed the correlation between histological components in livers and behavior by scanning electron microscopy and histochemical technique. Glycogen-rich group is well developed both the sinusoidal blood system and the bile ductal system, but not lipid-rich group. In addition, a group of streamlined and well moved fish has glycogen-rich liver, and a flat bottomed and poor moved fish that live in the bottom of ocean and river, has lipid-rich liver.