ANALYSIS OF NOVEL mRNA VARIANTS OF *PERIOD* GENE IN HONEYBEE, *APIS CERANA JAPONICA*

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The biological clock is regulated by clock genes and their protein products. *Period* is one of these clock genes, and the molecular biology of *period* is well studied in insects. Previously, we have reported two mRNA variants, per alpha and per beta, which are considered to be generated by alternative splicing. These variants might have some important roles in the biological clock and clock related behaviors of honeybees such as sun compass, time memory, and timing of mating flights. In this study, we report novel mRNA variants of *period* gene in honeybee, *Apis cerana japonica*. From the analysis of genomic DNA, these novel variants are also considered to be generated by the alternative splicing of the typical gt-ag introns. We kept the honeybee colony under the light-dark cycles (LD = 12:12), and analyzed the expression level of total *period* mRNA in brains and muscles by real-time RT PCR. Simultaneously, we analyzed the expression ratio of these *period* mRNA variants. The results showed that the expression pattern differ between brains and muscles.

CLOCK GENES EXPRESSION IN HEAD AND PERIPHERAL TISSUES OF *BOMBYX MORI*

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Circadian clocks in animals regulate the timing of molecular, physiological, and behavioral rhythms. Environmental features such as photoperiod and temperature cycles reset these biological oscillators, enabling anticipation of dawn, dusk, and season. In *Dorosophila melanogaster*, clock genes have been identified that are necessary for circadian rhythms. The clock genes expressing in pacemaker neurons of brain regulate the daily rhythms but several genes are expressed in peripheral tissue. Although the molecular mechanisms of circadian clock in the brains are well documented, the specific functions outside the brains are unknown. To investigate the circadian systems at molecular levels in head and peripheral tissues, we demonstrate expression analyses of *D. melanogaster* clock gene homologues in *Bombyx mori*. Northern blot analyses show that the clock genes are expressed in larval head, adult head, testis, ovary and flight muscle. The fact suggests the presence of independent photoreceptive circadian clocks throughout *Bombyx mori*.

PERCEPTION OF MOONLIGHT BY THE CULTURED PINEAL GLAND OF A LUNAR-SYNCHRONIZED SPAWNER, THE GOLDEN RABBITIFISH

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The golden rabbitfish, *Siganus guttatus*, is a definite lunar-synchronized spawner and repeats spawning at the first quarter moon during the spawning season. At present, it is obscure how cues from the moon are recognized by this species and conveyed as endogenous rhythm. The aim of the present study was to evaluate moonlight perception in the cultured pineal gland of this species. We measured melatonin secreted in culture medium under various conditions using time-resolved fluoroimmunoassay. Melatonin level in the medium during the light condition was higher than that during the dark condition. Under the constant dark conditions, melatonin increased during the subject night. Under the constant light condition, melatonin changed at low levels. After the pineal gland was exposed to moonlight conditions, melatonin levels decreased rapidly and significantly. Additionally, when continues culture of the pineal gland was done from the sunlight to the moonlight, increase of melatonin levels were inhibited. These results suggest that the pineal gland of golden rabbitfish can recognize lightness of the night.

LOCALIZATION PROFILES OF CIRCADIAN NEUROPEPTIDE PDF IN THE BRAIN OF FLIES, *MUSCA DOMESTICA*

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Pigment dispersing factor (PDF) is a neuromodulator peptide functioning for circadian rhythmicity. The precursor protein consists of the signal peptide, PDF-associated peptide (PAP), dibasic cleavage site, PDF, and amidation signal. In this study, by using several different monoclonal antibodies specific for each peptide and protein, we analyzed the localization of the precursor protein, PAP, and mature PDF in the brain of housefly Musca domestica immunocytochemically. Musca PDF precursor is likely degraded to PAP and PDF in its processing. For identification of mature PDF, it is a requisite to detect simultaneously the immunoreactive signals by both anti-PDF N-terminal and anti-PDF C-terminal amide antibodies. The signals by anti-PAP and anti- mature PDF were found in the cell bodies and axons. On the other hand, PDF precursor protein was found only in the cell bodies. These results indicate a clear profile of processing and amidation in the cytoplasm. Immunocytochemical analysis by a combination of antibodies will be also discussed to differentiate the PDF signals and other cross-reactivities.

MORPHOLOGICAL AND MECHANICAL DIFFERENCES IN PODIA OF THE SEA URCHIN *DIADEMA SETOSUM*

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We found two types of externally different coronal podia in *Diadema setosum*. The one on the oral side had a sucker at the tip and that on the aboral side were without a sucker. The suckered podia had a thick tube wall that was occupied mostly by a thick muscle layer. The observation in an aquarium revealed that the sea urchin used suckered podia for locomotion and in fixing their body on the substratum. The non-suckered podia were shorter and their tube wall was thinner with small amount of muscles. Their lumen was divided into two along the long axis by a septum except at the very tip. In sea urchins the podia with a septum have been regarded as respiratory in function. We examined all the podia on an ambulacrum from the oral end to the aboral end. The transition from suckered to non-suckered occurred abruptly at the place a little oral to the ambitus. The length of the septa, however, changed gradually, which suggested that the suckered podia near ambitus contributed also to respiration. The differences found in the mechanical activities of two podial types were also reported.

SCALING ANALYSIS OF PHYSIOLOGICAL TIME OF THE COLONIAL ASCIDIAN *BOTRYLLOIDES VIOLACEUS*

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It is known that the physiological time lengthens in proportion to the 0.25 power of body weight in homoiotherms. The scaling of the physiological time of poikilotherms is still an open question and we know nothing about the physiological time of colonial organisms. We investigated the scaling of time-related parameters of heartbeat in the colonial ascidian *Botrylloides violaceus*. Zooids in a colony are connected each other by a vascular system sharing the blood flow equally between them. The heart of ascidians reverses the direction of blood flow periodically. We measured the duration of a single heartbeat (*Tb*) and the duration of the blood-flow reversal (*Tr*) in the various-sized colonies (zooid number ranging 1 - 700) and in the larvae. *Tb* was constant irrespective of colonial size; *Tb* of larva was the same as that of adults. *Tr* of colony shortened in proportion to the 0.15 power of colony size. *Tr* of larva was shorter than that expected from the regression line of *Tr* of colonies.

LOCOMOTION PATTERNS AND ELECTRICAL ACTIVITIES OF TAIL MUSCLE BANDS IN THE ASCIDIAN *CIONA INTESTINALIS* LARVAE

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The larva of ascidians represents a minimal form of chordate bodies. Its motile tail has bilateral muscle bands, each composed of about 20 cells, and whose contraction patterns are controlled by a hundred of neuronal cells in total. This simplicity as well as the recent extensive accumulation of genomic data provides a good opportunity to investigate a detailed mechanism for regulating the complex locomotion patterns in chordates. Here, we report results obtained from a combinatorial study of the tail-beating patterns of *C. intestinalis* larvae using a high-speed video camera and an extracellular electrode. They swam at a rate of 4.5 + 1.6 mm/sec (Ave + SD, n = 22) and a frequency of 16.4 + 4.3 Hz (n = 26) under the free-swimming condition, which supported previous reports. The electrical recordings with a suction electrode were well consistent with the contractile patterns observed with the camera. The swimming patterns were significantly variable, depending on their growth degrees or on conditions such as light or mechanical stimulations. These observations would provide a good platform for further analyses on the neural circuitry for swimming pattern regulation.

STUDY ON EXPRESSION OF VISUAL PIGMENT GENES IN LIGHT-SENSITIVE IRIDOPHORES IN THE DERMIS OF NEON TETRA, *PARACHEIRODON INNESI*

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Lateral stripes of neon tetra show the diel color changes in vivo, from green in the daytime to violet at night. Irradiation causes a shift in the spectral reflectance to longer wavelengths in isolated skin of neon tetra, as is also the case in intact live fish. Each iridophore contains two stacks of light-reflecting platelets. A change in illumination induces in the inclination of platelets, resulting in an alteration in the distance between them. Immunocytochemical studies suggeted that a rhodopsin or a closely-related molecule is present in the iridophores (Lythgoe et al., 1984). Moreover, our previous observations showed that light at wavelengths near 520 nm was most