Diurnal rhythm in urea excretion of *Mugilogobius abei*

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Urea excretion of *M. abei* in 20% SW under starved conditions exhibits daily periodicity under light-dark cycles of 12 h light and 12 h darkness. Does this diurnal excretion originate from changes in urea synthesis or those in its extrusion? Nitrogenous compounds in tissues changed periodically, whereas enzyme activities related to urea synthesis (uricase and arginase) did not change significantly. These results suggest that the periodic urea excretion is not due to periodic urea synthesis but to periodic extrusion. Furthermore, diurnal periodicity of urea excretion was sustained even when the locomotor activity rhythm was disturbed artificially. Thus, it seems unlikely that periodic urea excretion depends on behavioral activities.

Extra-hepatic ureogenesis in the gobiid fish,*Mugilogobius abei*

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To make clear the main site of ureogenesis in *M. abei*, CPSIIImRNA expression and all enzyme activities in the ornithine-urea cycle (OUC) were examined. CPSaseIIImRNA expression and CPSIII activities could be detected in muscle,skin and gill, but negligible in liver. Furthermore, all other OUC enzyme activities were also detected in muscle,skin and gill. Thus, *M. abei* is able to produce urea mainly *via* OUC operating in multiple tissues as a mean for ammonia detoxi- fication.

FUNCTIONAL CLASSIFICATION OF CYTOPLASMIC DOMAINS OF BOVINE RHODOPSIN IN G PROTEIN ACTIVATION

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It has been reported that the 2nd, 3rd and 4th (extra) cytoplasmic loops are the interaction sites of rhodopsin for G protein transducin. We prepared bovine rhodopsin mutants whose cytoplasmic loops were replaced by those of other ligand-binding receptors and compared their ability for G protein activation in order to classify the loops according to their roles in G protein activation.

The mutants bearing the 3rd loop of other four types of Go-coupled receptors belonging to the rhodopsin superfamily showed the significant Go activation, indicating that the 3rd loop of rhodopsin possibly has a putative site(s) related to the interaction of G protein and that it is simply exchangeable with those of other Go-coupled receptors. The mutant having the 4th loop of Go-coupled muscarinic acethylcholine receptor also gained higher Go activation ability than that of wild-type, but showed slight decease in transducin activation ability. As reported previously, the mutants bearing the 2nd loop of other receptors, however, had little ability for G protein activation, indicating that the 2nd loop is not exchangeable and is essential for rhodopsin/G protein coupling. The systematic chimerical and point mutational studies indicated that three amino acids (Glu134, Val138 and Cys140) in the N-terminal region of the 2nd loop of rhodopsin are crucial for efficient G protein activation.

These results suggest the distinct roles of cytoplasmic loops, that is, the 3rd and 4th cytoplasmic loops of bovine rhodopsin have the similar role concerned with selective coupling to G protein subtypes, but the 2nd cytoplasmic loop plays the essential role to form the active structure in cytoplasmic domains.

A RHODOPSIN-RETINOCHROME SYSTEM IN THE RHABDOMERIC-TYPE PHOTORECEPTOR (LENS CELL) IN *Onchidium* DORSAL EYE.

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Onchidium sp. (Gastropoda, Mollusca) has numerous dorsal eyes (DEs) on the dorsal mantle. The DE has an inverted retina in which the photoreceptor cell is ciliary-type. A pear-shaped lens is located in the center of the eye and consists of several lens cells (LCs), which are rhabdomeric-type photoreceptor cells. The LC was characterized by massive microvilli in the distal portion that contained rhodopsin and retinal binding protein (RALBP). The proximal portion contained a large nucleus and abundant smooth endoplasmic reticulum. Many concentric lamellar bodies were clustering around the nucleus. The cytoplasm was stained with anti-retinochrome antibody. The present immunohistochemical study demonstrates the presence of a set of photopigments, rhodopsin, retinochrome and RALBP in the LC. The LC therefore possesses a rhodopsin-retinochrome system which has been established in the eyes of several cephalopod and gastropod species including the *Onchidium* stalk eye. It provides a definitive evidence that the LC is a photoreceptor cell, although it functions as the dioptric apparatus.

Role of amino acid residue(s) conserved in cone visual pigments

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Most vertebrates have two types of photoreceptor cell, rods and cones, which are responsible for twilight (scotopic) and daylight (photopic) vision, respectively. This difference might originate from the differences in molecular properties between rod and cone visual pigments. Recently, we reported that Glu 122 conserved in rhodopsins is one of the amino acid residues responsible for the differences. Replacement of Glu 122 of rhodopsin by Gln, which exists in the corresponding site of green-sensitive cone pigment, exhibits molecular properties close to that of cone pigments. Namely, the mutant shows faster regeneration from 11-cis-retinal and opsin, and faster decay of physiologically active intermediate (MetaII) than wild type rhodopsin.

The replacement of the residue, however, did not completely convert molecular properties of rhodopsin into that of cone visual pigment. In this study, we selected the amino acid residues conserved in all the known cone visual pigments but different from those of rhodopsins. Mutants of chicken rhodopsin and green-sensitive cone visual pigment at the sites are expressed, purified, and their molecular properties are investigated. As a result, one of the mutants, in which proline residue in 4–5 extracellular loop was changed, showed altered protein stability as well as lifetime of meta-intermediates. The strict conservation of this residue strongly suggests that rod visual pigment evolved from cone visual pigment.

Frequency of Color Vision Defects in Crab-eating Monkeys (*Macaca fascicularis*)

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Old World primates have trichromatic color vision owing to three types of cone visual pigments maximally sensitive to short (S), middle (M) and long (L) wavelengths of lights. Most color vision defects in humans are LW/MW abnormal that are caused by unequal crossing-over between L and M visual pigment genes. We previously reported that only three crab-eating monkeys of the 3153 macaque monkeys are protanopic (L pigment absent) and that frequency of dichromats in this species are lower than that in humans (Onishi *et al. Nature.* 402, 139–140 (1999)). To understand the mechanism leading to the difference between humans and crab-eating monkeys, we further identified frequency of prota/deuteranomalous trichromats by sequencing exon 5 of L and M genes that code amino acids responsible for L/M spectral tuning. Of 130 samples examined, no anomalous trichromats were found, although about 6% of humans are such color vision defects. Additional genetic analysis showed that frequency of individuals that have multiple L/M genes caused by unequal crossing-over is lower than that in humans, although the sequence of the visual pigment genes is similar.