

These results suggest that *OI*-G $\beta$ 6 is not colocalized with *O*/PD-C in cone outer segments. Medaka cones maybe have the system to prevent PD from localizing to cone outer segments. It is therefore possible that G $\beta$  $\gamma$ -PD system functions as a light-dependent regulator in cone cells, except for outer segments.

#### THE ANALYSIS OF THE GENES INDUCED AT THE EARLIEST PERIOD IN NEWT RETINAL REGENERATION

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Japanese common newts (*Cynops pyrrhogaster*) have an ability to regenerate their neural retina even as adults. After surgical removal of the retina from adult newt eye, the remaining retinal pigment epithelium (RPE) cells lose their pigment granules and transdifferentiate into retinal progenitor cells. Then, they proliferate and differentiate into various retinal cells. The transdifferentiation of RPE cells into retinal progenitor cells is quite important for the retinal regeneration and does not occur during normal development of newts. However, little is known about this process, for example what kinds of molecules are responsible for this process. We attempted to examine the change of mRNA expression in RPE cells by differential display method.

Two days after the removal of newt retina, the expression of some genes were upregulated. We focused on one of such genes, designated as CpDD28k, whose orthologs are present in various vertebrates but their function is unknown. Our immunohistochemical experiments indicated that the expression pattern of CpDD28k changed during the retinal regeneration.

#### VISUALIZATION OF VISUAL PATHWAYS IN THE ASCIDIAN *CIONA INTESTINALIS* LARVA USING WHEAT GERM AGGLUTININ (WGA) TRANSGENE

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Ascidian larvae share a basic body plan with vertebrates. The larva has a central nervous system with about 100 neurons, which is simple enough to allow every neural pathway to be known. In this study, we attempted to visualize the visual pathways of the larva by expressing wheat germ agglutinin (WGA) transgene in the photoreceptor cells under the control of the arrestin gene (*Ci-arr*) promoter. *Ci-arr-WGA* transgene was introduced into fertilized eggs by electroporation. Immunofluorescence labeling with anti-WGA antibody revealed that WGA transgene products in the photoreceptor cells were transported along their axons and transsynaptically transferred to second or third-order neurons. In order to discriminate the first-order neurons from the higher-order neurons a transgene construct containing both sequences of *Ci-arr-WGA* and *Ci-arr-EGFP* was introduced. Immunofluorescence labeling revealed that WGA and GFP transgene products were expressed in the photoreceptor cells and WGA transgene products were transported along their axons and transsynaptically to the second-order neurons in the brain vesicle and then the motor neuron in the visceral ganglion in ascidian larvae.

#### IDENTIFICATION OF GLUTAMATERGIC NEURONS IN LARVAE OF THE ASCIDIAN *CIONA INTESTINALIS*

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Ascidians are primitive chordates, and their tadpole-like larvae share a basic body plan with vertebrates. The ascidian larva has a remarkably simple central nervous system with about 100 neurons. The neural network of the ascidian larva would provide the fundamental knowledge to understand that of vertebrates. Previously, we succeeded in visualizing the neural network of the ascidian larva using an antibody against synaptotagmin. However, individual neurons in the ascidian larva are poorly identified. In order to identify the glutamatergic neurons in the larva of the ascidian *Ciona intestinalis*, we prepared an antibody against vesicular glutamate transporter. The antibody clearly stained axon filaments and revealed the detailed structure and distribution of glutamatergic neurons in whole-mount specimens. Glutamatergic neurons were detected in the brain vesicle, the trunk epidermal neurons and the caudal epidermal neurons. In the brain vesicle, the presynaptic terminals of ocellus photoreceptors were glutamatergic like that of vertebrate. Role of glutamatergic neurons will be discussed based on comparison with those of GABAergic and cholinergic neurons in the ascidian larva.

#### A NOVEL PHOTORECEPTOR CANDIDATE EXPRESSED IN THE CHICKEN RETINA AND PINEAL GLAND

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The chicken pineal gland is a photosensitive circadian clock tissue, in which the photoreceptor gene *pinopsin* is expressed. We searched a novel candidate for non-opsin type circadian photoreceptor in the pineal gland, and we isolated a cDNA encoding a protein that is closely related to zebrafish cryptochrome 4. The cryptochrome (CRY) family includes blue-light photoreceptors in plants and bacteria. DNA photolyases in many organisms, and negative components of circadian clock in vertebrates. As the first characterization of the chicken *Cry4* (*cCry4*) gene, we examined temporal and spatial expression of its mRNA by RT-PCR analyses. *cCry4* mRNAs were expressed in a variety of tissues such as retina, pineal gland, brain, skin, kidney, liver and heart, among which *cCry4* mRNA was most abundant in the retina. In cultured pineal cells maintained in daily light/dark cycles or in constant darkness, the level of *cCry4* mRNA was higher in the light than in the dark, implying that *cCry4* transcription depends on the light condition like *pinopsin* gene transcription. These data suggest that cCRY4 is a novel photoreceptor in the chicken pineal gland and retina that are photosensitive tissues.

#### ZINC-BINDING PROTEINS EXPRESSED IN REGENERATING NEWT RETINAS

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Japanese common newts (*Cynops pyrrhogaster*) possess a remarkable ability to regenerate their organs. We attempted to clarify the molecular mechanism for regeneration of nervous systems, and investigated genes expressed in regenerating newt retinas. A cDNA library from regenerating newt retinas were constructed and sequences in this library were analyzed. We obtained several genes containing zinc-binding motifs defined as C2H2 domain or RBCC (Ring, B box and Coiled-Coil domain). It has been reported that some of these zinc-binding proteins interact with DNA/RNA and regulate cell differentiation as transcription factors. However, the exact roles of the most zinc-binding proteins are still unclear. We compared the amino acid sequences of these zinc-binding proteins among various vertebrates and investigated their roles in retinal regeneration.

#### LEARNING AND DISCRIMINATION OF COLORED PAPERS IN DIURNAL, NOCT-DIURNAL AND NOCTURNAL SPIDERS

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The diurnal spider *Hasarius adansoni* could discriminate the blue, green, yellow and red papers by their hue (Nakamura and Yamashita, 2000). In the present study, color discrimination of the noct-diurnal spider *Argiope amoena* and the nocturnal spider *Araneus ventricosus* were examined by heat-avoidance learning in association with colored papers, similar methods as used for jumping spiders. The arena for the experiment was divided into two halves by a pair of colored papers. In training sessions, one half of the arena was heated from the bottom by a hot plate, and spiders were individually trained to avoid the heated half. In subsequent memory tests without heat, they sometimes avoided the heat-associated colored papers. However, both *Argiope* and *Araneus* trained with a blue-white pattern or a green-white pattern could not discriminate the blue or green from black. It seems that both *Argiope* and *Araneus* discriminate the colored papers by their brightness.

#### THREE-DIMENSIONAL RECONSTRUCTION OF EXTRAOCULAR DERMAL PHOTORECEPTOR CELLS FROM SERIAL SECTIONS EXAMINED WITH HIGH VOLTAGE TEM USING DELTAVIEWER

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The dermal photoreceptor cells (DP), extraocular photoreceptors, are distributed abundantly in the dermis of the dorsal mantle papillae of *Onchidium*. Serial semithin sections of an osmium-impregnated juvenile were examined with high voltage TEM. The axons of DPs and glial cells were reconstructed using DeltaViewer software. A single axon arose from the lateral side of two DPs, and joined the same small nerve bundle which was accompanied by a glial cell. Reconstructed images can be freely rotated and the sectioned views of reconstructed images can be observed on the computer screen. Reconstructed images using DeltaViewer and OZ-95-32 will be presented.