(retinochrome) and Y/E-type rhodopsins (invertebrate Gq and Go-rhodopsins as well as peropsin). Therefore, we analyzed Tyr-113 and Glu-181 mutants of Y/Y type *rhodopsins*, amphioxus Go-rhodopsin and peropsin. The result strongly supported the idea that counterion switching from Glu-181to Glu-113 in the course of molecular evolution was an important event for the *rhodopsin* divergence.

**PREPARATION OF A MOUSE MODEL HAVING GREEN-SENSITIVE CONE VISUAL PIGMENTS IN ROD PHOTORECEPTOR CELLS**

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In most vertebrates, there are two types of photoreceptor cells, rods and cones, which mediate scotopic and photopic vision, respectively. Because both cells have signal transduction proteins whose functions are similar but whose amino acid sequences are different from each other, the difference in photoresponse patterns between rods and cones should originate from the different properties of these proteins. Our investigations revealed that the difference in molecular properties between rod and cone visual pigments correlate well with the difference in photoresponse pattern between rod and cone photoreceptor cells. Thus the next investigation would be to make clear the physiological relevance of the difference in molecular properties between rod and cone visual pigments. To address this question, we have tried to prepare a mouse model having cone visual pigments in its rod photoreceptor cells using a gene targeting procedure. Then we have succeeded in preparing a mouse model that expresses functional green-sensitive cone visual pigments in its rod photoreceptor cells.

**MUTATIONAL ANALYSES OF THE CYTOPLASMIC DOMAIN OF METABOTROPIC GLUTAMATE RECEPTOR**

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G protein-coupled receptors identified so far are classified into several families. In family 1 including rhodopsin, there is considerable evidence for the conformational changes leading to G protein activation. In contrast, much less is known about other families. Recently, to comparatively investigate the G protein activation mechanism between rhodopsin and metabotropic glutamate receptor (mGluR) belonging to family 3, we analyzed bovine rhodopsin mutants having the cytoplasmic loop of mGluR. The rhodopsin mutant whose third cytoplasmic loop (Loop3) was replaced with the second cytoplasmic loop (Loop2) of mGluR activated G protein, which showed that Loop2 of mGluR has a similar role with Loop3 of rhodopsin. In this study, to analyze the function of Loop2 of mGluR, we prepared the alanine-replaced mutants of the loop. mGluRs are classified into several subtypes and we used Gi/Go-coupled subtype. Several mutations in the N-terminus of Loop2 caused the decrease of G protein activation ability. These residues tend to be conserved in other mGluR subtypes and are supposed to be important for G protein activation regardless of mGluR subtypes.

**REGIONAL DISTRIBUTIONS OF A1 AND A2 CHROMOPHORES OF THE FISH EYES ANALYZED BY HPLC**

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A visual pigment consists of an opsin and a chromophore. Four chromophores are present in nature; retinal (A1), 3-dehydroretinal (A2), 3-hydroxyretinal and 4-hydroxyretinal. The visual pigment of teleost fish possesses two types of chromophores, A1 and A2. In order to research the relationship between the photoreceptive function of the visual pigments and the chromophore distributions in different habitats, we analyzed the chromophores of visual pigments extracted from several fish eyes using high performance liquid chromatography (HPLC). Analyses were performed for totally 110 species fish including 26 species of pure fresh water fish, 4 species of limb fresh water fish, and 6 species of migration fish and 74 species of seawater fish. The 74 fish and 6 migration fish from oceanic region possessed only A1, however, 4 shore fish and 19 river fish possessed both A1 and A2. Additionally, we found that there were some species of fresh water fish in the river possessing only A1 or A2. Our results indicate that the fish from oceanic region possess only A1, however, the distributions of those A1 and A2 chromophores of the fish from river region are different from the previous reports.

**ANALYSIS OF G PROTEIN βSUBUNIT WITH SPECIFIC ANTISERUM IN MEDAKA RETINA**

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It has been reported that a light sensitivity of photoreceptor cells is decreased by an interaction between βγ subunit of G proteins (Gβγ) and phosducin (PD) at light adapted states. Tetrapods have only one type of PD in their photoreceptor cells, but we have been reported that teleost have two types of PDs, selectively distributed in either cone or rod.

We have previously reported a novel cone-specific subtype of Gβ (Ol-Gβ6) in the retina of medaka (*Oryzias latipes*). Here, we made antiserum raised against partial peptide of the predicted protein product of Ol-Gβ6. our Western blot analysis revealed that this antiserum recognize a 36kD protein in medaka retinal homogenate. On the other hand, it was suggested that this antiserum did not crossreact to Gβ1 subtype of medaka.

Medaka has a special set of Gβ and PD in their cone photoreceptor cells, so it is possible that regulation of phototransduction cascade of teleost cones is different from those in cones of other vertebrates. Our antiserum will be a useful tool to reveal the special molecular mechanism of regulation of teleost cones.

**ANALYSIS OF GENE EXPRESSION IN REGENERATING NEWT RETINA**

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Japanese common newts (*Cynops pyrrhogaster*) have an ability to regenerate their injured organs. Even in adult, newts can reconstruct neural retina from retinal pigment epithelial (RPE) cells. After surgical removal of original retina from adult newt eyes, the remaining RPE cells lose their pigment granules and transdifferentiate into neuroblasts. They proliferate and differentiate into various retinal neurons. Finally neural retina is regenerated.In order to elucidate the molecular mechanisms of retinal regeneration, we attempted to investigate genes expressed in regenerating retina. We constructed a cDNA library of the regenerating retina at 18 or 19 days after the surgical removal of the original retina, and isolated 112 clones. Our expression sequence tag (EST) analysis indicated that 78 clones have similarities to the genes previously identified and 17 clones seem to encode proteins related to cell growth and differentiation. Then, we investigated the expression of some of these genes in mature retina and regenerating retina by *in situ* hybridization. These results suggested that genes expressed in regenerating retinal cells are different from those in mature retinal cells.

**FUNCTION OF RAB5 ON SYNAPTIC VESICLES**

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Rab5, a member of small GTPase, is well known as a regulator of endocytic vesicular transport from plasma membrane to early endosomes. In neurons, Rab5 is also found on synaptic vesicles as well as on endocytic vesicles. However, function of Rab5 on synaptic vesicles remains unclear. In this study, we elucidate the function of Rab5 on synaptic vesicles with *in vivo* and *in vitro* experiments using *Drosophila* photoreceptor cells. Functional inhibition of Rab5 with Rab5N142I, a dominant negative version of *Drosophila* Rab5, induced enlargement of synaptic vesicles. This enlargement was suppressed by enhancing synaptic vesicle recycling under light illumination. In addition, synaptic vesicles prepared from Rab5N142I-expressing flies exhibited homotypic fusion in vitro. These results indicate that Rab5 functions to keep the size of synaptic vesicles uniform by preventing their homotypic fusion. In contrast, Rab5 was not involved in the endocytic reformation of synaptic vesicles, contrary to expectation from its conventional function. Furthermore, we electrophysiologically and behaviourally showed that the function of Rab5 is essential for efficient signal transmission across synapses.

**VISUAL PIGMENTS IN TWO PATHWAYS OF PHOTO-SIGNALS IN THE LAMPREY PINEAL ORGAN**

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The lower vertebrate pineal organ transforms photic information into humoral and neural messages through photoneuroendocrine and photoreceptor-ganglion pathways, respectively. The immunohistological and electrophysiological studies have suggested that six types of the photoreceptor cells existed in the lamprey pineal organ. However, the pineal visual pigments have not been demonstrated in the lamprey. We tried identifying the pineal visual pigments and show their localization in the

pineal organ. As the results, a red-sensitive pigment, rhodopsin and a pineal UV pigment roughly localized at pineal stalk, ventral wall and dorsal wall of the end-vesicles, respectively. To understand the function of their visual pigments, especially UV pigment, we discussed the sensitivity of the pineal photoreceptors and the transmission ratio of the pineal window.

### UV-SENSITIVE PHOTORECEPTORS IN THE PINEAL ORGAN OF THE RIVER LAMPREY, *LAMPETRA JAPONICA*

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It has been known that wavelength discrimination in incident light is one of the functions in the pineal organ of lower vertebrates. Although the previous studies suggested that UV-sensitive photoreceptor cells were involved in the wavelength discrimination, the mechanism or physiological significance is still uncertain. In order to obtain the clue to understand the wavelength discrimination, we tried to identify the UV-sensitive pigment in the lamprey pineal and characterize it histochemically and electrophysiologically. We found a novel UV-pineal pigment that is different from vertebrate UV-visual pigments. The immunohistochemical and electrophysiological analyses revealed that the pineal cells containing the UV-pineal pigment showed the hyperpolarization in response to light, and the maximum response was at around 380 nm. These findings demonstrated that the novel UV-pineal pigment is involved in the photoreception in the UV-sensitive pineal photoreceptor cells.

### CHARACTERIZATION OF UV SENSITIVE OPSIN IN THE PINEAL ORGAN OF THE LAMPREY

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Several electrophysiological studies suggested the presence of ultraviolet (UV) sensitive cells in the pineal organ of some teleosts and the lamprey. However, the molecular basis of extraretinal UV photoreception has yet to be understood. Recently we isolated a UV sensitive opsin from the river lamprey, which exhibits its absorption maximum at 370nm when it is expressed in the cultured cells. Interestingly, phylogenetic analysis showed that the pineal UV opsin does not cluster with the vertebrate visual UV opsins, which are involved in the UV vision in some vertebrates. Then we conducted further spectroscopic analysis of the pineal UV opsin to compare its characteristics with the visual UV opsin. As results, we found that the pineal UV opsin showed unique characteristics upon irradiation, which was different from those of all the known vertebrate opsins. These results arise the possibility that the pineal UV opsin and the visual UV opsin drive a distinct phototransduction cascade.

### G PROTEINS EXPRESSED IN THE EYE OF *LAMPETRA JAPONICA*

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So far, $G_t$ class G protein has been found only in the higher vertebrate. There are two different cell-type specific $G_t$ proteins in rod and cone cell, respectively. In the lamprey, which belongs most primitive vertebrate, there are two types of visual cells and they would be proto-types of rod and cone cell. In order to know what kind of G protein(s) are there in the lamprey visual cells, we cloned G protein cDNA fragments from lamprey eye. Three different cDNA fragments of G protein alpha subunit were obtained; one $G_o$ class and two $G_t$ classes. *In situ* hybridization study showed the expression of one type of $G_t$ class G protein in the retina. The expression of another type of $G_t$ class was not observed, probably due to its low expression level. The expression of $G_o$ class was observed in non-visual cells in the retina. The results suggest that specific G proteins are expressed depend on the cell types even in the eye of the most primitive vertebrate, lampry.

### ELECTROPHYSIOLOGICAL PROPERTIES OF SYSTEM N EXPRESSED ON RETINAL MUELLER CELLS OF TIGER SALAMANDER (*AMBYSTOMA TIGRINUM*)

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We previously reported that the glutamine transporter System N catalyzes the flux of glutamine (Gln) and asparagine (Asn) across the plasma membrane of Mueller cells in the tiger salamander retina. We also found that the uptake of Gln or Asn via this transporter is associated with the generation of membrane currents. Here we investigated the ionic mechanism underlying these Gln- and Asn-associated membrane currents. Amino acid-evoked currents were recorded under whole-cell voltage-clamp conditions in Mueller cells isolated from retinae of the tiger salamander. The I-V curve of Asn-evoked current obtained at $pH_o$ 8.0 was roughly linear. Reversal potentials ranged between –20 to +40 mV across the cell population. The reversal potential of Asn-evoked currents in individual cells depended upon intrapipette $Na^+$ and $H^+$ concentrations. Raising intrapipette $Na^+$ and $H^+$ concentrations moved the reversal potential to more negative values. When current recordings were made using a pipette containing of a high concentration of Asn (50mM), no steady current was detected. These observations suggest that the current mediated by an activation of the System N is carried by both of $Na^+$ and $H^+$.

### NMR MICROIMAGING AND SPECIFIC GRAVITY OF DIAPAUSE AND NON-DIAPAUSE PUPAE OF LARGE AND SMALL WHITE BUTTERFLIES, *PIERIS BRASSICAE* AND *P. RAPAE CRUCIVORA*

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Specific gravities of diapause and non-diapause pupae of *Pieris brassicae* and *P. rapae crucivora* were measured using microbalance. During 7 days after pupation, the specific gravities of both diapause pupae were more than 1.0, so they sunk in water. On the other hand, both non-diapause pupae of all ages floated on water, that is, the specific gravities were less than 1.0. NMR microimaging studies supported this observation; a cavity existed between the internal abdomen and the thorax of pupae and its size was correlated to the diapause condition. Non-diapause pupae had a larger cavity than a diapause ones. Therfore, the specific gravities of non-diapause pupae were smaller than those of diapause ones.

### HEMOLYMPH CIRCULATION IN LOCUSTS VISUALIZED WITH SYNCHROTRON X-RAY IMAGING

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Locusts are known to migrate for a long distance. The flight fuel is diacyglycerol carried by lipophorin, a major circulating lipoprotein in insects. Lipophorin exists as high-density lipophorin (HDLp, $\rho = 1.10$g/ml) in resting locusts. At the commencement of long-distance flight, lipophorin starts to transport diacylglycerol from the fat body to the flight muscle. The lipophorin in flight contains about five times more diacylglycerol than HDLp, which results in the transformation of HDLp to low-density lipophorin (LDLp, $\rho = 1.05$g/ml). We observed the hemolymph circulation in such locusts using a synchrotron beam at SPring-8.

### CUTICULAR ENCYSTMENT IN LARVAE OF PLUSIINAE LOOPER: INDUCTION BY POLYDONAVIRUS FROM BRACONID WASPS

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Plusiinae loopers (Lepidoptera: Noctuidae) has unique defnse system, or Cuticular encystment, in addition to cellular reaction against the foreign substances that invade into the hemocoel. If endoparasitoid oviposit eggs in Plusiinae loopers, the parasitized larva forms a cyst on the dorsum of the penultimate segment. After hatching, the parasitoid larvae enclosed in the cyst and finally expelled from the host. Parasitoid wasp's symbiotic virus (Polydonavirus) which was injected with eggs at the time of parasitoid's oviposition, might induce the formation of the cyst. Furthermore, the forming process of cuticular cyst would be report histologically.

### ALLORECOGNITION IN SELF-DEFENSE AND FERTILIZATION OF THE ASCIDIAN, *HALOCYNTHIA RORETZI*

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A solitary ascidian *Halocynthia roretzi* shows two events of allorecognitions; strict self-incompatibility in fertilization and a cytotoxic reaction called Contact Reaction (CR)(Fuke, 1980). A monoclonal antibody against the plasma membrane of coelomic cells in *H. roretzi* that inhibits the CR. This antibody recognized three glycoproteins of 210, 165 and 120 kDa in vacuolated cells and hyaline amoebocytes.