REGULATORY MECHANISM FOR STABILITY OF ACTIVE INTERMEDIATE OF PINOPSIN.

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Pinopsin is a chicken pineal photoreceptive molecule, likely contributing to photoentrainment of the circadian clock. The amino acid sequence of pinopsin suggested that it would be more similar in photochemical properties to cone visual pigments than to rhodopsin (rod pigment). We found, however, that the physiologically active meta II intermediate of pinopsin is much more stable than those of cone visual pigments, and its lifetime is similar to metarhodopsin II [Nakamura et al., Biochemistry 38, 14738-14745 (1999)].

In this study, we investigated the amino acid residue(s) responsible for this unique property of pinopsin by using site-directed mutagenesis. For this purpose, we focused on pinopsin-specific structural features (Ser171, Asn184 and the lack of two residues between 190 and 191), all of which are located in the second extracellular loop. The meta II of the 171/184 double mutant of pinopsin was as stable as that of wild-type pinopsin. In contrast, meta II lifetime was shortened (one third) by exchange of 6 amino acids stretch (188-193) with corresponding 8 residues of chicken green-sensitive cone pigment. Consistently, meta II of a green-sensitive pigment mutant, in which corresponding 8 amino acids stretch was exchanged in to the 6 residues of pinopsin, was more stable than that of the wild-type pigment. Our results strongly suggest an important role of the specific sequence and/or the length of the second extracellular loop in stabilization of metapinopsin II.

A NOVEL PHOTOTRANSDUCTION CASCADE INVOLVED IN THE PHOTIC INPUT PATHWAY TO CIRCADIAN CLOCK OSCILLATOR

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The photosensitive chicken pinealocyte contains a circadian clock entrainable to light cycles. Light has two distinct effects on the cell: one is an acute suppression of melatonin secretion and another is a phase-shifting effect (entrainment) of the clock. The acute effect is blocked by pertussis toxin (PTX)-treatment but the phase-shifting effect is not. Therefore, these pathways are likely to involve two G-proteins with diverged sensitivities to PTX. To elucidate the phototransduction pathways downstream of a pineal photoreceptor, pinopsin, we searched for G-protein α-subunits expressed in the pinealocytes, and isolated six kinds of cDNAs for $Gi_2\alpha$, $Gi_3\alpha$, $Go_1\alpha$, $Go_2\alpha$, $Gt_1\alpha$ (rod-type transducin), and $G_{11}\alpha$. In immunohistochemical analyses, $Gt_1\alpha$- and $G_{11}\alpha$-immunoreactivities were observed at pinopsin-immunopositive outer segment membranes of the pinealocytes. Trypsin protection assay demonstrated that pineal $Gt_1\alpha$ (PTX-sensitive) is activated by light, suggesting that $Gt_1\alpha$ mediates the acute pathway. On the other hand, by using an immunoprecipitation technique, we found that $G_{11}\alpha$ (PTX-insensitive) associates with rhodopsin in the dark and dissociates in response to light stimuli. To see whether $G_{11}\alpha$ is involved in the phase-shifting effect, we investigated the signaling cascade via $G_{11}\alpha$ by transfection of the pinealocytes with a gene for Gq/11-coupled m1 muscarinic acetylcholine receptor. A transient activation of $G_{11}\alpha$ by carbachol-treatment of the transformed cells induced an evident phase-shift of the melatonin rhythm, which was indistinguishable from that induced by light. These results suggest strongly that opsin–G_{11} coupling contributes to the photic entrainment of the chicken pineal circadian clock.

ROLE OF A NOVEL *DROSOPHILA* RAB-RELATED PROTEIN, RABRP1, IN THE VESICLE TRANSPORT

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Rab proteins of the small GTP-binding protein superfamily are essential for the regulation of vesicle transport in the cell. In the previous study, we identified a novel member of *Drosophila* Rab proteins, DRabRP1, and demonstrated it was selectively distributed in the sensory organs and gonads. Immunohistochemical analyses have elucidated that DRabRP1 is localized to the SRC in the photoreceptor cell and pigment granules in the pigment cell in the retina. In this study, we have constructed the transgenic, dominant negative RabRP1 mutant that synthesizes the dominant inhibitor protein, RABRP1(N146I), under the control of *Drosophila* major rhodopsin (*ninaE*) promoter. In this mutant, autophagosome-like vesicles were frequently observed just beneath the rhabdomeric microvilli in R1-6 photoreceptor cells. In addition, abnormally darkened multivesicular bodies and large electron-dense vesicles also accumulated between the subrhabdomeric and central regions of the photoreceptor cell. These results suggest that DRabRP1 may possibly be involved in the biogenesis or functionality of ER- and lysosome-related organelles. Analysis of another kind of mutant that expresses anti-sense RabRP1 RNA is now in progress.

PUTATIVE MAMMALIAN PHEROMONE RECEPTOR MEDIATED THE ACTIVATION OF VOMERONASAL NEURONS BY URINARY FRACTION

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To search for the biological activity of mammalian putative pheromone receptors, the mouse receptor gene was isolated and introduced in the adenovirus expression vector. The constructed receptor-expressing adenovirus was infected to the rat primary culture of vomeronasal organ (VNO). The mouse urinary fraction was applied to these VNO cells, then receptor-mediated activation of the cells was monitored by calcium imaging. These cells were specifically activated by male urinary fractions. This results indicate that putative pheromone receptor mediates the activation of vomeronasal neurons by crude urinary fractions, a major source of mouse pheromones.

TWO DISTINCT ROLES OF RAB5 IN THE VESICLE TRANSPORT SYSTEM OF THE *DROSOPHILA* PHOTORECEPTOR CELL

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The *Drosophila* photoreceptor cell is composed of three functional domains. The photoreceptive domain carrying rhabdomeric microvilli accepts light signal and induces cell depolarization. The somatic domain contains a nucleus and organelles involved in the protein synthesis and membrane transport. The axonal domain is filled with numerous synaptic vesicles, whose contents are released in response to the cell depolarization. Rab GTPases contribute to the formation and maintenance of such domain structure through regulating the exocytic and endocytic processes in the cell.

In order to investigate the regulation mechanism of the endocytic system in the *Drosophila* photoreceptor cell *in situ*, we cloned *Drosophila* homologues of mammalian Rab proteins (RAB4, RAB5 and RAB7), each of which functions in different step of endocytic processes. To elucidate the function of RAB5 in the different domains of the photoreceptor cell, we first carried out the immunohistochemical analyses by the use of the EGFP-tagged RAB5 exclusively expressed in photoreceptor cells. The results indicated that RAB5 is localized to the synaptic vesicles (SVs) in the axonal domain as well as to the multivesicular endosomes (MVBs) in the somatic domain. We previously demonstrated that overexpression of RAB5(N142I), a dominant negative version of RAB5, inhibited the formation of MVBs, while it had little effect on SV endocytosis. Here, we report that both the dominant negative and constitutively active (RAB5(Q88L)) versions of RAB5 induce the formation of irregularly sized SVs. This suggests that RAB5 in *Drosophila* photoreceptor cell would have two distinct functions; regulation of endocytic processes in the somatic domain, and keeping the size of SVs uniform in the axonal domain.

Ligand-specificity of the Drosophila gustatory sweet taste receptor TRE

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By electrophysiological and behavioral analysis of the gustatory sugar sensitivity, Drosophila sweet taste receptor TRE has been shown to have ligand-specificity to trehalose. We analyzed ligand-specificity of this receptor for various saccharides by comparing wild-type and the null mutants of Tre. A smaller but significant associated decrease of sugar sensitivity was found for some saccharides in the null mutants. General structural features involved in the ligand-specificity of TRE are analyzed and discussed.