2P268 High-throughput analysis elucidates the complex pattern of sensory-motor integration in thermotaxis behavior of *C. elegans*

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The mechanism of sensory-motor integration for adaptive behaviors is a major problem in neuroscience. Nematode *C. elegans* show adaptive migration behavior on thermal gradient, as known as thermotaxis. Worms can detect thermal stimuli by the use of thermal receptor neurons and choose appropriate direction according to the environmental information from the neurons. We tracked a mount of paths of worms on thermal gradients and quantified the behavior using video analysis and analyzed the data based on a mathematical model. The analysis shows that worms have different mechanisms for migration behaviors. They changes the migration mechanisms depending not only on the environmental temperature but also on the steepness of thermal gradient. From the thermal conditions of the observed behaviors, we inferred the way of coding thermal information in thermal receptor neurons and the way of determination of behaviors. We argue the selection mechanism of the behavioral patterns and propose new model for thermotaxis.

2P269 Cyclic nucleotide-gated channels are involved in phototransduction of dermal photoreceptors in *Lymnaea stagnalis*

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Cyclic nucleotide-gated channels are involved in phototransduction of dermal photoreceptors in *Lymnaea stagnalis*. In the pond snail *Lymnaea stagnalis* a tactile or photic (shadow) stimulus induces an avoidance behavior that is part of the whole body withdrawal (WBW) response. Since RPeD11 is assumed to be the key interneuron controlling WBW, we focused on the activity of RPeD11. Presenting a shadow stimulus to the mantle area evoked an EPSP in RPeD11 in semi-intact preparations (without ocular photoreceptors). This EPSP is monosynaptic and is mediated by dermal photoreceptors in the mantle area. That is, the EPSP is present in hi Mg²⁺/hi Ca²⁺ saline while it disappears in Ca²⁺ free saline. Here, we tested our hypothesis that cyclic nucleotide-gated (CNG) channels are involved in the dermal photoreceptor-mediated transduction of the shadow stimulus. The response to the shadow stimulus recorded in RPeD11 was abolished by 500 μM cis-diltiazem, which blocks cGMP-activated conductance of CNG channels. On the other hand, the shadow response elicited in RPeD11 was preserved by 2-amino ethyldiphenyl borate (2-APB), a transient receptor potential channel blocker. Consistent with the electrophysiological data, cis-diltiazem blocked the shadow-evoked withdrawal response whereas 2-APB did not block the withdrawal response evoked by the shadow stimulus in intact freely behaving *Lymnaea*. Together, these findings support the hypothesis that the second messenger in dermal photoreceptors involves CNG and not transient receptor potential channels.

2P270 活性化したトランスデューシンは微小管上を1次元格子拡散する

1A1420 One-dimensional lattice diffusion of activated transducin on the microtubules in rod outer segment

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One-dimensional lattice diffusion of motor proteins on microtubules has recently generated great amount of interest as a protein transport mechanism driven solely by thermal energy. We report here that the light-dependent translocation of retinal G protein transducin is carried about by this one-dimensional lattice diffusion in rod photoreceptor outer segment (ROS). The ROS is connected to the inner segment (RIS) by connecting cilium, and it has been known that the transducin exhibits massive translocation between ROS and RIS dependently on light conditions. To visualize the translocation of transducin in ROS, we applied single fluorescent molecule imaging technique to the α subunit of transducin ($G\alpha_t$). The $G\alpha_t$ exhibits high lateral diffusivity in the dark-adapted disk membrane, while, in the presence of GTP, light causes the segregation of activated $G\alpha_t$ ($G\alpha_t^*$) to the rim of disks and forms dynamic aggregates of $G\alpha_t^*$. The side-view imaging of ROS shows that the aggregated $G\alpha_t^*$ exerts one-dimensional random walk in longitudinal direction along the microtubules just beneath the plasma membrane with the diffusion coefficient of $0.22 \mu\text{m}^2\text{s}^{-1}$. Since it has been implicated that the lattice diffusion can carry their cargos to the end of microtubules without any consumption of ATP in much higher velocity than that of ATP-driven active transport in short range of distance, such a translocation mechanism would be favorable for the

photoreceptor to achieve randomized delivery and retrieving of $G\alpha_t$ without any loss of energy.

2P271 錐体視物質のメタ III 中間体の性質 Characterization of Meta III Intermediate of Cone Visual Pigment

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Photoactivated visual pigment undergoes the photobleaching process in which Meta II intermediate activates G-protein (transducin). Meta I and Meta III intermediates, the precursor and the thermal product of Meta II, respectively, are also physiologically important because they are relevant to the activation and deactivation of the visual pigment. While the behavior of Meta I is well studied, little is known about Meta III in cone visual pigment. In the present study, we characterized Meta III intermediate of chicken green-sensitive cone visual pigment (cG).

UV-visible absorption spectroscopy at room temperature showed that Meta III intermediate of cG (cG-Meta III) is formed along with cG-Meta II within 10 ms, while metarhodopsin III is formed from metarhodopsin I/metarhodopsin II equilibrium. cG-Meta II and cG-Meta III independently decay to apoprotein (opsin) and all-trans-retinal with the time constants of 4 s and 170 s. Upon the decay of cG-Meta II, intrinsic tryptophan fluorescence increased, indicating that all-trans-retinal is released from opsin. However, tryptophan fluorescence was slightly decreased upon the decay of cG-Meta III. Transducin C-terminal peptide slows the decay of cG-Meta III, but the kinetics of the quench of transducin activation agreed with the decay of cG-Meta II. These findings indicate that cG-Meta III binds to but does not activate transducin. In its decay product, Schiff base linkage of the chromophore is hydrolyzed but the retinal is retained in the chromophore binding pocket.

2P272 海藻由来アセタブラリアロドプシン II の光化学反応 Photochemical reactions of *Acetabularia rhodopsin II* from marine plant

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In the past decade, archaeal rhodopsin homologues have been found in many microorganisms, including proteobacteria, cyanobacteria, fungi, dinoflagellates, and green algae. *Acetabularia* rhodopsin (AR) is the first discovered rhodopsin from a marine "plant", *Acetabularia acetabulum*, which is a unicellular alga, but gigantic in size being about 5-10 cm tall. *Acetabularia* opsin gene was firstly cloned by Tsunoda *et al.* (*Biophys. J.* 2006), and AR turned out to be a light-driven H⁺ pump. Later, Jung re-cloned and obtained another new opsin homologue, which we named ARII. By using novel cell free expression system (Shimono *et al.* *Protein Sci.* 2009), we successfully obtained ARII in the presence of all-trans retinal. The absorption maximum of DDM-solubilized ARII located at 533.5 nm. The isomeric state of retinal was predominantly all-trans and no light-dark adaptation was observed. Photo-induced H⁺ release/uptake reaction was detected for Egg-PC reconstituted ARII by using pH-sensitive indium tin oxide (ITO) electrode. Moreover, *Xenopus* oocyte expressing ARII showed photo-induced membrane current. Thus, ARII was concluded to be a light-driven H⁺ pump. Flash-photolysis experiments were performed at neutral pH in the presence of 0.05% DDM. The photocycle of ARII was terminated with a halftime of 30 ms and included six spectrally distinct photo-intermediates, which were named as K, L, M, N, O and ARII' in analogy to the intermediates of bacteriorhodopsin. In the latter half of the photocycle, M, N and O were appeared in equilibrium state.

2P273 細菌型緑色光吸収タンパク質、SRI の構造変化・色制御・情報伝達機構の解析

Structural change, color tuning and signal transfer mechanism of the green-absorbing bacterial sensory rhodopsin, SRI.

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Microorganisms utilize light not only as energy sources but also as signals by which sensory rhodopsin I (SRI) works as a dual photoreceptor for both positive and negative phototaxis in bacteria and archaea. The signal relay mechanism from SRI to its cognate transducer protein, HtrI, has become a focus of interest, in part because of its importance to the general understanding of communication between integral membrane proteins. Because of the instability of SRI, little is known about the molecular mechanism(s) of interac-