2P348 視細胞円板膜上でのトランスデューション及びロドプシンの一分子追跡
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2pD03 Single molecule tracking of transducin and rhodopsin in retinal disk membrane.
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Phototransduction system in vertebrate rod photoreceptor cell is a typical trimeric G protein signaling system. The G protein in this system, i.e. transducin (T α β γ), and its upstream receptor rhodopsin have been believed to undergo diffusion on the disk membrane. Although traditional collision-coupling model has been thought to be able to explain the molecular mechanism of transducin activation by rhodopsin (Meta II), precise motions of rhodopsin and transducin have never been clarified in single molecular level. Here, we studied the motion of both rhodopsin and transducin in frog disk membrane by using single molecule imaging technique. Transducin α subunit (T α) was labeled with Hylite750 at ⁶⁶Cys. Rhodopsin was monitored by Cy7-labeled Fab' of antibody 1D4. Single molecule tracking experiments on a TIRF-microscope revealed that T α undergoes slightly faster diffusion than rhodopsin in the dark, and light stimulus reduces the T α 's diffusion rate. In contrast, the diffusion rate of rhodopsin was insensitive to light, and considerably smaller than that estimated from classical macroscopic studies. Immediately after the addition of GTP following light exposure, T α started to diffuse with much higher diffusion rate. These observations indicated that collision of transducin and rhodopsin is done in a condition in which the diffusion of both proteins are considerably restricted. In term, activated T α seems to be freed from the constraint(s) and starts running to activate its effector, PDE β .

2P350 E113Q/M257Y 変異体の分子動力学計算によるロドプシン活性化におけるヘリックス間水素結合の役割の研究
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The role of interhelical hydrogen bonds in the rhodopsin activation studied by MD simulation for a constitutive active mutant E113Q/M257Y
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Visual pigment rhodopsin is the only GPCR whose crystal structure is solved. A double mutant E113Q/M257Y can activate G-protein in the dark state (J. M. Kim et al, PNAS 2004, 101, 12508), which implies that the dark state of the mutant might be comparable to the wild type (WT) activated state, Meta II. In the present study, we performed molecular dynamics (MD) simulations for this mutant and the WT rhodopsin. The WT and the mutant rhodopsin were embedded in the hydrated lipid bilayer membrane. First, we performed a MD simulation for the WT rhodopsin, whose initial structure was taken from X-ray structure (PDB code: 1U19). Then, the mutant MD simulation was performed, in which the initial structure was obtained by substituting the corresponding residues of the WT MD snapshot. From these simulations, we found characteristic features described below. First, hydrogen bond networks around Met257 in the WT underwent significant modifications in the mutant, which involves changes in hydrogen bonds formed with neighboring internal water molecules. Second, compared with the distance between the counterion Glu113 and the retinal Schiff base in the WT, the corresponding distance (Gln113-Schiff base) in the mutant was longer by about 1 Å. Third, the structures and dynamics of transmembrane helices and intracellular loops were different between the two simulations. These facts suggest that weakening of the interhelical interactions in the mutant induce largers-scale structural changes which are likely relevant to the G-protein binding.

2P349 カエル視細胞のロドプシンは二量体化によって脂質ラフトへ移行する
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2pD04 Rhodopsin dimerization induces rhodopsin translocation into lipid rafts in bullfrog photoreceptor.
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It has been known that lipid raft participates in many cellular processes including signal transduction. Our biochemical studies have clarified that there is lipid raft-like detergent-resistant membrane (DRM) in the disk membrane of vertebrate rod photoreceptor, and that the trimeric G protein, transducin (T α β γ), is recruited to the DRM upon photic stimulus of rhodopsin in the absence of GTP. Thus, the rhodopsin in the lipid raft was strongly suggested to have a priority to bind GDP-T α β γ . Here, we examined the motion and the state of rhodopsin in the disk membrane of frog (*Rana catesbeiana*) rod photoreceptor by single-molecule tracking technique using TIRF-microscope and biochemical studies of DRM. Comparison of the tracking data of Cy7-Fab' of anti-rhodopsin CT IgG (1D4) and the Alexa 700-labeled IgG (1D4) itself suggested that the rhodopsin is at the equilibrium between monomer and dimer in the disk membrane. Dimerized rhodopsin showed slower diffusion rate (0.05 μ m²/s) than monomer (0.15 μ m²/s). To clarify the relationship between rhodopsin dimerization and lipid raft in the disk membrane, we examined the effect of artificial crosslinking of rhodopsin by IgG (1D4) to the partition of rhodopsin to the DRM. It was revealed that rhodopsin forced to dimerize by IgG goes to DRM, while Fab'-bound rhodopsin doesn't. These data show that rhodopsin dimerization induces rhodopsin translocation into lipid rafts. We will also discuss the physiological role of dimerized rhodopsin and its translocation to the lipid rafts.

2P351 Caged-ATPを用いた様々な条件下でのホタル生物発光の時間分解測定
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Time-resolved measurement of firefly bioluminescent reaction triggered by Caged-ATP under various conditions
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A new method to study dynamical processes of firefly bioluminescence has been developed. Luciferin, luciferase, caged-ATP and MgSO₄ were dissolved in HEPES buffer (around pH = 7.8) in the dark, and the solution was irradiated by a Nd:YAG laser(355 nm). The luminescence was then generated by ATP released from caged-ATP. Temporal variation of its intensity was measured with a photon counting system. The time-resolution of the measurement system was 3ms or 10ms. When the concentrations of luciferin, luciferase, caged ATP, and MgSO₄ are 5x10⁻⁶ M, 5x10⁻⁶ M, 8x10⁻⁴ M, and 5x10⁻³ M, respectively, a very fast peak followed by a monotonous decay was clearly observed. This is probably the first report of the time resolved measurement of bioluminescence as far as the authors know. The time course is well reproduced by a sum of two exponential functions. The relaxation time of the fast decay is about 5 ms, whereas that of the slow decay was several ten ms depending on pH of the solution and the luciferin concentration. The relaxation time of the slow component increases with the concentration of glycerin which increases viscosity of the solution. The time-resolved measurement of Raman spectra is under way in order to clarify the origin of these two kinds of decay.