the 13-*cis* form in the dark and light conditions. This observation clearly demonstrates the important role of Pro at position 206 for photochromic sensor. We further mutate this position to other amino acids, and the results of the systematic analysis of P206X ASR will be presented.

[1] Kawanabe et al. *J. Am. Chem. Soc.* 129, 8644 (2007).

2Q1548 ウシロドプシンの細胞質側ループを持つプロトンポンプ型ロドプシンカメラの性質

Chimeric Proton Pumping Rhodopsin Containing the Cytoplasmic Loop of Bovine Rhodopsin

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G-protein-coupled receptors (GPCRs) transmit various stimuli to the intracellular signaling systems, and rhodopsin (Rh) is the most studied GPCR. Microbial rhodopsins, similar heptahelical membrane proteins, do not activate G-proteins, but it is known that they can activate G-protein by introducing the third cytoplasmic loop of bovine Rh. We recently reported that such chimeras of bacteriorhodopsin (BR) and *Natronomonas pharaonis* sensory rhodopsin II (SRII) similarly activate G-protein (37,000 times lower than that of bovine Rh) [1]. The activation mechanism is intriguing, because SRII-chimeras have two-orders of magnitude slower photocycle than BR-chimera. This fact implies that the photocycle (the lifetime of the M intermediate) is not the simple determinant of activation efficiency [1].

Last year, we reported between two proton-pumping rhodopsins, G-protein activation was observed for the chimeras of *Gloeobacter* rhodopsin (GR), but not for those of proteorhodopsin (PR). Here we studied the detailed G-protein activation mechanism using various GR chimeras. No activation was observed for the chimeras containing the second loop of bovine Rh. To increase the activation efficiency, we are attempting combination of these chimeras and introduction of the specific ERY motif, which is well conserved among class A GPCRs. Activation mechanism of GPCRs will be discussed based on the observed activation efficiencies and photocycling kinetics for the GR chimeras. [1] Nakatsuma et al. *Biophys J.* 100, 1874-82 (2011).

2Q1612 ポンプ機能変換をめざしたプロテオロドプシンへの変異導入 Functional conversion of green-proteorhodopsin by site-directed mutagenesis

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Nature created two kinds of light-driven ion pumps, bacteriorhodopsin (BR) and halorhodopsin (HR) functioning as outward proton and inward chloride pumps, respectively [1]. BR contains two negative charges at D85 and D212 near the retinal chromophore, the former of which is replaced by Thr in HR. We previously converted BR into a chloride pump by a D-to-T mutation at position 85 [2]. This finding implied that BR and HR share a common transport mechanism, and the ionic specificity is determined by the Schiff base region. However, it is known that HR cannot pump protons by the reverse T-to-D mutation. Therefore, ion-pumping mechanism is not so simple. In addition, chloride binding to D212N BR restores the proton-pump function, in which strongly hydrogen-bonded water molecule plays a crucial role [3].

In this study, to investigate the molecular mechanism of transporting rhodopsin, we tried functional conversion of green proteorhodopsin (PR) using site-directed mutagenesis. It should be noted that the functional conversion from proton to chloride pump has been reported only for BR, and it is intriguing to test other proton-pumping rhodopsins. We expressed D97T/S and D227T/S PR in *E. coli*, which correspond to D85 and D212 in BR, respectively. Ion-pumping measurements using cell suspensions and various spectroscopic characterizations are now in progress, and functional determinants will be discussed.

[1] Kandori, *Mol. Sci.* 5, A0043 (2011).

[2] Sasaki et al. *Science* 269, 73 (1995).

[3] Shibata et al. *Biochemistry* 46, 7525 (2007).

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Optogenetics gives a new trend in life science which enables us to control neuronal excitability and animal behavior by light. Channel rhodopsin, halorhodopsin and their mutants are widely used to control excitability of cell membrane. As the field developed, it is rapidly needed to improve their ion permeability and selectivity, and variety of acceptable light color. In this study, we intend to develop a novel light-driven ion channel by preparing fusion proteins combining microbial-type rhodopsins and a potassium ion channel, KcsA. We choose *Anabaena* Sensory Rhodopsin; ASR, *pharaonis* Halorhodopsin; pHR, *Gloeobacter* Rhodopsin; GR, which can be expressed at sufficient level. KcsA is an ion channel whose molecular mechanism has been well studied in atomic detail. Therefore, we can design protein molecules to cause domain interaction based on their X-ray crystal structures. We have succeeded to express ASR-KcsA fusion protein in *E. coli*. Visible absorption with λ_{max} around at 550 nm confirmed a proper folding of ASR domain. We have also succeeded to obtain ion-exchange induced difference IR spectra similar to that obtained from KcsA alone. This suggests that KcsA domains form a pore by assembling into a homo-tetramer. Hereafter, we will prepare the other fusion proteins combining pHR or GR with KcsA and compare their expression levels to select a suitable construct for further optimization. We also plan to assay ion pump activity for studying properties of ion transmission of the fusion proteins.

2Q1636 低 pH 条件下におけるプロテオロドプシンの速いプロトン放出について

The fast proton release of proteorhodopsin at low pH

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Proteorhodopsin (PR) is a microbial rhodopsin found in marine eubacteria. Light absorption induces the photoisomerization of a retinal and undergoes the photochemical reaction cycle (photocycle). PR acts as an outward light driven proton pump. So far, the photo-induced proton transfer scheme of PR during its photocycle at neutral or alkali pH was investigated in detail, while that at low pH was not well-known. In this study, we investigated the photo-induced proton transfer of PR under low pH (<4) condition. The flash light-induced proton uptake or release on PR was measured by a tin-oxide (SnO₂) transparent electrode. The fast proton release was observed under pH ~ 4.0. We also performed the same measurement on several PR mutants at the position of Asp97 and Asp227. As a results, the fast proton release at low pH was not only observed in D227N mutant, implying that the amino acid residue concerned with the fast proton release is Asp227. Furthermore, this fast proton releasing signal was affected by the presence of chloride. This may suggest the chloride binding in the vicinity of Asp227. On the basis of these results, the plausible mechanism of the fast proton release of PR at low pH will be discussed.

2Q1624 KcsA と微生物型ロドプシンの融合タンパク質の調製と分析 P67 Preparation and Characterization of the Fusion Protein:KcsA and Microbial Rhodopsins