3P-216 ハロロドブシンポンプ機能におけるシッフ塩基近傍酸性残基の役割

The role of the acidic residue near PSB in Cl⁻ pump by *Natronomonas pharaonis* halorhodopsin

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Natronomonas pharaonis halorhodopsin (pHR) is an inward light-driven Cl⁻ pump. It is known that HR transports Cl⁻ bound in the vicinity of the proton of the protonated Schiff base (PSB) to cytoplasmic space (CP) during a photocycle reaction. There is the only negative charge due to Asp in the vicinity of PSB, but the role in Cl⁻ transport has yet to be elucidated. In the present study, to clarify the role of the residue in the pump function, we expressed pHR and Asp252 mutants substituted with Glu and Asn in *Xenopus* oocytes and determined the photo-induced Cl⁻ pump activities using the two electrode voltage-clamp technique. The outwardly directed photo-induced current due to the pumping was observed only in wild-type (WT) and D252E, whereas D252N did not show any photo-induced current, implying that the negative charge located near PSB is essential in Cl⁻ pump function. We then carried out the flash photolysis experiment to determine how the photo-intermediates alter in D252N. The analysis of the absorption kinetics showed that the photocycle of D252N contained a single red-shifted intermediate (λ_{max} at approx. 640 nm). The titration of D252N with Cl⁻ induced a red-shift of the absorption maximum, indicating that D252N had the capability of Cl⁻ binding. These results suggested that the red shifted photo-intermediate might be Cl⁻ bound form and could not proceed to a crucial intermediate to transport Cl⁻ to CP space. The molecular mechanism of the role of the negative charge in Cl⁻ pump is discussed here.

3P-217 大腸菌多剤排出輸送担体 EmrE のアンチパラレル構造

Anti-parallel membrane topology of a homo-dimeric multidrug transporter EmrE in *E. coli*.

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EmrE in *Escherichia coli* belongs to the small multidrug resistance (SMR) transporter family. It functions as a homo-dimer, but the orientation of the two monomers in the membrane (membrane topology) is under debate. We expressed various single-cysteine EmrE mutants in *E. coli* cells lacking a major efflux transporter. Efflux from cells expressing the P55C or T56C mutant was blocked by the external application of membrane-impermeable SH-reagents. This is difficult to explain by the parallel topology configuration, because Pro55 and Thr56 are considered to be located in the cytoplasm. From both the periplasm and the cytoplasm, biotin-PE-maleimide, a bulky membrane-impermeable SH-reagent, could access the cysteine residue at the 25th position in the presence of transport substrates and at the 108th position. These observations indicate the anti-parallel topology in the membrane⁽¹⁾.

The anti-parallel structure detected in our biochemical assay supports a recent crystal structure by Chen *et al.*⁽²⁾. Similar configuration is also observed in a hetero-dimeric SMR transporter EbrAB in *B. subtilis*⁽³⁾. It appears that the anti-parallel configuration is a general form in SMR.

1. Nara, *et al.*, 2007, *J. Biochem.*, **142**: 621-625.
2. Chen, *et al.*, 2007, *Proc. Natl. Acad. Sci. USA.*, **104**: 18999-19004.
3. Kikukawa, *et al.*, 2007, *Biochim. Biophys. Res. Commun.*, **358**: 1071-1075.

3P-218 バクテリオロドブシン L93A ミュータントのX線結晶構造解析

X-ray crystallographic studies of the L93A mutant bacteriorhodopsin

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Bacteriorhodopsin functions as a light-driven proton pump. Previous crystallographic studies of the hexagonal P622 crystal of wild-type bacteriorhodopsin (bR) have shown that rotation of the side chain of Leu93 assists water relocation across the retinal Schiff base in the K-to-L transition. It is interesting to investigate how this water relocation is affected by replacement of Leu93 by a smaller amino acid residue. In this study, we crystallized the Leu93-to-Ala mutant bacteriorhodopsin into a hexagonal P622 crystal with cell dimensions of a = b = 102.0 Å and c = 112.2 Å. Diffraction data at 2.7 Å resolution showed that the Leu93-to-Ala replacement caused no large alteration in the overall structure of bR and that the micro-cavity created by the replacement are occupied by four additional water molecules. When an O-like intermediate was accumulated by illumination, the crystal quality became worse. Nonetheless, it was observed that formation of this intermediate was accompanied by a large movement of the EF loop underwent.

3P-219 好アルカリ性好塩菌由来 *Natronomonas pharaonis* のハロロドブシンの結晶化

Crystallization of halorhodopsin from the haloalkaliphilic archaeon *Natronomonas pharaonis*

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Halorhodopsin (hR), an archaeal rhodopsin in Haloarchaea, uses light energy to pump chloride from the extracellular side to the cytoplasm. With the aim to obtain a 3D crystal that is suitable for investigation of light-induced structural changes, our group has previously produced a hR-overproducing mutant (strain KM-1) of the haloalkaliphilic archaeon *Natronomonas pharaonis*. This strain allowed us to prepare a large quantity of hR-rich claret membrane. Titration experiments with sodium chloride showed a negative cooperativity in the binding process of chloride ions the claret membrane. In this study, the membrane fusion method that was developed for crystallization of bacteriorhodopsin was used to crystallize hR-lipid complexes into a hexagonal crystal. Its spectroscopic data suggested that bacterioruberine, the second chromophore in the claret membrane, was incorporated into the 3D crystal. We are now improving the crystallization condition to obtain high-quality crystals.

3P-220 Tet-Off 遺伝子発現システムを用いた小脳学習における MAPK/ERK kinase1 の機能解析

Physiological analysis of MAPK/ERK kinase 1 in cerebellar Purkinje cells by using Tet-Off gene expression system

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Tetracycline-inducible gene expression (Tet-ON/Off) system allows reversal suppression of gene function and provides powerful tool to study molecular mechanism underlying learning and memory. In the present study, we focus on physiological roles of MAPK/ERK kinase 1 (MEK1) in cerebellum-related functions. By using combination of cerebellar Purkinje cells (PCs)-specific promoter L7 and Tet-Off gene expression technology, we developed a new transgenic line called HA-dnMEK1 Tg mice. In HA-dnMEK1 Tg mice, PCs specifically expressed a dominant-negative MEK1 (dnMEK1) in a doxycycline (DOX)-dependent manner. In case of drinking Dox-containing water, PCs does not express dnMEK1, but normal water drinking induces dnMEK1 expression within PCs in the Tg mice. To explore the physiological function of MEK1 in PCs in motor learning, classical eyeblink conditioning was examined in HA-dnMEK1 Tg mice. Classical conditioning of eyeblink response is elicited by paired presentation of a conditioned stimulus and an unconditioned stimulus and represents a basic form of cerebellum-dependent motor learning. Normal water-induced expression of dnMEK1 in PCs severely retarded acquisition of conditioned responses (CRs) in this mutant mice, whereas Tet-treated HA-dnMEK1 Tg mice exhibited intact CR acquisition during conditioning. These results indicate that MAPK/ERK kinase in adult PCs is essential for eyeblink conditioning, suggesting that the signaling cascade from mGluR1 to MEK1 within cerebellar PCs is critical for motor memory formation.

3P-221 ヒスタミン H1 受容体発現レベルの IL-4 などの因子による調節

Regulation of the expression of histamine H1 receptors by IL-4 and other factors

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The level of histamine H1 receptor (H1R) in the brain and peripheral tissues is dynamically regulated under various physiological and pathological conditions, and these changes may be responsible for some pathogenesis, such as allergic rhinitis. We have examined the effects of various stimulants on the expression of H1R to seek for the factors that influence the expression of H1R. The factors that have been found to affect H1R level are histamine, muscarinic agonists, and adrenergic agonists. Histamine stimulation and muscarinic m3 receptor stimulation had dual effects on the regulation of H1R level, i.e., these stimulations down-regulated H1R by the degradation of the receptor protein, but up-regulated H1R by accelerating the H1R mRNA synthesis. On the other hand, β_2 -adrenergic receptor stimulation down-regulated H1R both by receptor degradation and by inhibition of the H1R mRNA synthesis. We have recently found that IL-4 stimulation increased H1R level by accelerating the mRNA synthesis. This activation was mediated by JAK-STAT6 pathway. On the other hand, activation of H1R mRNA synthesis by histamine was mediated by protein kinase C. The effect of IL-4 and that of histamine on H1R mRNA synthesis were additive, probably because these processes were mediated by distinct mechanisms. These results indicate a very important point in the pathogenesis of allergy, in which both the level of histamine and that of IL-4 are highly increased. These stimulants will work together to increase the H1R level and thus aggravate the symptoms.

3P-222 ライセニンチャネルのバイオセンサー担体としての応用に向けて Lysenin channel as a nanopore for biosensing applications

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Lysenin is a novel protein obtained from body fluid of earthworm, *Eisenia foetida*. It specifically binds to sphingomyelin (SM) in the cell membrane and induces cytolysis by forming membrane-spanning channel pores. Because of its specific binding properties to SM it has been used as a marker of membrane raft structures, since such structures are rich in sphingolipids and cholesterol. To date there are only a few studies that evaluate the electrophysiological properties of the lysenin protein channel, the detailed features of the single lysenin channel are essentially