additional amino acid chemoreceptor(s). None of the other MLPs with tandem PAS-like domains appeared to mediate amino acid taxis. We then turned our attention to MLPs with a single PAS-like domain. The overproduction of two members of this family, Mlp2 and Mlp3, in the $\Delta mlp24 \Delta mlp37$ strain enhanced taxis to serine in capillary assays, suggesting that these MLPs mediate attractant responses to serine. Interestingly, the expression of Mlp3, but not Mlp2, enhanced taxis to alanine, cysteine, glycine and threonine, implying the difference in specificity between these MLPs. However, we failed to detect direct binding of serine to these MLPs in isothermal titration calorimetry measurements with periplasmic fragments of Mlp2 and Mlp3, as well as membrane vesicles of an otherwise receptor-less strain of *Escherichia coli* expressing Mlp2 or Mlp3. This raises the possibility that these MLPs sense serine via periplasmic serine-binding protein(s).

3PT189 アミノ酸変異による苦味受容体 TAS2R16 機能の多様化

Functional diversity of bitter taste receptor TAS2R16 by amino acid substitution

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In mammals, bitter taste is mediated by TAS2Rs, which belong to the large family of seven transmembrane G protein-coupled receptors. Since TAS2Rs are directly involved in the interaction between mammals and their dietary sources, it is likely that these genes evolved to reflect species' specific diets during mammalian evolution (1, 2). Here, we investigated the sensitivities of TAS2R16s of various primates by using a cultured cell expression system in combination with behavioral tests (3). We found that the sensitivity of each primate species varied according to the ligand. Especially, the sensitivity of TAS2R16 of Japanese macaques to salicin, a bitter compound contained in the bark of Salicaceae (willow) plants, was much lower than that of human TAS2R16, which was supported by behavioral tests. The replacement of amino acid residue at position 86 caused dynamic changes in the sensitivities of TAS2R16s. These results suggest the possibility that bitter taste sensitivities evolved independently by replacing specific amino acid residues of TAS2Rs in different primate species to adapt to accessible food items they use. The amino acid residues responsible for the specificities of each ligand would be discussed in the presentation.

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3PT190 弾性ネットワークモデルを利用した β_2 アドレナリン受容体のアンサンブルドッキング

Ensemble Docking Simulation for β_2 Adrenergic Receptor Using Elastic Network Models

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G protein-coupled receptors (GPCRs) are membrane proteins involved in signal transduction and participate in many diseases. Therefore, the receptors are important targets in the field of drug discovery.

Protein structural information is useful to understand their function and enable us to develop new therapeutic molecules. However, these membrane proteins are not easy to crystallize. And it sometimes happens that rigid-body protein docking simulation for GPCR based on one ligand-receptor complex structure to discover new drugs lead to inefficient results with own flexible conformation. In this study, GPCR's conformational polymorphism into the virtual screening of β_2 adrenergic receptor (ADRB2) using elastic network model (ENM) and normal mode analyses (NMA). First, we build the ENM of ADRB2 and performed NMA. Next, we make virtual models based on ADRB2's elastic network normal mode analysis data. And we do docking simulation for the virtual model group to make up for the above shortcoming.

According to the results, the screening efficiency of virtual model group is improved by over 20% against that of the initial model.

3PT191 大腸菌走化性受容体 Tar システイン置換シリーズの化学架橋によるクラスター構造推定

Characterization of receptor clustering by cross-linking a series of cysteine-substituted mutants of the aspartate chemoreceptor Tar

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In the chemotaxis of *Escherichia coli*, the bacterium can respond to a wide range of attractant concentrations. The chemoreceptors, which exist as homodimers regardless of ligand binding, form complexes with the adaptor protein CheW and the histidine kinase CheA. The chemoreceptors form "trimers of dimers" with their cytoplasmic tips in contact with each other, which are supposed to be organized into a hexagonal array. To account for high sensitivity of the chemotaxis system, it has been proposed that attractant binding to a receptor dimer affects neighboring receptor dimers. Receptor clustering has also been implicated in signal gain control by methylation of specific glutamate residues that is responsible for adaptation to persisting stimuli. However, molecular mechanisms underlying such interactions between receptor dimers remain to be elucidated. In this study, we carried out *in vivo* chemical cross-linking assay to probe changes in the receptor array by using divalent maleimide cross-linkers and a series of site-directed mutant proteins of the aspartate chemoreceptor Tar with single cysteine residues on the surface of the dimer. The pattern of cross-linking was generally in agreement with our previous results of disulfide-crosslinking assay without a chemical cross-linker. Efficiencies of cross-linking were affected when an attractant or a repellent was added. We propose a plausible model of the receptor array, in which three trimers of dimers are in contact with each other in the periplasmic spacer.

3PT192 コレラ菌走化性解析のためのスウォームアッセイ法の開発

Development of a system for swarm assays to evaluate chemotaxis of *Vibrio cholerae*

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Vibrio cholerae, the causative agent of cholera, shows chemotaxis, which has been implicated in pathogenicity and survival in various environments. A classical biotype strain of *V. cholerae* has 44 homologs of methyl-accepting chemotaxis protein (MCP), which are named MCP-like proteins (MLPs). Among them, Mlp24 and Mlp37 are major chemoreceptors for amino acid taxis in *V. cholerae*. A mutant strain lacking *mlp24* and *mlp37* ($\Delta mlp24 \Delta mlp37$) shows significantly weaker attractant responses to amino acids than the wild-type strain in capillary assays. However, in swarm assays, no detectable difference is observed between the double mutant and the wild-type strains. The swarm assay is not only easier than the capillary assay but also suited for screening of mutants. In this study, we aimed at developing a system for swarm assays to evaluate chemotaxis of *V. cholerae*. We deleted the *mlp32* gene (previously named *are-2*), which encodes a homolog of the redox sensor Aer for aerotaxis of *Escherichia coli*. The resulting triple mutant strain ($\Delta mlp24 \Delta mlp37 \Delta mlp32$) showed significantly slower spreading than the wild-type and the double mutant strains in tryptone swarm plates. Introduction of a plasmid carrying the *mlp32* gene complemented the defect of the triple mutant strain in swarming. Furthermore, introduction of a plasmid carrying the *mlp24* or *mlp37* gene significantly enhanced swarming of the triple mutant strain, demonstrating that this strain can be used as a plasmid host to evaluate amino acid taxis of *V. cholerae* by the swarm assay.

3PT201 Seeking molecular and neural mechanisms of temperature response and resistance in *C. elegans*

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Animals have adaptation mechanisms against environmental temperature changes. To understand the molecular mechanism of temperature response, we are using simple model animal, nematode *C. elegans*. We recently found that *C. elegans* shows cultivation temperature-dependent cold-resistance, which is regulated through neural pathway. After cultivation at 25 degree, wild-type