3P204

In Vivo 架橋を用いた大腸菌走化性における受容体クラスターの構造機能連関の解析
○入枝 泰樹^{1,2}、本間 道夫¹、川岸 郁朗^{2,3}
¹Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ., ²Research Center for Micro-Nano Tech., ³Dept. of Frontier Biosci., Hosei Univ.
Probing structure-function relation of the bacterial chemoreceptor cluster by *in vivo* crosslinking
Hiroki Irieda (1,2), Michio Homma (1) and Ikuro Kawagishi (2,3). (1: Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ.; 2: Research Center for Micro-Nano Tech.; 3: Dept. of Frontier Biosci., Hosei Univ.)

Among various sensory systems, the chemotaxis of *Escherichia coli* is extensively studied. Environmental stimuli are sensed by methyl-accepting chemotaxis proteins (MCPs), including Tar for aspartate and Tsr for serine, which form homodimers and regulate the histidine kinase CheA. MCPs cluster at a cell pole together with CheA and the adaptor protein CheW. In this cluster, chemotactic signals are believed to be amplified via interactions between MCP dimers. However, the molecular mechanism underlying such MCP communications is obscure. Using *in vivo* disulfide crosslinking assays, we previously analyzed the dimer-to-dimer interactions. The Cys-mutant versions of Tar and Tsr formed interdimer crosslinked Tar-Tsr heterodimer. Moreover, aspartate binding to Tar affected the crosslinking of the Tar-Tsr heterodimer and the Tsr dimer, implying horizontal signaling via interaction between MCP dimers, which may be involved in signal amplification. To understand the precise mechanism of signal amplification, more detailed structural information of the cluster is indispensable. It is believed that three MCP dimers interact with each other in the periplasmic and cytoplasmic domains to form a hexagonal array. We are trying to purify and analyze trimers of dimers of MCP that are crosslinked at either periplasmic or cytoplasmic residues.

3P206

GPCRのモノマー・ダイマー動的平衡の解明；一分子追跡法による研究
○笠井 倫志¹、Eric Prossnitz²、楠見 明弘¹
¹京大・再生研、²ニューメキンコ大

3aC01

Single-molecule tracking revealed the complete kinetics of a GPCR monomer-dimer dynamic equilibrium
Rinshi S. Kasai (1), Eric R. Prossnitz (2), and Akihiro Kusumi (1). (1: Membrane Mechanisms Project, ICORP-JST, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; 2: Department of Cell Biology and Physiology, University of New Mexico, Albuquerque, New Mexico, U. S. A.)

By developing a single-molecule tracking methods, we determined the complete kinetics of monomer-dimer dynamic equilibrium of formyl peptide receptor (FPR), a GPCR. Although G-protein-coupled receptor (GPCR) dimers have been implicated in a variety of signaling pathways, the dimer fractions and lifetimes have remained totally unknown, and once these are known, the functional significance of GPCR dimers could be quantitatively assessed. Both the wild type and the internalization-deficient mutant D71A fused to mGFP, expressed in CHO cells, formed dimers at very similar levels (with a koff of 3.1/s). Since the fluorescent fraction of mGFP on FPR is unknown, first, the binding of a fluorescent (Alexa594) ligand, exactly labeled at one single site, was used to determine the true monomer/dimer ratio and the dissociation constant of the Alexa-ligand (Kd=3.0 nM) by theoretical fitting. (e.g., 47% of FPR was in dimers at the density of 2.3 copies/ μ m²). Then by incubating the CHO cells expressing different concentrations of D71A with 6.0 nM Alexa-ligand, the 2-dimensional equilibrium constant (KD) was determined, giving 2.8 copies/ μ m², providing a kon of 11 copies/ μ m²/s. By comparing these observations with the apparent monomer-dimer fractions of D71A-FPR expressed at the level of ~10,000 copies/cell, and also assuming that mGFP does not alter the monomer-dimer equilibrium, it was found that 86% of mGFP-FPR was fluorescent.

3P205

大腸菌アスパラギン酸受容体TarのHAMPドメイン内の1つのアミノ酸残基置換がそのクラスター形成と極局在に与える影響
○太田 徳子^{1,2}、入枝 泰樹^{1,2}、坂野 聡美^{2,3}、本間 道夫¹、川岸 郁朗^{2,3}
¹Div. of Biol. Sci., Grad. Sch. of Sci. Nagoya Univ., ²Research Center for Micro-Nano Tec., ³Dept. of Frontier Biosci., Hosei Univ.
Single residue substitutions in the HAMP domain of the bacterial aspartate chemoreceptor Tar affect its clustering and polar localization
Noriko Ohta (1,2), Hiroki Irieda (1,2), Satomi Banno (2,3), Michio Homma (1), Ikuro Kawagishi (2,3)(1: Division of Biological Science, Graduate School of Science, Nagoya University; 2: Research Center for Micro-Nano Technology; 3: Department of Frontier Bioscience, Hosei University)

In the chemotaxis of *Escherichia coli*, environmental stimuli are sensed by four transmembrane chemoreceptors (MCPs) and an MCP-like transmembrane protein (the redox sensor Aer). Regardless of their signaling states, MCPs and Aer exist as homodimers and form a huge cluster at a cell pole with a trimer of MCP (and Aer) dimers as a building unit. Receptor clustering has been implicated in signal amplification and gain control. However, molecular mechanisms of receptor clustering and polar localization are only poorly understood. In this study, we examined the formation of receptor cluster by using localization-deficient Tar mutants that have single amino acid substitutions in the HAMP domain which connects the transmembrane helix to the adaptation/signaling domain. We found that polar localization of Tar mutants were rescued by the co-expression of Aer, which cannot localize to cell poles by itself. *In vivo* cross-linking assays to probe the "trimer of dimers" suggested that the most severe Tar mutation impaires the formation and/or structure of the trimer of dimer. Thus, the ability to form a cluster can be affected by a structural changes in a region (e.g. the HAMP domain) other than the "trimer contact" which is at the tip of the cytoplasmic domain. These results also support the notion that effective clustering is required for the polar localization of MCP (and presumably Aer).

3P207

グルタミン酸受容体のシナプスへの出入り：1分子法と多分子法による研究
○中田 千枝子¹、土方 博子¹、吉田 広人¹、長谷川 理恵²、岡部 繁男³、楠見 明弘¹
¹JST-ICORP/京大・再生研、²阪大・産研、³東大院医・神経細胞生物
Turnover of glutamate receptors in the synapse of cultured hippocampal neurons: single molecule and bulk studies.
Chieko Nakada (1), Hiroko Hijikata (1), Hiroto Yoshida (1), Rie Hasegawa (2), Shigeo Okabe (3) and Akihiro Kusumi (1). (1: JST-ICORP/Inst. Frontier Medical Sciences, Kyoto University; 2: Institute of Scientific and Industrial Research, Osaka University; 3: Graduate School of Medicine, University of Tokyo)

The postsynaptic density (PSD) of the neuronal synapse is a highly ordered structure consisting of a large variety of species of specific proteins. Recent studies have shown that the PSD constituent molecules exchange with a bulk pool. To elucidate the mechanisms by which PSD modifies its molecular organization in response to synaptic activities, we examined the dynamics of glutamate receptors. Using TIRF microscopy, we observed YFP-tagged NR2A or NR2B expressed in cultured hippocampal neurons at the level of single or clustered molecules and obtained the following results. 1, In the plasma membrane of resting cells cultured for 7 days, about 15% of YFP-NR2A molecules are in clusters containing several to hundreds of N-methyl-D-aspartate receptors (NMDARs). 2, In contrast, the majority of YFP-NR2B-containing NMDARs were rather uniformly mono-dispersed over the somatodendritic cell membrane and exhibited translational diffusion, leaving 5% in clusters. 3, About 80% of YFP signals in clusters were colocalized with synapse markers. 4, FRAP experiments showed 58 % and 50 % of the fluorescence signals of YFP-NR2A and 2B recovered at ~10 min after photobleaching, respectively, indicating that NMDARs incorporated in synaptic clusters exchange with those outside, with a half time of recovery on the order of minutes. These results suggest that although the clustering tendency of surface NR2A-containing receptor is different from that of NR2B, the behavior of both receptors in synaptic clusters is similar in terms of molecular exchanges.