cylindrical structure with diameter and height are 10.6 and 7.5 nm, respectively. In the cylinder bottom, each monomer bended inside with angle of approximately 55 degrees, was attached to a small cylindrical structure with outer/inner diameters of 4.2/1.5 nm and the height of 4.3 nm, respectively.

3TA5-06 電子線結晶解析によって明らかになったヒト赤血球膜蛋白質バンド3膜貫通ドメインの立体構造

The three-dimensional structure of human erythrocyte band 3 membrane domain determined by electron crystallography

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A 55 kDa C-terminal membrane domain of band 3 mediates electroneutral exchange of chloride and bicarbonate across erythrocyte plasma membranes. It has been revealed that the anion-transporting is carried out by conformational change between inward- and outward-open forms, induced by binding of the substrates. Although band 3 has been studied for a long time, its structural information is very limited so far. Here, we report the three-dimensional structure of the human erythrocyte band 3 membrane domain determined by electron crystallography using two-dimensional crystals at 7.5 Å resolution. The band 3 membrane domains used for 2D crystallization were fixed to outward-open conformation by cross-linking with 4,4'-diisothiocyanatodihydrostilbene-2,2'-disulfonic acid before purification. Band 3 membrane domains exist as dimers in the crystals. A unit cell with the dimension of 194.5 Å x 182 Å contained six dimers, and those dimers could be classified two types of dimers; two structurally homodimers and four structurally heterodimers. The existence of the several forms indicates that some flexibility remains in the band 3 membrane domain in spite of "fixing" to outward-open form. The dimer had dimensions of about 110 x 50 Å, and a height of about 75 Å. Several large protrusions were recognized on the hydrophilic surface. Although it was not possible to count all 14 transmembrane segments expected from hydropathy prediction, several helices were obviously recognized and two major subdomains were recognized in band 3 monomer. From present data, the structural futures are discussed.

3TA5-07 分子動力学的手法を用いたアクアポリンに関する研究 Molecular Dynamics Simulations of Aquaporin

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The aquaporin (AQP) family conducts water molecules and expresses at the epithelial membrane. It is known that mercury is an inhibitor of water permeation of AQP family. But the inhibition mechanism has not been clarified yet. We performed two 40 ns MD simulations of bovine aquaporin-1 (AQP1) with mercury (Hg-AQP1) and without mercury (Free AQP1) to clarify how mercury inhibits water permeation of AQP1. During each last 10 ns MD simulation, we observed that the water permeation events in Free AQP1 and Hg-AQP1 occurred of 23 times and 0 time, respectively. We also found that mercury-binding is not influence on framework of whole structure but induces the collapse of orientation of residues at ar/R region. In Free AQP1, backbone oxygen atoms of Gly190, Cys191, and Gly192 lined and were oriented to the channel surface of water pore. Carboxyl group of Glu144 formed hydrogen bonds with backbone nitrogen atoms of Gly192 and Ile193 and holds the position of Gly190, Cys191, and Gly192 at around ar/R region. In Hg-AQP1, however, SHg⁺ of Cys191-SHg⁺ residue was oriented to the outside of water pore and collapse of orientation at ar/R region occurred. As a result, backbone oxygen atoms of Gly190 and Gly192 were oriented to the outside of water pore and carboxyl group of Glu144 forms hydrogen bond with backbone nitrogen atom of only Gly192. It suggests that the collapse of orientation at ar/R region is the origin of inhibition of water permeation. Our results indicate that the water inhibition mechanism of AQP1 by mercury is not simple occlusion of water pore by mercury but conformational changes at ar/R region induced by mercury.

3TA5-08 膜タンパク質アクアポリンの構造と進化 The Structure and Evolution of Aquaporin

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Aquaporins is the ubiquitous membrane protein which transports water molecule (in a case some small molecules could go through the channel). There are many isoforms in a single body and between species. Since the channel protein controls the behaviour of water, the defect of the genes gives serious effects and causes disease. Thus we think the analysis of the protein structure may be helpful for the medical research of the disease. Then we analyzed the structures of these isoforms using available data from the Protein Data Bank and some useful tools. Here we report the duplication point of the membrane protein and the evolutionary change of the protein structure after the duplication. It might suggest the general way of the evolution of membrane proteins.

3TP1-01 GPCR のオリゴマー化研究のためのウェブサービス Web services to investigate GPCR oligomerization

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G-Protein Coupled Receptors (GPCRs) are one of the most important targets for pharmaceutical medicines. Recent study revealed that many GPCRs are able to form homo- and/or hetero-oligomers, and the functional importance of the oligomerization has been extensively investigated. Contrary to that, however, the molecular mechanisms of the oligomerizations are not fully understood yet, due to the lack of structural data for GPCR complexes. Therefore, accurate predictions of the interfaces would accelerate investigations of the molecular mechanisms of oligomerization. To predict the interfaces for GPCR oligomerization, we launched a web service, GRIP (G-protein coupled Receptors Interaction Partners : <http://grip.cbrc.jp/GRIP/index.html>). As far as we know, it is the only service designed to specifically predict the interfaces for GPCR oligomerization. In addition, we have recently released a database for providing information about GPCR oligomerization (<http://grip.cbrc.jp/GDB/index.html>). In this session, we will explain these services.

3TP1-02 Akt 経路のローパスフィルタ特性による EGF 受容体と下流のリン酸化シグナル強度の逆転

Decoupling of receptor and downstream phosphorylation in Akt pathway by its low-pass filter characteristics

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In communication systems, signals in which information is encoded as temporal patterns, are selectively transmitted and decoded by characteristics of signal processing modules. However, temporal coding of cellular signal transduction has been poorly investigated in living organisms. We examined how the Akt pathway, involved in regulation of protein synthesis and cell growth, transfers and decodes the temporal information of upstream signals to downstream signals. We modeled the epidermal growth factor (EGF)-dependent Akt pathway in PC12 cells based on experimental results. We predicted responses of the Akt pathway by various temporal patterns of EGF and then validated them. We found counterintuitive results that peak amplitudes of receptor and downstream phosphorylation are decoupled; a weak sustained phosphorylation of EGF receptor (EGFR), rather than a strong transient phosphorylation, strongly induced phosphorylation of S6, a downstream molecule of Akt. This indicates that signal transfer efficiency in the Akt pathway differs depending on the temporal patterns of phosphorylation of EGFR. By use of frequency response analysis, we found that the transient and sustained phosphorylation of EGFR relatively consists of higher and lower frequency waves, respectively, and that the Akt pathway exhibits low-pass filter (LPF) characteristics. The higher frequency waves were not efficiently transferred downstream compared to the lower frequency waves. Thus, the Akt pathway serves as LPFs so that this pathway can decouple EGFR and downstream phosphorylation. These results indicate that a signal transduction pathway can selectively transfer and decode information, which is encoded as specific frequency waves in receptor phosphorylation, to the downstream molecules and exert cellular functions.

3TP1-03 高性能粒子反応拡散シミュレーションが明らかにする信号伝達経路における時空間相関の役割: MAP キナーゼを例に

High-performance particle reaction-diffusion simulations reveal roles of spatio-temporal correlations in signaling pathways: a study on the MAP kinase system

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To address the pressing computational need to precisely model cellular biochemistry with microscopic details, we have been developing a high-performance Brownian Dynamics method called eGFRD (enhanced Greens Function Reaction Dynamics). With this novel simulation algorithm, we studied a dual phosphorylation cycle of mitogen-activated protein kinase (MAPK) in which the enzymes act according to a distributive mechanism. We find that the response of this network can differ dramatically from that predicted by a mean-field analysis based on the chemical rate equations. In particular, rapid rebindings of the enzyme molecules to the substrate molecules after modification of the first site can markedly speed up the response, and lead to loss of ultrasensitivity and bistability. In essence, rapid enzyme-substrate rebindings can turn a distributive mechanism into a processive mechanism. We argue that slow ADP release by the enzymes can protect the system against these rapid rebindings, thus enabling ultrasensitivity and bistability.