Shimadzu synthesis system (Transdirect) prepared from Sf21 cells using either canine pancreatic microsome or insect-cells microsome lysate. Either lysate expressed BK channels efficiently. We also report the effects of DTT on BK channel activity and its incorporation into the lipid bilayer. This cell-free system derived from insect cells is potentially a useful tool to synthesize eukaryote membrane channels.

3P-197 AVT 刺激した腎上皮細胞の上皮型ナトリウムチャネルにおけるクロライドイオンの影響

Effect of chloride ion on epithelial sodium channels (ENaC) in AVT-stimulated renal epithelial cells

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It is well known that arginine vasotocin (AVT) stimulates the sodium reabsorption by increasing the number of ENaC in the apical membrane of renal epithelial cells. It is also indicated that Cl⁻ regulates cell functions such as cell growth and neurite elongation. However, the effect of Cl⁻ on the AVT-stimulated ENaC-mediated current is unclear. In the present study, we examined effect of Cl⁻ on activity of AVT-stimulated ENaC in renal epithelial A6 cells. AVT significantly increased the ENaC-mediated currents in A6 cells. Lowering the Cl⁻ concentration of the apical solution by replacing Cl⁻ with gluconate diminished the stimulatory action of AVT on the ENaC-mediated current, while the diminishing effect of lowering Cl⁻ on the AVT action was not observed under a low Cl⁻ condition for the basolateral solution. We therefore examined effect of lowering the apical Cl⁻ concentration on activity (*N*Po) of ENaC by cell-attached patch clamp technique. Under a low apical Cl⁻ condition, the current-voltage relationship of ENaC shifted leftward about 50 mV and *N*Po of ENaC was lower than that under a normal condition. In addition, these effects of lowering Cl⁻ on ENaC were also observed under a low Cl⁻ pipette solution with a normal bath solution. These results suggest that the apical chloride ion is a key molecule for AVT to show its stimulatory action on the ENaC-mediated sodium reabsorption in renal epithelial cells. Supported by JSPS 19590212, 20390060, The Salt Science Research Foundation 0736, 0837 and KPUM RAS project.

3P-198 トランスポゾンランダム挿入法を用いた1型リアノジン受容体の構造機能解析

Structure-function analysis of type 1 ryanodine receptor by transposon-based random insertion

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Type 1 Ryanodine receptor (RyR1) is an intracellular Ca²⁺ release channel in the sarcoplasmic reticulum in skeletal muscle and plays an important role in excitation-contraction coupling. A relatively small C-terminal region (500-1,000 residues) of the RyR constitutes an ion channel and the other N-terminal region forms a large cytoplasmic assembly (4,000-4,500 residues) which serves as the regulatory domain. The structure-function relationship of RyR1, however, remains unclear, especially in the cytoplasmic assembly due to its large size. A transposon-based random insertion approach is a powerful tool to study the structure-function relationship of large proteins. In this study, we applied this method to RyR1 by inserting GFP using Tn5 transposon. The cDNA for GFP-tagged RyR1 was transfected in HEK293 cells and the expression was confirmed by green fluorescence and the anti-RyR1 antibody. Ca²⁺ release channel function was examined by caffeine-induced Ca²⁺ release, [³H]ryanodine binding, and single channel recordings. The GFP-tagged RyR1 was affinity-purified by FKBP12, and the structure was determined by negative staining electron microscopy. We are currently trying to identify the location of GFP in the three-dimensional structure of RyR1 by nanogold labeling. The structure-function relationships of RyR1 will be discussed.

3P-199 大腸菌機械受容チャネル MscL の温度感受性変異体の単離

Isolation of temperature sensitive mutants of the mechanosensitive channel MscL in *Escherichia coli*

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MscL, the mechanosensitive channel of large conductance of *E. coli*, is a homopentamer of a subunit that has two transmembrane helices, M1 and M2. MscL is gated directly by membrane tension, but little is known about the structural bases of the energetic barrier on gating. In this study, the region responsible for the gating barrier was surveyed by isolating temperature sensitive (TS) mutants. Mutant MscL was generated by random mutagenesis and screened for the mutants that depressed the cell growth at 42 °C but not at 25 °C in comparison with wild type MscL. We so far have obtained 25 TS mutants out of approximately 43,000 transformants. Although the temperature sensitivity varied among mutants, the cell expressing G22S, I25M, and I92T MscL showed severest temperature sensitivity and hardly grew at 42°C. Gly-22 and Ile-25 are located in M1 whereas Ile-92 is located in M2. These three residues are located closely inside the MscL crystal structure and do not face the membrane lipid. The present result indicates that the change in the interaction among these three residues decreases the gating barrier of MscL.

3P-200 モデルチャネルとK⁺チャネルにおけるイオン透過機構の理論的研

究

Theoretical Study on Ion Permeation Mechanism in Model Channel and K⁺ Channel

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We investigated the dynamics of K⁺ ions through an anion-doped carbon nanotube (ANT) in terms of the molecular dynamics (MD) simulation. The free energy, energy, and entropy profiles about the ion permeation through ANT are calculated. It is found that the free energy profile is mostly determined by the entropy term, that is, the entropy dominates the dynamics of ion permeation through ANT. The molecular origin of the entropy is revealed; the constraint of the orientation of a water molecule makes the entropy barrier at the entrance of ANT. The dependency of the free energy, energy, and entropy on the charge on ANT is also analyzed. The rate of ion permeation is found to sensitively depend on the charge on ANT. The maximum channel conductance is obtained at a certain charge value, where the balance of the energy and entropy makes the low free energy barrier.

We also study the ion permeation mechanism through the K⁺ channel, Kv1.2, embedded in the DOPC membrane. The role of the concentration is investigated, and the MD simulation with the electric field to observe the ion fluxes is performed. The several mechanisms of ion permeation found in the model system are examined for this real system.

3P-201 調節性容積減少 (RVD) によって引き起こされる細胞内 Cl⁻ 濃度低下は細胞内シグナルとして機能する

Physiological importance of regulatory volume decrease: reduction in intracellular Cl⁻ concentration acting as an intracellular signaling

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Regulatory volume decrease (RVD) occurs after hypotonicity-caused cell swelling. RVD is caused by activation of ion channels and transporters, which cause effluxes of K⁺, Cl⁻ and H₂O, leading to cell shrinkage. Recently, we showed that hypotonicity stimulated transepithelial Na⁺ reabsorption via elevation of epithelial Na⁺ channel expression in renal epithelia A6 cells in an RVD-dependent manner, and that reduction of intracellular Cl⁻ concentration ([Cl⁻]_i) stimulated the Na⁺ reabsorption. These suggest that RVD would reveal its stimulatory action on the Na⁺ reabsorption by reducing [Cl⁻]_i. However, the reduction of [Cl⁻]_i during RVD has not been definitely analyzed due to technical difficulties involved in halide-sensitive fluorescent dyes. In the present study, we have developed a new method for the measurement of [Cl⁻]_i change during RVD by using a flow cytometer with a halide-specific fluorescent dye, MQAE. The [Cl⁻]_i in A6 cells in an isotonic medium was 43.6 ± 3.1 mM. The RVD caused drastic diminution of [Cl⁻]_i (from 43.6 to 10.8 mM). Under RVD-blocked conditions with NPPB (Cl⁻ channel blocker) or quinine (K⁺ channel blocker), we did not detect the reduction of [Cl⁻]_i. Based on these observations, we conclude that one of the physiological significances of RVD is the reduction of [Cl⁻]_i, and that RVD shows its action via reduction of [Cl⁻]_i acting as an intracellular signal regulating cellular physiological functions. Supported by JSPS 19590212, 19790168, 20390060, and Salt Science Research Foundation 0736, 0837.

3P-202 クラミドモナスの細胞内機械受容チャネル MSC1 と MSC3 の特徴

Characterization of the intracellular mechanosensitive channel MSC1 and MSC3 in *Chlamydomonas*.

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Mechanosensitive channel is important for the cell to sense to a variety of mechanical stimuli such as osmotic pressure, touch, and gravity. The bacterial mechanosensitive channel of small conductance, MscS, serves as safety valve to protect the cell from hypo-osmotic shock. Genes that encode MscS-like protein also are present in some eukaryotes. *Chlamydomonas* possesses three genes encoding MscS-like protein (MSC1, MSC2, MSC3). We have recently reported that MSC1 is a mechanosensitive channel present in the intracellular space of *Chlamydomonas*. Since the localization, kinetics and the ion selectivity of MSC1 are different from MscS-like proteins; it is possible that the MscS homologues of *Chlamydomonas* have the function diversity from *E. coli* MscS. To explore the functional difference among multiple MscS homologues, we characterized MSC3 and compared the properties with MSC1. MSC3 was expressed heterogeneously in *E. coli* cells and the channel activity was analyzed by patch clamp. When a negative pressure was applied to the patch membrane, the channel current with a conductance of 0.3 nS was elicited. As like MSC1, MSC3 displayed pronounced hysteresis: MSC3 opened at high pressure when the pressure was increased whereas MSC3 closed at low pressure when the pressure was decreased. Antibodies against the carboxyl terminus of MSC3 recognized small spots in the cytoplasm and the chloroplast. These findings suggest that both MSC1 and MSC3 is mechanosensitive channel in the intracellular space of *Chlamydomonas*.