2P300 ヒト臍帯内皮細胞における TRPV2 ノックダウンによる伸展刺激依存的応答の抑制効果
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TRPV2 knockdown suppresses the stretch-induced Ca²⁺ increase and subsequent cellular responses in HUVEC
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Mechanical stimulation to vascular endothelial cells plays a physiologically important role in cardiovascular system. We previously reported that intracellular Ca²⁺ increased transiently in response to stretch through the activation of mechanosensitive (MS) cation channel in human umbilical vein endothelial cells (HUVEC). However, neither the identity nor the mechanism of activation of the plasma membrane influx pathway that mediates stretch-induced change in [Ca²⁺]_i is known. Since HUVEC expresses several types of mechanosensitive TRP channels including TRPV2, we examined the effects of the targeting suppression of TRPV2 protein expression in HUVEC using TRPV2-specific morpholino-oligo. Western blot analysis and immunocytochemical staining confirmed that TRPV2 was efficiently knocked down by transfection with the morpholino-oligo. In these cells, the stretch induced Ca²⁺ increase measured by fluorescence imaging using fura2 was completely abolished. After the cells were subjected to 20% uni-axial cyclic stretch at 1Hz for 1h, neither a stretch-enhanced stress fiber formation nor a shift in the cell orientation transverse to the strain direction could not be observed. Finally, TRPV2-knocked down HUVEC did not show stretch-induced NO production. These observations strongly suggest that TRPV2 is a responsible ion channel for stretch-induced Ca²⁺ increase, which leads the cytoskeletal reorganization and NO production in HUVEC. Thus, TRPV2 would be a key component of MS channel complex in HUVEC.

2P302 アザイド存在下におけるハロロドプシンのプロトン輸送機構の解析
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Mechanism of proton transport via azide-bound halorhodopsin from *Natronomonas pharaonis*
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Natronomonas pharaonis halorhodopsin (pHR) is an inward light-driven Cl⁻ pump. pHR has a capability of binding for azide, which can confer bacteriorhodopsin (BR) like properties on the protein, including proton pump [Biochemistry (1996) 35:6604-6611]. In the present study, to clarify the mechanism of a proton movement in the pump function, the photo-induced proton pump activities of the azide-bound pHR (pHR^{az}) expressed in *Xenopus* oocytes were not only determined using the two electrode voltage-clamp technique, but also photo-induced proton uptake and release of pHR^{az} reconstituted with phospholipid were analyzed using SnO₂ electrode. Photo-induced outward currents were observed only in the addition of azide. In kinetical term of the photo-response of SnO₂ electrode, similar profile of the proton movement were observed: on illumination pHR^{az} fast releases proton, followed by the proton uptake. The relationship between the photo-induced proton current via pHR^{az} and the light intensity was linear; demonstrating that the movement of proton is driven by a single-photon reaction and that the steady-state current is proportional to the excited pHR^{az} molecule. The current-voltage relationship for pHR^{az}-mediated photo-induced currents was also linear. The reversal potential for the proton pump activity of pHR^{az} was about -180 mV. These properties are almost similar to BR expressed in *Xenopus* oocytes. Differences and similarities in the molecular mechanism of the proton movement between pHR^{az} and BR are discussed here.

2P301 固体NMRによる抗菌ペプチドアラメチシンの脂質膜中での配向と精密動的構造解析
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Dynamic structure and orientation of antibiotic peptide alamethicin in lipid bilayers by solid-state NMR spectroscopy
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Alamethicin is an antibiotic peptide extracted from *Trichoderma viride* and consisting of 20 amino acid residues. Alamethicin exhibits a voltage-gated ion channel activity in the lipid bilayers. It is shown that orientation and dynamic structure of α -helical peptide bound to magnetically oriented lipid bilayers can be determined. We will show here that same information can be obtained from non magnetically oriented lipid bilayers. [1-¹³C]Ala⁶, Val⁹, Gly¹¹, Val¹⁵, Aib¹⁶, Aib¹⁷, Gln¹⁸-labeled alamethicin analogs were synthesized by Fmoc-chemistry and incorporated into DMPC bilayers for investigating the structure and orientation of alamethicin in lipid bilayers. Dynamic structure of alamethicin in the fully hydrated lipid bilayers was investigated by analyzing the anisotropic and isotropic ¹³C chemical shifts of carbonyl carbons under the static and magic-angle spinning conditions. Isotropic ¹³C chemical shifts on obtained from DD-MAS experiments showed that alamethicin adopted helical forms. We analyzed the ¹³C chemical shift tensors of carbonyl carbons by considering the rotational motion of helix about the bilayer normal with the tilt angle ζ and the phase angle γ of the peptide plane. These ζ and γ angles can be determined using RMSD plot between calculated and experimental values. These results indicate that the N-terminal region adopts an α -helical structure with the tilt angles of 21° and the C-terminal region adopts a 3₁₀-helical structure with the tilt angles of 32° from the bilayer normal.

2P303 人工脂質二重膜を用いたCOPII小胞形成過程の可視化 ～GTP加水分解の役割について～
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Visualization of COPII vesicle formation process on artificial membrane. ~Role of GTP hydrolysis~
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In eukaryotic cells, coat protein complex II (COPII) is responsible for the vesicle formation from endoplasmic reticulum membrane. The released vesicles from ER membrane diffuse or are moved on cytoskeleton to Golgi complex in order to transport particular cargo proteins. This vesicle formation was reconstituted in vitro from a SNARE protein (Bet1p), a small GTPase (Sar1p), and two COP II coat protein complexes (Sec23/24p and Sec13/31p) [Sato, K. and Nakano, A., Nat. Struct. Mol. Biol., 2005]. The in vitro assay offers ways to investigate the fundamental questions on the mechanism of the vesicle formation: how each complex forms vesicle? What is the role of GTP hydrolysis? To access these tasks, a SNARE protein, Bet1p, was fluorescently labeled with Cy3, and reconstituted into a lipid bilayer formed on microscope stage. The single fluorescent intensity of the Cy3-labeled Bet1p in an artificial membrane was observed with a TIRFM. When Sar1p and COPII coat proteins were added to the system containing GTP, drastic change of the fluorescent intensity was observed: prebudding complexes (Cy3-labeled Bet1p, Sar1p and Sec23/24p) quickly bind together with Sec13/31p to form large cluster. The addition of GMP-PNP gave a strong change to this phenomenon. Fluorescent strength of the cluster was observed to decrease to 1/10. It suggests that Cy3-labeled Bet1p be concentrated by the GTP hydrolysis. In this poster, we will propose about the relation between the role of GTP hydrolysis and process of the vesicle formation.