1P389**Effect of cobalt treatment on mitochondrial DNA damage in HEK293 acutely exposed to H₂O₂**○Yasutomo Nomura¹, Hiroyuki Fujiwara², Toshifumi Takayama¹, Michihiko Sato³, Eiji Takahashi⁴, Yasukazu Hozumi⁵, Zhonggang Feng², Takao Nakamura¹¹Dept. Environ. Life Sci., Yamagata Univ, ²Dept. Bio-System Eng., Yamagata Univ,³Central Lab. Res. Edu., Yamagata Univ, ⁴Dept. Physiol., Yamagata Univ, ⁵Dept. Anat. Cell Biol., Yamagata Univ

Severity of ischemia/reperfusion injury accompanied with generation of reactive oxygen species is reduced by hypoxic preconditioning, the precise mechanisms of which are not completely understood. Recently, several authors suggested the pathway via hypoxia-inducible factor-1 α (HIF-1 α). The activation reduced the number of ischemia-induced apoptotic cells through the suppression of apoptosis signaling of cytochrome *c* release. Reactive oxygen species cause not only apoptosis via cytochrome *c*, but also mitochondrial DNA (mtDNA) damage. In this study, we determined cell growth and mtDNA damage in HEK293 cells exposed to H₂O₂ as pretreated with cobalt to mimic hypoxic preconditioning. When cells were treated with 0.2-0.4 mM H₂O₂, cell growth was suppressed, and mtDNA was damaged dependent on H₂O₂ concentration. When 0.4 mM H₂O₂ was exposed to cells where HIF-1 α was induced with 100 μ M CoCl₂, mtDNA damage was suppressed, and cell number 4 days after H₂O₂ exposure (15.7 % against no exposure) was significantly greater than that without cobalt treatment (3.7 %). When the amount of H₂O₂ remaining in the medium was determined, the rate constant for H₂O₂ depletion by the cobalt-treated cells clearly increased. This is consistent with the fact that heme oxygenase of antioxidant enzyme is a target gene of HIF-1 α . These results suggest that cobalt-induced HIF-1 α activity ameliorates suppression of cell growth through protecting mtDNA damage caused by H₂O₂.

1P391**Effects of nonbilayer lipid and membrane curvature on membrane association of PLC- δ 1 PH domain**

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In the previous work, we have shown that a conformational alteration of PH domain of PLC- δ 1 is induced at membrane surface as the PH domain forms complex with PIP₂ head group. The conformational change accompanied by an insertion of an amphipathic α -helix (α 2-helix) into the interface region of the membrane was found to facilitate the membrane association of the PH domain. Here, we investigated effects of nonbilayer lipid, PE on the structure and membrane binding affinity of the PH domain. PE have been proposed to facilitate membrane insertion of amphipathic molecules by providing insertion sites between rather small head groups. Solid state NMR spectra of the PH domain forming complex with PIP₂ in vesicles containing 0-80% PE indicated that the membrane insertion of the α 2-helix is inhibited in the presence of more than 30% PE in the membrane. This unexpected inhibitory effect of PE on the membrane insertion of the PH domain accompanied by an induction of a molecular motion of the domain would be ascribed to disturbance of the structure of lipid bilayer by the nonbilayer lipid. Although ³¹P-NMR of the vesicles revealed that the structure of the lipid bilayer is maintained in the presence of up to 40% PE, transient fluctuation of the local structure of the interface region could destabilize the membrane associated state of the α 2-helix. In order to evaluate effect of decrease in packing of lipid head group on protein insertion, influence of increase in membrane curvature on the structure of the PH domain was also investigated.

1P390**Effect of phosphatidylserine on function and structure of the PLC- δ 1 PH domain at the membrane surface**

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In this study, suppression of membrane binding affinity and simultaneous alternation of structure of the PLC- δ 1 PH domain at membrane surface induced by phosphatidylserine (PS) are investigated by using vesicle co-precipitation assay and solid-state NMR spectroscopy. PLC- δ 1 PH domain has been proposed to form complex with PI(4,5)P₂ in the plasma membrane with high affinity and specificity, and consequently regulate membrane localization of PLC- δ 1. Previously, we have shown that conformation of β 5/ β 6 loop of the PH domain is altered by association of PH domain with PI(4,5)P₂ vesicle by using solid-state NMR spectroscopy. At the physiological condition, the inner leaflet of the plasma membrane accessible by PLC- δ 1 contains PS at an amount of nearly 20% of the total phospholipids. Considering a potential physiological importance of PS in the inner leaflet, we examined binding affinities and conformations of PLC- δ 1 PH domain in the presence of PS. The results of vesicle co-precipitation assay indicated that dissociation constants of the PH domain-PC/PI(4,5)P₂ and -PC/PS/PI(4,5)P₂ (20% PS) vesicles were $1.62 \pm 0.33 \mu$ M and $2.77 \pm 0.48 \mu$ M, respectively. The solid-state NMR spectra revealed that the conformational change of β 5/ β 6 loop and consequent decrease in the interaction between the PH domain and the membrane surface are induced by 20% PS in the membrane. These effects of PS were suppressed by electrolytes suggesting that electrostatic interaction responsible for the effects of PS on the membrane associated state of the PH domain.

1P392**The plasma membrane shuttling of CAPRI is related to regulation of mast cell activation**○Tadahide Furuno¹, Rika Nakamura², Mamoru Nakanishi¹¹Sch. Pharm., Aichi Gakuin Univ., ²Grad. Sch. Pharm. Sci., Nagoya City Univ.

The Ca²⁺-promoted Ras inactivator (CAPRI), a Ras GTPase-activating protein (GAP), is involved in the inactivation of mitogen-activated protein kinase, extracellular signal-regulated kinase 2 (ERK2). However, a precise role of CAPRI in immune responses is still unknown. Here we studied the effects of cyan fluorescent protein-tagged CAPRI (CFP-CAPRI) expression on mast cell activation. CAPRI localized in the cytoplasm, and moved to the plasma membranes within 1 min after the addition of antigen to mast cells (RBL-2H3 cells). The translocation of CAPRI on the plasma membrane of the cells became the maximum at ~ 1.5 min, and then it returned to the cytoplasm from the plasma membranes. The translocation of CAPRI occurred invariably after the intracellular calcium ion concentration ([Ca²⁺]_i) had increased, and CAPRI never moved to the plasma membranes when the [Ca²⁺]_i did not increase. The nuclear import of ERK2 occurred after the re-localization of CAPRI to the cytoplasm, suggesting that the early phase of ERK2 activation is eliminated. Moreover, we found that antigen-induced degranulation and cytokine production was significantly suppressed in CFP-CAPRI-expressing cells. A mutant of GAP domain, CAPRI(R472S), showed a feeble translocation to the plasma membrane but did not affect the degranulation, ERK2 activation, and cytokine production. The results suggested that the translocation of CAPRI to the plasma membranes regulates crucially cellular responses in mast cells.