entered the FA zone via lateral diffusion in-on the PM, continuing diffusion even within the FA zone (diffusion coefficient is 1/3 of that outside the FA). 11% Of Rac1 molecules that entered the FA zone exhibited temporary or long-term immobilization in the FA zone, suggesting the binding to the FA island proteins. 19% Of Rac1 arrived at the FA zone directly from the cytoplasm and immobilized, suggesting that Rac1 is recruited to the FA protein islands both directly from the cytoplasm and via the general PM area.

1PT210 ERK リン酸化・脱リン酸化反応再構成系の ERK 濃度に依存した応答特性

Response characteristics of reconstructed ERK phosphorylation and dephosphorylation depending on ERK concentration

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ERK, the mitogen-activated protein kinase (MAPK) in the Raf-MEK-ERK cascade, has two phosphorylation sites which are phosphorylated by a kinase, MEK (MAPKK) and dephosphorylated by a phosphatase, MKP. Some theoretical studies have shown that two enzymes with opposing effect (e.g. kinase and phosphatase) can lead to zeroth-order ultrasensitivity which is an ultrasensitive response at the steady-states depending on the substrate concentration (Goldbeter, A. and Koshland, D., PNAS: 78 1981).

To show this type of ultrasensitivity experimentally, we reconstructed the ERK phosphorylations and dephosphorylations in *E. coli* cells, in which concentrations of ERK, MEK and MKP were independently controllable using extracellular chemicals. The attained protein concentrations were estimated by fluorescence intensities of Cerulean (a cyan fluorescent protein variant), Venus (a yellow fluorescent protein variant), and mCherry (a red fluorescent protein variant) each fused on ERK, MEK and MKP, respectively.

We investigated the dependence of ERK phosphorylation on MEK and MKP expression level. The ERK phosphorylation increased by the increase of MEK concentration and/or the decrease of MKP concentration. Thus, the response curve for ERK phosphorylation depended on the ratio of MEK to MKP concentration and could be described by the Hill equation. When the ERK expression decreased by a factor of 0.29, the Hill coefficient decreased by a factor of 0.69. This response is consistent with the theoretical prediction by the zeroth-order ultrasensitivity.

1PT211 マウス胚盤胞における細胞内カルシウム濃度とメカニカルストレス負荷の同時計測

Simultaneous investigation of intracellular calcium concentration and mechanical stimuli to mouse blastocysts

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Mammalian embryos are transported to the uterine cavity through the fallopian tube during cell cleavage and blastocyst development. Fertilized oocytes may be subjected to a mechanical influence. Dynamic embryo culture systems have been developed to generate and apply mechanical stimuli (MS) similar to those generated within the oviduct affecting the mammalian embryo. To investigate the responses of the mammalian embryos to MS, we developed a dynamic air actuation system to apply MS in a microfluidic channel by deforming a 0.1-mm membrane, and investigated changes of intracellular calcium ion concentration ($[Ca^{2+}]_i$) in mouse blastocyst applied MS. Both MS and ($[Ca^{2+}]_i$) were quantified in the microfluidic channel. ($[Ca^{2+}]_i$) was measured in a stained mouse embryo with Fluo-4 AM using confocal fluorescence microscopy. We captured a z-series stack of sections encompassing the entire embryo. When blastocysts were compressed, fluorescence intensity (FI) in the blastocyst also increased in response to the applied MS. Compressive force estimated from the shape of the blastocysts was approximately 0.5-2.0 μ N according to a force deformation curve for the mouse embryo. The sum of FIs increased by a factor of 1.1-1.2 times compared with those observed before MS. The increase in the sum of FI indicated that enhancement of ($[Ca^{2+}]_i$) would be induced by these MS. Molecular mechanosensing systems such as mechanosensitive ion channels (MSIs) would play an important role in responses to these MS.

1PT212 バイセクティング GlcNAc とフコースの導入が水中 N 型糖鎖の構造多様性に及ぼす影響

Effect of Bisecting GlcNAc and Core Fucosylation on Conformational Properties of Biantennary Complex-Type N-Glycans in Solution

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The introduction of bisecting GlcNAc and core fucosylation in N-glycans is essential for fine functional regulation of glycoproteins. In this presentation, the effect of these modifications on the conformational properties of N-glycans is examined at the atomic level by performing replica-exchange molecular dynamics (REMD) simulations. We simulate four biantennary complex-type N-glycans, namely, unmodified, two single-substituted with either bisecting GlcNAc or core fucose, and disubstituted forms. By using REMD as an enhanced sampling technique, five distinct conformers in solution, each of which is characterized by its local orientation of the Man α 1-6Man glycosidic linkage, are observed for all four N-glycans. The chemical modifications significantly change their conformational equilibria. The number of major conformers is reduced from five to two and from five to four upon the introduction of bisecting GlcNAc and core fucosylation, respectively. The population change is attributed to specific inter-residue hydrogen bonds, including water-mediated ones. The experimental NMR data, including nuclear Overhauser enhancement and scalar J-coupling constants, are well reproduced taking the multiple conformers into account. Our structural model supports the concept of "conformer selection", which emphasizes the conformational flexibility of N-glycans in protein-glycan interactions.

1PT213 新規フォトクロミック HDAC 阻害剤の設計・合成ならびに阻害作用 Design and synthesis of a novel photochromic HDAC inhibitor and its inhibitory effect

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Histone acetylation-deacetylation is one of the epigenetic regulations of gene expression in eukaryotes. Histone deacetylase (HDAC) catalyze deacetylation of lysine residues in N-terminal domain of core histones and regulates gene transcription and expression. Inhibition of them induces transcriptionally active chromatin, causing arrest of growth, differentiation and apoptosis. HDAC inhibitors are thought to be effective for neurodegenerative disorders, cardiac hypertrophy, inflammation and cancer. In this study, we designed and synthesized novel photochromic HDAC inhibitor, composed of photochromic region (azobenzene), linker, and zinc-interacting thiol, for photo-regulation of differentiation or apoptosis. Azobenzene moiety is placed in hydrophobic interacting region of inhibitor, therefore, light-induced structural change were thought to be affect affinity against HDACs. Synthesized photochromic HDAC inhibitor showed light-induced isomerization. Photochromic HDAC inhibitor showed micro-molar order HDAC inhibitory effect in vitro. The inhibitor composed of trans isomer azobenzene generated by visible-light irradiation inhibited deacetylase activity of HDAC with higher K_i than that composed of cis isomer azobenzene induced by ultraviolet-light irradiation. Development of photochromic HDAC inhibitor is thought to be useful for site-directed inhibition of HDACs.

1PT214 PC12 細胞シグナル伝達系における PLS 回帰を用いた解析 PLS regression analysis of Signal transduction network and phenotypes in PC12 cell

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PC12 cells, an adrenal chromaffin cell line, are a well-characterized model of nerve cells. This cell line exhibit several characteristic phenotypes such as differentiation, proliferation and apoptosis in response to different external stimuli. Previous studies reported that these various stimuli are redundantly mediated by a few common signal transduction factors such as Akt, CREB and MAPKs, which increase expression levels of some proteins known as Immediate Early Genes (IEGs). However, quantitative relationships between these factors and cell phenotypes are yet to be well characterized. To elucidate this underlying relationship between the temporal patterns of the signaling factors, IEGs and phenotypes we focus a statistical analysis. Statistical models are constructed to predict temporal patterns of IEGs/phenotypes from those of signaling factors such as Akt, CREB and MAPKs using Partial Least Squares (PLS) regression analysis. The expression patterns of signal transduction factors and IEGs were measured by QIC method previously developed by our group and the cell phenotypes are measured by some molecular biological experiments. Using these data a regression equation was calculated in terms of