MPTP opening. This suggests that after mitochondrial permeability transition, mitochondria need some changes to undergo swelling.

3L1358 移動性細胞における熱パルスに対するカルシウムダイナミクスの環境温度依存性

Sensitivity of Ca^{2+} dynamics to the heat pulse in migrant cells at various temperatures

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Cell migration is observed in many parts of living organisms. In the studies of WI-38 cells as a model of migrant cells, the migration is activated at specific positions of a cell where discrete, local and short-lived high- Ca^{2+} microdomains (Ca^{2+} flickers) are observed [Wei, C. *et al.*, *Nature* **457**, 901-905 (2009)]. These reports suggest that in migrant cells the changing pattern of Ca^{2+} dynamics plays an important role in the migration, e.g., the determination of the movement direction. On the other hand, we previously revealed that in HeLa cells Ca^{2+} dynamics can be induced by microscopic heat pulses, which must be as large as 2°C at 22°C and only 0.2°C at 37°C [Tseeb, V. *et al.*, *HFSP J.* **3**, 117-123 (2009)]. Here we studied the molecular mechanism of Ca^{2+} dynamics in migrant cells, e.g., WI-38 cells, responding to the microscopic heat pulse. We found that each microscopic heat pulse induces Ca^{2+} uptake into intracellular Ca^{2+} stores, i.e., endoplasmic reticulum (ER), by SERCA during heating (Ca^{2+} pump), and then Ca^{2+} release (Ca^{2+} burst) from the stores at the onset of re-cooling through IP_3 receptors (Ca^{2+} channel). Thus, the molecular mechanism of Ca^{2+} response to the heat pulse is similar to the HeLa cells. We also examined the sensitivity of WI-38 cells to the heat pulse applied at various ambient temperatures.

3L1422 Effects of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) P54 modification on oxidative stress-response

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O-linked *N*-acetylglucosamine (*O*-GlcNAc) modification is one of the post-translational modifications of proteins at serine/threonine residues. *O*-GlcNAcylation occurs on numerous nucleocytoplasmic proteins and regulates protein stability, subcellular localization and protein-protein interactions. This modification which is reversibly controlled by *O*-GlcNAc transferase and *O*-GlcNAc-specific *N*-acetylglucosaminidase (*O*-GlcNAcase) plays an important role in the dynamic regulation of various cellular functions as well as protein phosphorylation. In order to clarify the role of *O*-GlcNAc modification in the cell response caused by oxidative stress, we examined the effect of *O*-GlcNAc modification on the expression of heat shock protein 70 (HSP70) and the activation of heat shock factor 1 (HSF1), a transcription factor of HSP70, in HeLa cells exposed to sodium arsenite (NaAsO_2).

The induction of HSP70 expression and the translocation of HSF1 to the nucleus were observed in NaAsO_2 -exposed HeLa cells. Enhancement of cellular *O*-GlcNAc levels by the addition of *O*-(2-acetamido-2-deoxy-*D*-glucopyranosylidene)-amino-*N*-phenylcarbamate (PUGNAc), a potent inhibitor of *O*-GlcNAcase, suppressed the elevation of cellular HSP70 caused by NaAsO_2 -exposure, but did not affect nuclear translocation of HSF1.

These results suggest that the enhancement of cellular *O*-GlcNAc levels may disturb the expression of HSP70 in the following stage of translocation of HSF1 to the nucleus.

3L1434 Aberrant cAMP responses in huntingtin-null cells in *Dictyostelium*

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Cyclic AMP (cAMP) plays a key role in *Dictyostelium* aggregation; an increase in extracellular cAMP concentrations elicits further synthesis and secretion of cAMP thereby creating a gradient that directs chemotaxis. The response is mediated by a G-protein coupled receptor, however the exact nature of regulation following receptor-ligand binding remains unclear. Recently, *Dictyostelium* homologue of Huntingtin was isolated from a yeast two-hybrid

screen. Here, we study cAMP waves and the cAMP-induced cAMP relay response in a null-mutant of huntingtin gene. We show that mutant cells do exhibit waves and oscillations of cAMP. However the onset of signaling is markedly delayed compared to wild type cells. Live imaging at the single cell level with a FRET-based cAMP sensor revealed that when stimulated continuously with cAMP, pre-aggregating stage cells showed markedly diminished response. For aggregating stage cells, null-cells did respond with similar amplitude however with altered kinetics. The initial spike of the intracellular cAMP concentration after micromolar cAMP stimulation in mutant cells took 5 min to complete compared to 3 to 4 min in wild type cells. From these observations, we suggest that Huntingtin plays a role in adaptive dynamics of the GPCR-mediated response and that aberrance in cAMP synthesis in the null-cells leads to its abnormal developmental phenotypes.

3L1446 ダイナミックマイクロアレイによる異種マイクロビーズの操作

Handling of different types of microbeads in a dynamic microarray

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The advancement of microfluidic platforms for bead-based analysis impacts a broad range of biomedical fields. System miniaturization results in a variety of benefits, low reagent volumes and rapid diffusion times. As a result, there have been many kinds of microarray for bead-based chemical reactions. Recently, the device for paired bead-based reactions has been developed to analyze reactions between different types of beads. However, the device which enables us to achieve triple-hetero-bead-based reactions has not been reported. Triple-hetero-bead-based reaction is desired for further cell communication and differentiation analysis using triple types of cells (encapsulated in or attached on hydrogel beads). Compared with paired microbeads, three different kinds of microbeads in contact have notable characteristics: Multiple contacting modes (ABC, CAB, BCA). Therefore, control of the contacting mode is significant for control of chemical reactions. Here, we present a parallel microfluidic approach for contacting three different microbeads in an array and changing their contacting mode (e.g. $\text{ABC} \Rightarrow \text{CAB} \Rightarrow \text{BCA} \Rightarrow \text{ABC}$). This method would enable us to achieve not only bead-based chemical reactions using triple microbeads but also temporal/spatial control of reactions such as rearrangement of cells after arraying and stop/start of chemical reactions.

3L1458 Analysis of bactericidal activity on *Escherichia coli* by Ag nanoparticle in medium and by its surface coating in sub millimeter chamber

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The use of Ag nanoparticles in a wide variety of consumer products for bacterial sterilization has been growing as the Ag particles are the most applicable nanomaterials. Because of recent Europe's deadly E coli outbreak, bactericidal products will receive more attention than ever. However, surprisingly the microscopic mechanism of the bactericidal activity caused by the particles has not well understood, especially by the particles in "paint" as surface coating despite of its a long lasting biocide with low volatility. We have been investigating the bactericidal activity of Ag nanoparticle on E coli, NBRC3972 in two methods. Method 1 is the direct introduction of Ag nanoparticles into growth medium. Method 2 is coating surface of Petri dish by perhydropolysilazane containing Ag nanoparticles. Furthermore, homebuilt sub millimeter scale chambers made of PDMS are prepared to confine the motion of E. coli within the optical microscopic range for better understanding Ag bactericidal properties on both methods. The densities of Ag particles in medium are carefully selected from 100 to 1000 ppm and colony count was tested on these dishes over 8 hrs. We will discuss the result of colony count and analysis by observations in detail.

3L1522 細胞サイズ空間はタンパク質発現を顕著に加速する：その脂質組成 P55 依存性

Marked acceleration of gene-expression in a cell-sized droplet covered by a lipid layer

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We investigate the effect of cell-sized confinement on gene-expression and its