force microscopy (AFM) and an AFM tip, etched by focused ion beam, with several hundred nanometers in diameter. We called the etched tip “nanoneedle”. For example, highly-efficient DNA transfer into a mesenchymal stem cell could be achieved by inserting a plasmid DNA-adsorbed nanoneedle. The insertion efficiencies vary according to cell type. We have found that the stress fibers and actin filaments comprising the plasmalemmal undercoat are important determining factors as to whether a nanoneedle can be successfully inserted into a cell. Some cell species show quite low insertion efficiencies less than 50% even by using very thin nanoneedle of 200 nm in diameter. To improve the insertion efficiency of a nanoneedle, we attempt to form fibrous structures on a cell surface by addition of materials. We applied a layer-by-layer technique to prepare nanometer-sized films on a cell surface through the alternate immersion into solutions containing a component of extracellular matrix. It was demonstrated that both nanofilms consisting of fibronectin-gelatin and fibronectin-dextran sulfate had effects on increment of insertion efficiencies of nanoneedle into cells.

2P224 1C1505 GPI アンカー型タンパク質のタンパク部分相互作用による動的ホモダイマー形成とラフト相互作用による安定化
Lipid-stabilized dynamic homo-dimers of GPI-anchored proteins based on ectodomain protein interactions in the steady-state cell membrane

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The dynamic organization of GPI-anchored proteins (GPI-APs), putative raft-associated proteins with a phospholipid anchoring chain, in the plasma membrane has been investigated, at their low physiological expression levels of 1000 - 5000 copies/cell, using single fluorescent-molecule tracking, single molecule FRET, bimolecular fluorescence complementation (BiFC), and chemical crosslinking. Virtually, all of the GPI-APs are mobile, and CD59, DAF, and GFP-GPI continually form transient (140-300 ms lifetime) homo-dimers based on ectodomain interactions, stabilized by cholesterol- and GPI-anchoring-dependent lipid interactions which were detectable only in the presence of ectodomain protein interactions. Such transient dimers were not formed between different GPI-APs, suggesting the specific protein interaction is a key step for homo-dimerization and lipid-based stabilization. Upon ligation, some CD59 molecules formed stable oligomers greater than trimers, which recruit cholesterol and glycosphingolipids to form oligomer-based rafts, which in turn recruit raftophilic, signaling molecules, triggering intracellular Ca²⁺ responses. Meanwhile, mGFP-GPI, which is incapable of forming natural oligomers, induces these events much less efficiently, upon stimulation. We propose that the plasma membrane is in the metastable state, where only small stimulation-induced modulations of the delicate balance of molecular interactions trigger the formation of stable receptor-cluster rafts, followed by molecular recruitment and intracellular signaling.

2P225 T 細胞活性化における受容体シグナル分子動態の 1 分子解析
Single molecule analysis of dynamics of T cell signaling molecules on activation

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We have developed a microscopic system and technologies that allow immunological responses and signaling processes to be visualized at the single-molecule level. We have clearly visualized the initial process of signaling activation in T cells, from the moment of signaling activation. We reported that T cell activation is initiated and sustained in TCR (T cell receptor)-containing microclusters generated at the initial contact areas and that the activation signals precede immunological synapse formation. Microclusters containing TCR, ZAP-70 (kinase) and SLP-76 (adaptor) are continuously generated in the periphery. Microclusters containing TCR, ZAP-70 and SLP-76 migrate toward the center of the contact cell surfaces, but SLP-76 disappeared completely and ZAP-70 was mostly lost before the center. We calculated the number of TCR molecules from the fluorescence intensity of single EGFP-CD3z molecules. Newly generated microclusters at the periphery contained about 50 molecules of CD3zeta. Here, we focus on the difference among the dynamic behavior of TCR, ZAP-70 and SLP-76. The number of TCR molecules in microclusters increased linearly with time at the early phase and the periphery. However, that of downstream protein molecules showed different behavior. We discuss these features of microclustering with respect to molecular interactions and mechanisms.

2P226 神経-免疫相互作用におけるカルシウム応答と接着力の関係
Relationship between Calcium Response and Adhesion Strength in Neuro-Immune Interaction

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We previously showed that cell adhesion molecule 1 (CADM1) promoted attachment and interaction between nerves and immune cells (mast cells). Recently, we found that there are at least three kinds of isoforms derived from splicing variants in CADM1, which are named CADM1b, CADM1c, and CADM1d. Here the relationship between calcium response and adhesion strength mediated by CADM1 isoforms has been studied in nerve-mast cell interaction. Both Neuro2a cells (cultured neuroblastoma cells) and mast cells expressed CADM1c. When Neuro2a cells were cocultured with mast cells, the responding rate of mast cells attached to Neuro2a cells was 40% after specific stimulation of Neuro2a cells. Femtosecond laser application to estimate adhesion strength showed that the detachment of mast cells from Neuro2a cells occurred within 23.6 μ m away from the focal point in average. Transfection with CADM1d into Neuro2a cells increased the responding rate of mast cells to 66% and decreased the distance from focal point causing the detachment to 14.3 μ m in average. Transfection with CADM1b affected neither the responding rate nor the detachment distance. In Neuro2a cells expressing CADM1d-RFP, the responding rates of attached mast cells were 63% and 38% in the contact points with and without RFP, respectively. These suggested that the efficiency of intercellular signal transduction was intimately related to the adhesion strength in nerve-mast cell interaction.

2P227 酸化ストレスによるアポトーシスにおける O-結合型 N-アセチルグルコサミン (O-GlcNAc) 修飾の影響
Effect of O-linked N-acetylglucosamine (O-GlcNAc) modification on oxidative stress-induced apoptosis

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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 cytoplasmic and nuclear proteins and regulates protein stability, subcellular localization of proteins 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 induction of apoptosis and the expression of heat shock protein 70 (hsp70) by sodium arsenite (NaAsO₂)-exposed HeLa cells.

Exposure to NaAsO₂ led to dose-dependent apoptosis, which was shown as chromatin condensation. Increased cellular O-GlcNAc levels caused by the addition of O-(2-acetamido-2-deoxy-D-glucopyranosylidene)-amino-N-phenylcarbamate (PUGNAc), a potent inhibitor of O-GlcNAcase, enhanced NaAsO₂-induced apoptosis. Furthermore, the elevation of cellular hsp70 expression by NaAsO₂ was suppressed by treatment with PUGNAc. These results suggest that the increase of O-GlcNAc modified proteins disturbs the intrinsic property of protective response to oxidative stress in HeLa cells.

2P228 GPCR のダイマー・モノマー平衡：2 分子相補蛍光の 1 分子イメージングによるダイマーの直接検出
Dimer-monomer equilibrium of a GPCR: direct dimer detection by single-molecule bimolecular fluorescence complementation (SM-BiFC)

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In many GPCR signaling cascades, receptor dimerization, even before ligand binding, is considered to be critically important. However, the dynamic monomer-dimer equilibrium of any receptor has never been characterized, hampering our fundamental understanding of signal transduction processes in the plasma membrane. Here, we fully characterized this dynamic equilibrium in live cells at 37 °C, using N-formyl peptide receptor (FPR), a chemoattractant GPCR, as an important paradigm, by developing a single fluorescent-molecule imaging method. To directly detect FPR dimers at the molecular level, we em-