1SEA-01 GPI アンカー型タンパク質とガングリオシドの1分子追跡により明らかになったラフト組織化

Single-molecule tracking of GPI-anchored proteins and gangliosides revealed raft organization

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Raft domains have been drawing extensive attention as a signaling platform. However, raft structure and function are still very controversial. Here, the dynamic organization of raft molecules in the plasma membrane has been investigated, using single fluorescent-molecule tracking. Virtually all of the GPI-APs (CD59, DAF, Thy-1, GFP-GPI) and gangliosides (GM1, GM2, GM3, GD1b) are mobile, and continually formed transient (100-300 ms lifetime) homodimers based on ectodomain interactions, which were stabilized by raft-lipid interactions. Furthermore, we found that GPI-AP homodimers recruited gangliosides, and ganglioside homodimers recruited GPI-APs. Our results suggest that the transient homodimers are likely one of the basic unit for raft organization.

1SEA-02 細胞質分裂におけるスフィンゴミエリンラフトの役割

A role for sphingomyelin-rich lipid domains during cytokinesis

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Real-time observation of cell division with a sphingomyelin (SM) specific protein, lysenin, revealed that SM is concentrated in the outer leaflet of the cleavage furrow at the time of cytokinesis. Superresolution fluorescence microscopy analysis indicates a transbilayer colocalization between the SM-rich domains in the outer leaflet and phosphatidylinositol-4,5-bisphosphate (PIP2)-rich domains in the inner leaflet of the plasma membrane. The depletion of SM disperses PIP2 and inhibits the recruitment of the small GTPase RhoA to the cleavage furrow, leading to abnormal cytokinesis. These results suggest that the formation of SM-rich domains is required for the accumulation of PIP2 to the cleavage furrow, which is a prerequisite for the proper progression of cytokinesis.

1SEA-03 Lipid Rafts and Membrane Proteins Collaborate to Organize and Shape Biological Membranes

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By concentrating lipids and proteins, lipid rafts enable diverse cellular processes. Using model membranes and cells, our work has demonstrated two mechanisms by which proteins and rafts work together to organize membranes. First, we have precisely quantified the capacity of rafts to concentrate proteins, demonstrating that the stability of protein-lipid assemblies relies on a subtle balance between the enthalpy of membrane phase separation and the free energy of protein diffusion. Building on these findings, we have revealed that steric and electrostatic interactions among proteins confined within rafts can generate strong, highly localized membrane surface forces that drive membrane bending, helping to shape endocytic vesicles and other curved membrane structures.

1SEA-04 リポソームの膜内相分離における外場の影響

Phase separation on cell-sized liposomes in the presence of external force

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Lipid domains in cell-sized liposomes upon phase separation have been extensively studied as a model of lipid rafts in cell membranes. In spite of distinct properties between liposomes and cells, comparing them should elucidate physical conditions important for lipid rafts. For example, the steric repulsive force between bulky lipids like glycolipids inhibits the size growth and stabilizes smaller domains. In this talk, we mention about the effects of external force and viscoelastic cytoplasm on phase separation of lipids. Both effects drastically vary the domain structures according to the kinetics of phase separation and the shape deformation involved. We believe that such bottom-up approach is also necessary for comprehension of complex cellular systems.

1SEA-05 パターン化人工膜を用いた膜タンパク質のラフト親和性解析

Micropatterned model membrane for studying the affinity of proteins to lipid raft

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Controlled association of membrane proteins to lipid rafts is believed to play important functional roles. We describe a methodology to evaluate the affinity of proteins to lipid raft by using a micropatterned model membrane composed of polymeric and fluid bilayers. The fluid bilayer has patterned regions of liquid-ordered (Lo) and liquid-disordered (Ld) bilayer domains. We observed that rhodopsin and transducin were localized in Ld domain according to their basal affinities to the disordered lipid phase. We anticipate that we can quantify the affinities of membrane proteins to lipid rafts by measuring their distributions in the micropatterned membrane. This technique should help to elucidate the functional roles of lipid rafts in the native biological membrane.