3H1034 Smooth muscle differentiation related transcription factor CRP2 directly regulates physical properties of actin filaments

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Actin filaments form many types of structures and dynamically regulate the cell morphology and movement, and furthermore, they play a mechanosensory role for extracellular stimuli. Thus, actin filaments are the most important physical components of a cell and their modulation factors have the potential to directly regulate the cell fate. In this study, we clarified that smooth muscle related transcription factor, CRP2 (cysteine-rich protein 2), directly bound to actin filaments and regulated their physical properties. The CRP2 molecule has a dumbbell-like structure with 3 tandem clots as indicated by SAXS (small angle X-ray scattering) analysis. CRP2 is directly associated with F-actin through its N-terminal LIM domain and Gly-rich region. CRP2-bound actin filaments showed higher persistent length as compared with unmodified actin filaments. By induction of smooth muscle cells differentiation, intracellular CRP2 localizes to actin stress fibers as they are formed. Furthermore, smooth muscle cells with active CRP2 expression showed higher mechanical stiffness. Now we speculate that actin-bound CRP2 plays direct roles in the stabilization of smooth muscle cells differentiation by regulation of the physical properties of actin filaments. This study was supported by KAKENHI (24700454).

3H1046 細胞接着班における p130Cas と Src の動的相互作用を介した遊走制御機構の解明

A distinct role for the dynamics of p130Cas at focal adhesion via interaction with Src in the regulation of cell migration

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Cell migration is involved by the orchestrated regulation of remodeling of actin cytoskeleton, actomyosin contraction and continuous focal adhesion turnover. The phosphorylation of a focal adhesion protein p130Cas (Cas) has been reported to regulate cell migration through the modulation of focal adhesion turnover. However, it is still unclear how individual Cas molecules contribute to the regulation of cell migration. The fluorescence recovery after photobleaching (FRAP) method was used to spatiotemporally analyze the dynamics of various forms Cas mutants at focal adhesion in migrating cells. We find that displacement of Cas molecules from adhesion sites is accelerated by their phosphorylation but hampered by association with Src, although the Src-binding of Cas at focal adhesion promotes Cas phosphorylation. Genetically-engineered stabilization of Cas association with Src using the bimolecular fluorescence complementation (BiFC) method, which sustain Cas phosphorylation but mitigate Cas displacement from adhesion complexes, result in retarded cell migration. Myosin II inhibition that impedes displacement of Cas molecules from adhesion sites also decreases cell migration, despite sustained Cas phosphorylation. Our findings indicate that both association with Src and dissociation from Src are the key elements for Cas to activate Cas-phosphorylation signaling and thereby to drive cell migration.

3H1058 Spatiotemporal regulation of signaling by active cytoskeletal remodeling

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Biochemical reactions performed by signaling and sorting complexes, both on the surface and within the living cell, is often subject to the local regulation by the active cytoskeleton. We have recently proposed that in many cases, this spatiotemporal regulation is due to the interplay between active contractility and remodeling of the cytoskeleton which results in transient 'asters', focusing of passive molecules to form clusters inside its core. This mechanism of clustering gives rise to a significant increase in the local concentration of signaling proteins, which enhances reaction efficiency and output signal. The dynamic cytoskeletal elements that drive focusing behave as quasi-enzymes catalyzing the chemical reaction. These ideas are applicable to the cortical actin dependent clustering of cell surface proteins such as lipid-tethered GPI-anchored proteins, Ras-proteins as well as many proteins that have domains to interact with the actin-cytoskeleton. We discuss the implications of actin

dynamics by introducing an 'activity temperature', which can work as a regulatory parameter for signaling replacing the details of cytoskeletal dynamics. We show this using time-temperature-transformation (TTT) and Arrhenius plots while 'activity temperature' facilitate and/or inhibit (slow down) chemical reactions.

3H1110 Arp2/3 とアクチンを内包した油中液滴のフィロポディア様変形 Filopodia-like protrusions in water-in-oil droplets induced by Arp2/3 and actin polymerization

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When cell moves, cytoplasmic protrusions called filopodia spread out in the direction of movement. Filopodia consist mainly of bundled actin filaments and are elongating by the actin polymerization. In the initial growth, the following mechanism has been proposed. First, Arp2/3 complex is anchored to the cell membrane via the VCA domain of WASP, which activates Arp2/3 and nucleates actin polymerization. Arp2/3 transmits the force of actin polymerization to the membrane, protruding it. To verify this hypothesis, many experiments have been carried out using cells, however, the minimal components and physical conditions indispensable for the membrane protrusion are still unclear. In this study, we used purified proteins and cell-sized water droplets, surrounded by a phospholipid monolayer, in water-in-oil emulsions. Inside the droplets we encapsulated fluorescently labeled G-actin, Arp2/3, and VCA, which was designed to bind to the inner surface. After the promotion of actin polymerization, distribution of actin became inhomogeneous, and filopodia-like protrusions were occasionally observed. In the absence of Arp2/3, water droplets maintained a spherical shape. To understand the mechanism of such deformations, we plan to visualize Arp2/3 and VCA in addition to actin and clarify the relationship between the deformation and the localization of these three proteins. From these results, we will discuss the mechanism of filopodia formation and regulation.

3I0912 ヘモグロビンのアロステリック平衡のリアルな描像

A realistic picture of allosteric equilibrium of hemoglobin

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In general, the molecular mechanism of protein allostery has been explained by assuming an equilibrium between the T (tense) and R (relaxed) states within the framework of the two-state allosteric model. However, it has long been recognized by hemoglobin (Hb) experts that such a simple two-state model fails to represent the relationship between structure and function in Hb in quantitative terms. To overcome this situation, some researchers including us continue to pursue the elusive "intermediate allosteric states" for decades. Here, we present crystallographic evidence that the Hb molecule assumes many kinds of distinct quaternary conformations that exist in equilibrium. In this study, we have utilized a novel Hb crystal form, which contains three independent Hb molecules with different quaternary structures. Significantly, this crystal allows large-scale conformational changes of the containing three molecules in response to the changes in ligation state and solution conditions. By using a series of three isomorphous crystals obtained from different conditions, nine distinct Hb conformations can be characterized by X-ray crystallography and oxygen equilibrium measurements. Notably, the nine conformations are distributed continuously over the whole conformational space of Hb, ranging from T to R2 through R, with various intermediate forms also present. These results suggest a mechanism for the allosteric control of a protein's function by changing the delicate balance among the multiple states on its energy landscape.

3I0924 ヘムをヘム垂線のまわりに 90° 回転した時の共鳴ラマンスペクトル変化: ヘムオキシゲナーゼ

Effects of Heme Rotation around the Heme Normal by 90° on Resonance Raman Spectra of Heme Proteins; Observation for Heme Oxygenase

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To understand the protein effects on resonance Raman spectra of heme proteins further, we report here the resonance Raman spectra of two proteins in which their heme orientations are different with regard to the rotation around the heme normal by 90° in the same protein pocket. We employed heme oxygenase (HO)