stimulus/response relationships, which explicitly includes various molecular interactions influencing phosphorylation kinetics.

We applied this model into the MAPK cascade which is well conserved in eukaryotic cells and in various signal transduction pathways; thereby, we have unambiguously indicated that the noise-reduction effect can be fully explained through both multistep and multisite phosphorylation mechanisms in the reaction cascade. Furthermore, our formulation may provide a powerful scheme to directly simulate even big network cascades at the steady state, without solving a large set of simultaneous differential equations.

3P-179 張力およびコフィリンはアクチンフィラメントのらせん構造に影響を与えるか？

Do force and cofilin modulate the twisting motion and rotational rigidity of actin filament?

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A number of reports show that mechanical tension affects the structural dynamics of actin cytoskeleton, however, little is known about the underlying biophysical mechanisms. We recently demonstrated that mechanical tension in a single actin filament inhibited its severing by cofilin using an in vitro assay system, suggesting that mechanical status of actin filament would modulate the binding and/or activity of cofilin. As earlier studies revealed that the binding of cofilin to the actin filament altered the pitch of twist of the filament, it is suggested that external forces will change the mechanical properties of the filament leading to an altered binding affinity to cofilin. Thus detailed analyses on the conformational changes of actin filament by cofilin binding and the effect of the mechanical tension on the changes are important for understanding the negative effect of mechanical tension on the actin severing activity of cofilin. To this end, we developed an assay system, where one end of the filament was immobilized on a coverslip, while the other free end was attached to a phalloidin conjugated magnetic bead that can be pulled by a magnet to tense the actin filament. We optically observed the rotational Brownian motion of the bead, which reflects the twisting motion of the actin filament and the rotational rigidity of the filament. We are currently analyzing the effects of tensile forces and cofilin on the twisting motion and rotational rigidity of the actin filament.

3P-180 細胞運動における樹状アクチンネットワーク形成に関する数理解析

Formation of dendritic actin filament network in a cell motility by numerical analysis

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Actin dynamics is one of the essential mechanisms for cell motility. Recent studies revealed a lot of proteins required to control actin dynamics in cells, i.e. G-actin for actin assembly and disassembly, profilin for actin turnover, Arp2/3 for filament branching, ADF/cofilin for filament severing, capping protein for terminating filament elongation and so on. Although these protein mechanisms regulating cell motility have been gradually clarified, it is still unclear how they relate to the whole movement of the cell. Therefore, it is important to develop a numerical model for cell motility in which whole movement of the cell is analyzed in connection with its underlying molecular mechanisms.

In this study, for the first step to develop the numerical model mentioned above, a stochastic mathematical model of actin dynamics is constructed based on the dendritic-nucleation hypothesis. Using the proposed reaction rate equations and the reported rate constants, actin dynamics is numerically simulated using Gillespie method. From the analysis, the effect of the concentration distribution of proteins will be discussed.

3P-181 Ras から Raf への信号伝達の 1 分子キネティクス解析

Single molecule kinetic analysis of signaling from Ras to Raf in living cells

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The active form of a small GTPase Ras (RasGTP) recruits cytoplasmic kinase Raf to the cell membrane for activation through phosphorylation by unknown kinases. Raf has two binding domains for Ras, Ras binding domain (RBD) and Cys rich domain (CRD). Using single-molecule imaging of GFP-fused Raf in living cells, we have found that Raf binds to RasGTP via both RBD and CRD initially and then dissociates through an intermediate.

Here, we investigated the dissociation kinetics precisely to discuss the entity of the intermediate and molecular mechanism of signaling from Ras to Raf. Dissociation of each Ras-binding domain was a first-order reaction with single rate constant, while dissociation of RBDCRD was a consecutive reaction containing an intermediate suggesting that the intermediate was Raf bound to Ras through one of RBD and CRD. We constructed a dissociation reaction model for RBDCRD by using the determined off-rates of RBD and CRD, and then obtained the best-fit values of the association rate constants. Raf and RBDCRD showed different durations of association with RasGTP. We, then, extended the dissociation model for RBDCRD into model for Raf by incorporating phosphorylation reaction. The results of modeling suggested that complex formation between Raf and its kinase is rapid (< 30 ms) and that efficiency of phosphorylation is high (> 80%) for Raf once bound to Ras. Association between CRD and RasGTP seems to be important for presentation of the catalytic domain in Raf to membrane kinases concentrated nearby RasGTP.

3P-182 C 端 LIM 領域が zyxin の接着斑への機械的力に依存した局在の責任部位である

The C-terminal LIM region of zyxin is responsible for the force-dependent recruitment of zyxin to focal adhesions

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The transmembrane adhesive molecules integrins are clustered at focal adhesions (FAs) forming tight bindings to extracellular matrices (ECM), while they link to actin cytoskeleton at their cytoplasmic domains. FAs serve as sites for actin polymerization, and accumulated F-actin presumably strengthens the integrin-actin cytoskeleton linkage. On the other hand, the linkage between integrin and actin cytoskeleton is regulated by intra- and extracellular mechanical environments. We recently revealed that mechanical forces induce actin polymerization at FAs, and the force-dependent recruitment of zyxin to FAs is involved in this process. However, the molecular interaction responsible for the zyxin localization is unrevealed. In the present study, we examined the molecular mechanism underlying the zyxin recruitment to FAs. The N-terminal region of zyxin alone, which has a binding site for the FA protein α -actinin, did not localize to FAs, suggesting insufficiency of the α -actinin-zyxin interaction for the zyxin recruitment to FAs. In contrast, the C-terminal region of zyxin, which consists of three tandem LIM domains, was sufficient to localize to FAs. As in the case of full-length zyxin, the localization of the C-terminal region of zyxin to FAs were diminished by the myosin II inhibitor blebbistatin, and were restored by the 50%-uniaxial stretch of the blebbistatin-treated cells. These results suggest that the C-terminal LIM region of zyxin is responsible for the force-dependent recruitment of zyxin to FAs.

3P-183 3D マイクロバタニング培養基板を用いた細胞と細胞外マトリクスとの力学的相互作用の解析

Analysis of Mechanical Interaction between Cell- Extracellular Matrices by 3D micro patterned culture substrate

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Mechanical cell-extracellular matrix (ECM) interactions plays an important role in the interactions of cells with the extracellular matrix (ECM) in many tissues, such as adhesion, proliferation, differentiation, and survival by activating intracellular signal transduction pathways. Recently, many researchers studied mechanical interactions between cells and their substrates because they clearly direct how cells organize in their environment. However, the molecular mechanisms on mechanical interactions between cells and their ECMs remain incomplete owing to the difficulty to find out which molecule cues the signal of force transduction. To answer this question, it is necessary to measure the force exerted to the all adherent point and amount of activated molecules recruited to all that point at same time. Recent development of traction forces microscopy allows quantitative measurement of traction forces through the deformation of flexible poly acrylamide substrates embedded with fluorescent beads. However, these continuous deformable substrate methods have fundamental limitation that the methods must calculate an inverse problem which measurement of cellular force of each adherent point is still not accurate. In this study, to measure the forces exerted by single focal adhesions directly in real time and in live cells, we have developed a method based on the simultaneous visualization of the displacements and the corresponding focal adhesions using two-photon fabricated 3D micro-patterning.

3P-184 細菌べん毛モーター MotA/MotB 複合体構造解析のための試料調製

Preparation of the MotA/MotB complex of the bacterial flagellar motor for structural analysis.

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The flagellar motor of *Salmonella* and *E. coli* is a proton-driven rotary nanomachine composed of about a dozen proteins with copy numbers ranging from several to more than one hundred. Among those are six proteins that form the rotor and the stator: FliF, FliG, FliM and FliN form the rotor; MotA and MotB form the stator. MotA and MotB are cytoplasmic membrane proteins, and four MotA and two MotB molecules form a stator complex, which is thought to localize around the basal body and be anchored to the peptidoglycan layer as well as to the basal body. The stator complex functions as a proton channel and couples proton flow across the cytoplasmic membrane with torque generation. FliG, FliM and FliN form the basal body C ring, which functions as the rotation-switching complex. It is known that torque is generated by interactions between the stator complex and the switching complex, but the torque-generation mechanism by proton motive force still remains unclear due to the lack of atomic-resolution structural information of the rotor and stator complexes. We have been trying to prepare the stator complex for high-resolution structural analyses by X-ray crystallography and electron cryomicroscopy. We will report our strategy and progress of the structural analysis.