suggest that D-loop contributes to the actin-myosin interaction when myosin is in the ADP/Pi state, and the modifications of D-loop strongly interfere with the force generation by myosin II and the processivity of myosin V.

1P-172 組み換えエンテロキナーゼの発現

Expression of the active recombinant enterokinase light chain

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Enterokinase is a serine protease produced by cells in the duodenum wall and secreted from intestinal glands, which plays a critical role in mammalian digestion through activation of trypsinogen. The enzyme exhibits a high specificity for the sequence of (Asp)⁴-Lys (DDDDK) and allows the release of the carboxyl-terminal fusion partner without leaving unwanted amino acid residues on its N-terminus. Therefore, the enterokinase cleavage sequence is present in many expression vectors that express recombinant N-terminal fusion proteins. Enterokinase consists of a disulfide-linked 115 kDa heavy chain and a 35 kDa light chain (EK_L), which is a catalytic subunit. To express the recombinant EK_L with fusion partners, disulfide oxidoreductase and secretion signal gene, various hosts have been tested, such as *Escherichia coli*, *Pichia pastoris*, *Aspergillus niger*, and *Saccharomyces cerevisiae*. Enterokinase was first expressed as a secretory protein inside these hosts and then discharged outside the cell.

We successfully expressed the active recombinant EK_L without secretion signal gene by using cold-shock expression vector, pCold I (Takara Bio), in *Escherichia coli*. At this meeting we report in detail the purification method and the properties of the purified enterokinase.

1P-173 浮遊培養細胞の凝集がMDCK細胞のアノイクスに与える効果

Effects of cellular aggregation on anoikis of MDCK cells in suspension culture

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Normal epithelial cells require contact with extracellular matrix to survive. When epithelial cells are detached from the matrix, they induce apoptosis, which is termed anoikis. Anoikis plays important roles in the physiological induction of apoptosis during development and maintenance of tissue homeostasis in the organism. Preceding studies have indicated that cell-cell interactions (thus cellular aggregation) in suspension culture suppress anoikis in several cell lines. Here we studied the effects of cellular aggregation on anoikis in MDCK cells, a model cell line for normal epithelial cells. The extent of cellular aggregation in suspension culture was controlled by several methods. In any case, the indices of cell death positively, while the indices of cell viability negatively, correlated with the extent of cellular aggregation in the suspension culture. Characteristics of apoptosis were observed mainly in single cells. The extent of apoptosis in the single cells depended on the suspension condition. Extensive apoptotic characteristics were observed in the single cells that had been maintained in suspension in conditions where spontaneous cellular aggregation was allowed. When the cells had been maintained in suspension in conditions where cellular aggregation was suppressed, single cells in the resulting cell culture retained few apoptotic characteristics. The results suggest that cellular aggregation in suspension culture facilitates anoikis of MDCK cells.

1P-174 細菌べん毛蛋白質輸送におけるプロトン駆動力の2成分の異なる使われ方

Different usage of the two components of proton motive force in coupling of bacterial flagellar protein export with proton translocation

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The bacterial flagellum, which is responsible for motility, is a biological nanomachine consisting of a rotary motor, a universal joint and a helical screw. Most of the flagellar component proteins are translocated into the central channel of the growing structure for self-assembly at the distal end by the flagellar protein export apparatus.

FliI ATPase forms a complex with its regulator FliH and delivers export substrates into the export gate made of integral membrane components of the export apparatus. Recently, we have shown that the FliH-FliI complex facilitates only the initial entry of export substrates into the export gate upon mounting onto the platform of the export gate, with the energy of ATP hydrolysis being used to disassemble and release the FliH-FliI complex from the protein about to be exported and that the rest of the successive unfolding/translocation process of the long polypeptide chain is driven by proton motive force (PMF). However, how the PMF is utilized for the export process remained unknown.

In wild-type cells, the membrane potential was found to be essential for flagellar protein export while the chemical potential of proton was not. In contrast, both components of PMF are critical in a *fliH-fliI* bypass mutant background. D₂O significantly reduced secretion rates of the substrates in the bypass mutant, suggesting a unique mechanism in which the two components of PMF are used differently to couple protein unfolding/translocation with proton translocation.

1P-175 結晶構造と変異体解析から明らかになったサルモネラ菌べん毛ロッドキャップ蛋白質FlgJのペプチドグリカン加水分解機構

Peptidoglycan hydrolyzing mechanism of FlgJ, a putative flagellar rod cap, revealed by the crystal structure and mutational analyses.

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The axial structure of the bacterial flagellum consists of three parts: the filament as a helical propeller; the hook as a universal joint; and the rod as a drive shaft connecting the hook and the MS ring. The construction of the axial structure, which occurs at its distal end, requires cap complexes attached to the growing end. FlgD and FliD are cap proteins necessary for hook and filament growth, respectively. Because the rod penetrates the peptidoglycan (PG) layer, the rod cap is thought to have a PG-hydrolyzing activity. FlgJ is a putative rod cap protein. The N-terminal region of FlgJ interacts with the rod proteins and the C-terminal region shows a sequence similarity to muramidase, such as autolysin, muramidase2 and AcmA. To understand the mechanisms of rod formation, we solved the crystal structure of a C-terminal fragment of FlgJ at 1.7 Å resolution using diffraction data collected at Spring-8 beamline BL41XU. A half of the putative active site structure of FlgJ shows remarkable similarity to that of hen egg white lysozyme (HEWL), although no significant sequence similarity is found between them. From the structure and following mutational analyses, we identified that the E184 is essential for the PG-hydrolyzing activity. The remaining half of the active site is rather disordered, suggesting that some control mechanism for the hydrolyzing activity is present in FlgJ.

1P-176 細胞の自発運動・走化性におけるイノシトールリン脂質の自己組織化現象

Self-organization of phosphatidylinositol lipids signaling involved in spontaneous cell movements and chemotaxis

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Phosphatidylinositol (PtdIns) lipids metabolic system plays an important role for cell polarization, migration and tactic responses. Intracellular PtdIns lipids dynamics are regulated by phosphoinositide-3 kinase (PI3K), phosphate tensin homolog (PTEN) and phospholipase C (PLC). PI3K and PTEN are localized exclusively with each other on membrane at the opposite sides of cells, where PI3K and PTEN catalyze the production and degradation of PtdIns(3,4,5)P₃, respectively and PLC degrades the PtdIns(4,5)P₂. Such enzymatic activities are essential for pseudopods formation and cell migrations. However, the intrinsic dynamics of PtdIns metabolic system in resting-state is unclear. Here we report the self-organized oscillatory translocation, expressed as traveling waves, in the PtdIns lipids metabolic system of *Dictyostelium discoideum*. The fluorescent-labeled pleckstrin homology domain of Akt/PKB, a PtdIns(3,4,5)P₃ reporter, and PTEN were observed simultaneously by a confocal microscope, showing self-organized traveling waves along the membrane even in the absence of external signals. Enzymatic activities of PI3K, PTEN and PLC were essential for the generation and maintenance of traveling waves. In the presence of cAMP, cells showed oscillatory localization of PtdIns(3,4,5)P₃ on membrane facing to the higher concentration. These results demonstrate that the spontaneous cell polarization and migration are regulated by the self-organized PtdIns metabolic system in the resting-state, and external signal may bias the cell direction.

1P-177 アクチンフィラメントのねじりとコフィリン結合阻害の熱力学的関係

Thermodynamics study on torsion induced inhibition of cofilin binding to actin filament

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Actin filament under tensile force survives for longer period against severing by cofilin than that without the force. This mechanosensitivity of actin-cofilin binding leads to selective formation and maintenance of stress fibers in cell, however its mechanism remains elusive. Because actin-cofilin binding causes an increase in the torsion angle of the double helical filament, affinity of actin-cofilin relates to torsion of the filament. We propose a thermodynamics model for the mechanosensitivity of actin-cofilin binding, at which difference in the chemical potential between cofilin distributed in cytosol and it binding to the filament is expressed as a function of the torsion angle of the cofilin-bound filament on the basis of Gibbs-Duhem relation. We found a critical torsion angle at which the amount of cofilin binding to the filament balances to that of dissociating from it. The predicted critical angle is about -164[deg/2.75nm]. The sign of the chemical potential difference becomes negative over the critical angle, while the sign is plus below the angle. Therefore, untwisting the double helix of the cofilin-bound filament causes the cofilin dissociation from the filament. Further, the double helical filament likely induces the stretch-twist coupling. Thus, the mechanism of mechanosensitivity of actin-cofilin binding can be explained: tensile force induces the change in the torsion angle, yields the plus sign of the chemical potential difference, and then, results in that the dissociation kinetics of actin-cofilin becomes dominant.

1P-178 極低温電子顕微鏡によるサルモネラ菌べん毛フック繊維連結部の構造解析

CryoEM structure of the hook-filament junction of *Salmonella*

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The bacterial flagellum is a biological nanomachine for the locomotion of bacteria.