Cooperative binding of cofilin and HMM to immobilized F-Actin on a glass surface


In amoeboid (crawling) cells, cofilin and myosinII are localized in the anterior and posterior regions, respectively. In the last meeting, we reported the exclusive binding of cofilin and HMM to F-Actin at the cooperative binding condition, and concluded that this exclusive binding is correlated with their differential localization. However, quantitative analysis in that experiment was difficult because of the bundling of F-Actin. In this study, therefore, cooperative and exclusive binding of cofilin and HMM was analyzed for immobilized F-Actin on a glass surface. The results showed that cooperative binding of cofilin and HMM were also observed on immobilized F-Actin. Now we are analyzing the exclusive binding of cofilin and HMM to immobilized F-Actin.

Proton permeation mechanism through the channel of flagellar motor stator complex MotA/B

Yasutaka Nishihara, Akio Kitao (IMCB, Univ of Tokyo)

Bacterial flagellar motors are powered by ions (proton in Escherichia coli and sodium ion in Vibrio alginolyticus) but the molecular mechanism is still unclear. The motor consists of a rotor and stators, and the latter that comprises MotA and MotB proteins in E. coli acts as a torque-generating unit.

To examine the structural changes coupled with ion permeation through the stator channel, we performed steered molecular dynamics calculations using the model structures which we had modeled with the experimental data. Our results showed that the channel gating was controlled by Leu46 on MotB and that helix movements were induced by ion permeation. We will also discuss the motions of the cytoplasmic domain in MotA induced by these structural changes.

Observation of MSP filaments in cell-free extract from Ascaris sperm by high-speed atomic force microscopy

Katsuya Shimabukuro, Takamitsu Haruyama, Ryoko Chijimatsu, Hiroki Konno (Ube Nat. Coll. Tech., Bio-AFM, Kanazawa Univ.)

Crawling movement in eukaryotic cells requires dynamic assembly and disassembly of cytoskeleton. In Ascaris sperm, motility is powered by an unique cytoskeletal protein, Major Sperm Protein (MSP), instead of actin which is commonly used for amoeboid motility. To understand the MSP dynamics at single filament level, we have observed MSP filaments attached to the aminosilane-coated mica by high-speed AFM. Despite of high protein concentration (~ 1 - 2 mg/ml) in cell-free extract from Ascaris sperm, filaments with 200-nm length and 9-nm diameter on average were clearly visible. Portions of filaments that were not attached to the surface fluctuated substantially, indicating that MSP filaments are highly flexible unlike actin filaments.

The Mg\textsuperscript{2+} binding site of the ATP synthase ε subunit from Bacillus subtilis derived by Molecular Dynamics simulations

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ATP synthases are the main producer of ATP, the universal energy source, in all living cells, from microbes to humans. The mechanism of ATP hydrolysis inhibition differs in bacteria and mitochondria, driven by either the [ATP] dependent subunit ε in bacteria or the pH dependent inhibitor protein IF\textsubscript{ε} in mitochondria. The ATPase inhibitory extended state in bacteria is found at low [ATP], while the ATP binding non-inhibitory down state is observed at high [ATP]. In addition the conformational change from the extended to the down state of subunit ε from Bacillus subtilis has been shown to be [Mg\textsuperscript{2+}] dependent along with its [ATP] dependency. In this work we predict the Mg\textsuperscript{2+} binding site of the ε subunit from Bacillus subtilis, using Molecular Dynamics simulations.

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