mechanism through the channel. However, the energy coupling mechanism remains unknown. Here we show that a D33E mutation in MotB dramatically alters the proton conductivity of the MotA/B complex in response to the viscous drag force. Motor speeds of the *motB*(D33E) mutant and its suppressor *motA*(V35F) *motB*(D33E) mutant fluctuated considerably at low loads but not at high loads whereas the rotation rate of the wild-type motor was almost constant. Interestingly, at low load, pausing events were more frequently observed in both mutants. These results suggest that the D33E mutation results in a low load-sensitive inactivation of the stator complex. We propose that the stator complex acts as a mechano-sensitive proton channel to conduct protons in response to changes in the amount of load.

2P168 Observation of *Halobacterium salinarum* Flagellar Rotation by Fluorescence Microscope

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Halobacterium salinarum which grow in saturated or nearly saturated salt condition is classified into extreme halophile in Archaea. The mechanism and structure of *H. salinarum* flagellar motor are not well known. Alam and Oesterhelt (J. Mol. Biol., 1984) studied movement of Halobacteria by means of dark field optical microscope. They reported that flagella bundle did not spread apart at tumbling and this manner is not like *Salmonella Thyphimurum*. The diameter of flagella of *H. salinarum* is about 12 nm and is thinner than *Salmonella Thyphimurum*. Therefore it is difficult to distinguish individual flagella filament with dark field optical microscope. Here we observe rotation of *H. salinarum* flagella by fluorescence microscope. *H. salinarum* is stained with fluorescence dye, Alexa Fluor 488. The cell suspension was added to 0.4 mg of Alexa Fluor 488, 110 mM of NaHCO₃ and 4 M of NaCl. The suspension was stirred gently for 1 h in the dark at room temperature. *H. salinarum* was separated and resuspended by centrifugation in 100 μ l of buffer described above containing 10⁻⁴% Brij 35. According to these procedures, *H. salinarum* flagellar filaments were stained individually and can be visualized by fluorescent microscope. We observed flagella bundle fly apart at some moment. The staining condition should be improved, because living cell number was small in this condition. We aimed to reveal the mechanism of flagella rotation in *H. salinarum*.

2P169 部位特異的架橋法によるべん毛 III 型輸送装置への FliH のターゲティング機構の解析 Analysis of FliH targeting to the flagellar type III protein export apparatus by site-directed crosslinking

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The flagellar type III protein export apparatus translocates most of its components from the cytosol to the growing distal end of the flagellum where they self-assemble. The flagellar specific ATPase FliH forms a heterotrimeric complex with a dimer of its regulator FliN in the cytoplasm. This FliH₂-FliN complex delivers export substrates to the export gate by the specific interaction between FliH and a C ring protein, FliN, and helps the initial entry of export substrates into the export gate. It was previously revealed that W7 and W10 of FliH are essential for the interaction with FliN and are also required for efficient localization of the FliH-FliN complex to the export gate. However, the molecular details of FliH-FliN interaction and which components of the export gate interact with FliH remain to be elusive. Here, we studied these interactions of FliH by using an in vivo site-directed photo-crosslinking that identifies the interacting residues at high spatial resolution. A photo-reactive phenylalanine synthetic analog (p-benzoyl-phenylalanine) was introduced at position 7 and 10 of FliH and the crosslinking reaction was induced by UV irradiation. Crosslinked products were detected when FliN or an export gate component, FliA, was co-expressed with FliH, indicating that FliH interacts with FliN and FliA via hydrophobic residues, W7 and W10. We are currently attempting to apply the crosslinking technique to the structural analysis of FliH-bound flagellar basal body to catch the elementary steps of protein export process.

2P170 マイコプラズマの“あし”が認識するシアル酸オリゴ糖の構造 Structure of sialyloligosaccharides recognized by *Mycoplasma* leg

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Mycoplasma, pathogenic bacteria bind to solid surfaces and glide in the direction of the membrane protrusion at a pole. This gliding motility occurs by a unique mechanism different from known biomotility systems. In gliding, *my-*

coplasma legs bind a sialyloligosaccharide fixed on a solid surface, which is also the target of many pathogenic factors. Interestingly, *mycoplasma* leg can release sialyloligosaccharide unlike other sialic acid binding proteins. In the present study, we examined the structure of sialyloligosaccharide recognized by *mycoplasma*, to elucidate the mechanism of gliding motility. *Mycoplasma mobile*, a fish pathogen has been reported to glide on the glass coated by fetuin, a protein attached with sialyloligosaccharide, and can be detached from glass surface when free sialyllactose is added (Nagai R & Miyata M (2006) Journal of Bacteriology 188, 6469-6475). In the present study, we used this method to examine the affinity of 16 compounds of synthetic sialyloligosaccharides to *M. mobile* and *M. pneumoniae*. The results showed that *M. mobile* recognizes the disaccharide of N-acetylneuraminic acid α -(2,6)-linked to galactose and *M. pneumoniae* recognizes the trisaccharide of N-acetylneuraminic acid α -(2,3)-linked to galactose with the highest affinities among the compounds examined in this study. The information about the structure of binding target for gliding may enable us, (i) the visualization of leg movement and (ii) the structural study of binding domain of leg.

2P171 マイコプラズマモービルの滑走タンパク質 Gli521, Gli123 のネガティブ染色を用いた電子顕微鏡による構造解析 Negative staining electron microscopy Gli521, Gli123 involved in *Mycoplasma mobile* gliding

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Mycoplasma mobile, a pathogenic bacterium glides on solid surfaces at the speed of four microns per second, with a unique mechanism. Four proteins, Gli123, Gli349, Gli521 and, P42 are known to be involved in the gliding mechanism. Gli521 possessing the role of “gear” is divided into three domains, hook-rod-oval from the N terminus and forms a shape of “?” under the rotary-shadowing electron microscopy (Nonaka T, Adan-Kubo J, Miyata M. (2010) Journal of Bacteriology 192: 636-42). In the present study, we analyzed the shape of isolated Gli521 molecule by negative-staining electron microscopy and image averaging, and found the following facts. (i) The rod domain has the additional three bending points. (ii) The oval domain, probably interacting with Gli349 has a cleft at the center. (iii) The dimensions are slightly smaller in the negative staining than those in the rotary shadowing method. We also analyzed the isolated Gli123 protein, “mount” and found that two oval molecules from a dimer of 20 $\times$ 15 nm, consistent with the result from gel filtration.

2P172 キネシンの α 1 ヘリックスの構造変化 Conformational change of α 1 helix of kinesin

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It remains to be clarified at submolecular or atomic level how cooperative nucleotide and microtubule binding at two kinesin motor domains is controlled. We have used site-directed spin-labeling and electron spin resonance (ESR) spectroscopy to monitor a nucleotide dependent conformational change of monomeric kinesin. Nitroxide spin label was placed in cys-lite kinesin5A (K349 monomer) at amino acid residue 62, 66, 67, 70, or 72. These residues exist in α 1 helix and those C-terminal residues form a part of a nucleotide-binding pocket, so that the mobility of the label is a good indicator of changes in steric hindrance around the side chain due to nucleotide dependent conformational changes. When microtubules (MTs) were added, all spectra were broadened. These data indicated that mobility of the helix was restricted by binding of kinesin to MTs. When ADP or AMPPNP was added, no spectrum change was detected. These data suggested that upon transition from the ADP form to AMPPNP form, no conformational changes occurred at the helix. On the other hand, the residues 67 increased mobility but residues 66 decreased upon transition from ADP-binding to non-nucleotide form, suggesting that the helix rotated by releasing ADP. N-terminal of α 1 interacts with neck linker. In order to see how the neck-linker affects conformational dynamics of the helix and nucleotide binding, we plan to measure ESR spectra and nucleotide affinity of the undock mutant where β -sheet between neck-linker and motor domain is broken.

2P173 軸糸外腕ダイニンが微小管上に整列する際のメカニズム Molecular mechanism in alignment of the axonemal outer dynein arm along microtubules

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The axonemal outer dynein arm (ODA) is known as a major force generator for the rhythmic flagellar/ciliary beating motion. The ODA is composed of three heavy chains, each of them having a motor function. The ODAs lo-