to E1 (high-affinity state to Ca^{2+}). Lipid molecules surrounding Ca^{2+} -ATPase may also move dramatically in response to the movements of the transmembrane helices. Visualization of lipid bilayer in the crystal of the membrane protein requires determination of Fourier terms at very low resolution. But it is difficult to solve the phases of the low resolution data below $15 \text{ \AA}$ by multiple isomorphous replacement due to changes in solvent contrast. We therefore used X-ray contrast variation method to visualize the lipid bilayer in the crystals of Ca^{2+} -ATPase. Complete diffraction data were collected from 200 to $3.2 \text{ \AA}$ resolution for solvent electron density from 0.35 to $0.45 \text{ e/\AA}^3$. The obtained structure of the lipid bilayer shows that (i) dynamics of the lipid bilayer is associated with the movements of the transmembrane helices, (ii) the polar head groups of phospholipids are not observed underneath the hydrophobic side of the amphipathic helix, and (iii) Glu309 at the entry of the calcium binding cavity can be accessed by solvent molecules in E1- 2Ca^{2+} , but not in E2.

1TA3-08 T細胞認識における PUFAs の影響

1P-195 Effects of disruption of lipid rafts by PUFAs on T cell recognition

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T lymphocytes recognize antigens by molecular aggregation of T cell receptor (TCR), coreceptor and signal transducing molecules. Pivotal molecular association often takes place in lipid rafts on the plasma membrane. Since the affinity of TCRs to their ligands, MHC-peptide complexes is ranked among the weakest molecular interactions found in Biology the efficiency of molecular association is likely to be affected by the physical condition of lipid rafts. We found that addition of long chain PUFAs (poly unsaturated fatty acids) from fish influences T cell recognition substantially. PUFAs have been known to be pivotal for cold adaptation of fish. Mice fed with long chain PUFAs are more resistant to develop contact dermatitis. Although immunosuppressive activity of PUFAs has been known to be largely due to influence of their metabolic products on lipid second messengers in a cell we found that isolated long chain PUFAs have a profound activity to interfere with phase separation of lipid rafts even on liposomes. Significance of physical nature of lipids on the efficacy of antigen recognition by T cells is discussed.

1TA3-09 1分子イメージングによるGPCRダイマーの解離定数の決定

First determination of the dimer dissociation constant of GPCR in the living cell membrane by single-molecule imaging

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A variety of G protein-coupled receptors (GPCRs) may exist as homo- or heterodimers even prior to ligand engagement. Unliganded dimers may be critical for GPCR functions, by enhancing the dynamic range of GPCR sensitivity to ligands and allowing crosstalk between different signaling pathways. Dimers have been found by biochemical, FRET or BRET experiments, but these studies remain qualitative, and no report on the critical parameter, i.e., the dimer dissociation constant, has been published thus far. Therefore, in the present research, we intend to do just that. Using formyl peptide receptor (FPR) as a paradigm for GPCR, labeling FPR using a formyl peptide (ligand) precisely labeled at 1:1 ratio, and single fluorescent-molecule imaging method and theory we developed previously to determine the monomer/dimer ratio of FPR, including the effect of the presence of non-fluorescent FPR molecules, the FPR monomer/dimer ratios were estimated at FPR expression levels between $0.3 - 2.2$ copies/square microns. Based on these ratios and the theory developed here, two-dimensional dissociation constant (KD) for FPR was calculated to be 2.3 copies/ μm^2 . Furthermore, these FPR dimers were found to be forming and dispersing continually, with the exponential decay lifetime of approximately 100 ms, using dual-color simultaneous single-molecule tracking of FPR-mGFP and FPR-Halo7, developed by Koyama et al. (2005, BJ). From these values, the dimerization rate (on-rate) was calculated to be $0.043 \mu\text{m}^2/\text{s}/\text{copies}$.

1TA4-01 微小管のチロシン付加とグルタミン酸付加によるモータータンパク質の交通ナビゲーション

MTs tyrosination and polyglutamylation for the traffic navigation of motor proteins

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Neurons form distinctive axonal and dendritic compartments that are important for directional signaling, but the mechanisms that discriminate between axons and dendrites remain elusive. Previous studies have demonstrated that the kinesin-1 motor domain is capable of distinguishing the axon from dendrites. Here we found that the amino acid substitutions in the beta5-loop8 region transformed truncated kinesin-1 from a uni-destination (that is, the axon-specific destination) to a bi-destination (that is, axons and dendrites) state. Furthermore, tyrosinated

tubulins that are abundant in somatodendrites prevent the wild-type kinesin-1 from binding to microtubules, whereas the bi-destination-type kinesin-1 does not have this inhibition. Consistently, inhibition of tubulin tyrosination in rat hippocampal neurons resulted in the distribution of truncated kinesin-1 in both axons and dendrites (Nature Neurosci Konishi and Setou 2009). Together with our previous report of polyglutamylation for KIF1A (PNAS Ikegami, Setou et al., 2007) Our study identifies previously unknown linkage between tubulin modification and motor trafficking.

1TA4-02 KIF1A分子モーターの一方向性の運動発現機構のシミュレーション研究

Mechanism of unidirectional move of KIF1A motor studied by coarse-grained simulations

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KIF1A is a single-headed motor which can move unidirectionally along a microtubule (MT) using the chemical energy produced by ATP hydrolysis. Experimental studies revealed that KIF1A makes the biased Brownian movements {Okada et al., 2000}. However, how KIF1A generates the translational movement from chemical reaction cycle still remains to be elucidated.

To address this questions we try to reproduce translational movement by coarse-grained simulation based on Go-like model (Okazaki et al., 2006) that takes into account of conformational change {Kikkawa et al., 2006} and ATP hydrolysis cycle. In particular, to discuss how the mechanical stepping couples to the chemical reaction, we apply multiple-basin energy landscape.

We found one condition that reproduces the unidirectional movement and the following two phenomena that are critical for biased motions:

- (1) If the bounded nucleotide on KIF1A changed from ATP to ADP, 1D-free diffusion of KIF1A along MT can start easily and smoothly.
- (2) In ADP bound state, KIF1A makes a biased diffusive motion along MT.

To generate unidirectional movement in simulations, we found that the friction and mass of cargo (bead, vesicle) must be much (at least 20 times) larger than those of an amino acid. In the meeting, we will also suggest the similarity of stepping mechanism between one-headed KIF1A motor and two-headed conventional kinesin.

1TA4-03 滑走するバクテリア、Mycoplasma mobile のステップ検出

1P-142 Step detection of gliding bacterium, *Mycoplasma mobile*

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Mycoplasma, a flask-shaped bacterium $0.8 \mu\text{m}$ long, glides on surface at a speed of $2-4.5 \mu\text{m/s}$ by a unique mechanism. We proposed a working model, "power stroke" model, where the *Mycoplasma* repeatedly catches and releases sialic acid adsorbed on the solid surface, by "legs" which are sticking out from the base of membrane protrusion of *Mycoplasma*.

On the surface of *Mycoplasma*, about 450 legs are clustering. To reduce the working leg number, the direct binding target, sialic acid was added to the medium. Detach of *Mycoplasmas* from the glass surface was prevented by adding gelatin. Under these conditions, stepwise movements were observed using high speed camera, and it was more obvious when we traced a 100 nm fluorescent bead attached to the cell. The step size was about 80 nm under various concentrations of sialic acid, while the gliding speed depended on the concentration with extension of dwell time. The obvious step was not observed when gliding was disturbed by a group of inhibitory antibodies. The 80 nm step size is comparable to the putative leg length, 50 nm , suggested from electron microscopy.

1TA4-04 Mycoplasma mobile の滑走運動を阻害するモノクローナル抗体の標的部位

Target sites of monoclonal antibody inhibiting *Mycoplasma mobile* gliding

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Mycoplasma mobile, a pathogenic bacterium of a freshwater fish glides on solid surfaces with a unique mechanism at an average speed of 2.0 to $4.5 \mu\text{m/s}$. We found that four gliding proteins are involved in the gliding mechanism, including Gli521 and Gli349, weighting 521 kDa and 349 kDa , respectively.

Previously, we isolated 11 and 24 scFv monoclonal inhibitory antibodies, respectively against Gli349 and Gli521. In the present study, we chemically synthesized Gli521 gene divided into 13 parts and Gli349 gene divided into 9 parts, expressed recombinant proteins in *Escherichia coli*, and decided the binding sites of antibodies by western blotting. Gli521 consists of three domains, (hook)-(rod)-(oval) from C terminous, forms trimer, "triskelion", and plays a role of crank in the gliding mechanism. Three antibodies bound to hook, and four bound