Understanding tissue dynamics during embryo development requires knowledge of active mechanical forces exerted by cells and passive response by the neighboring cells. Apoptosis, or programmed cell death, is the process that remodels tissue by eliminating cells that are no longer biologically necessary. During the apoptotic process, the dying cell is squeezed out from the original tissue by mechanical forces produced within the cell and within the neighboring non-apoptotic cells. Those neighboring cells are also deformed during the cell elimination, indicating that the mechanical stress in the surrounding tissue is increased. Combining *in vivo* imaging, laser microsurgery, quantitative image analysis, and theoretical modeling, we deduced the tension of the tissue and found a correlation between cell death and mechanical force. In this presentation, we will present two discrete developmental phenomena during *Drosophila* morphogenesis as the examples of tissue dynamics where apoptosis could play an active mechanical role.

3N1458 血管平滑筋ミオシン I による F アクチン束化に対するリン脂質、カルシウム及びガリモジュリンの作用

F-actin bundling by smooth muscle myosin I and the regulatory roles of phospholipids, calcium ion and calmodulin

Kai Sunada, Syougo Tokuda, Masaru Yosida, Tatsuya Kwai, Yoh Okamoto (Muran Institute of Technology, Division of Applied Science)

It has been reported that actin cytoskeletal dynamics are occurring during the activation of smooth muscle contraction.(S.J.Gunst and W.Zhang(2008)) We have isolated and characterized a single head myosin from vascular smooth muscle (Hasegawa Y. et al.(1996,1998)). We have found the myosin I induced F-actin bundling at physiological ionic condition.(Okamoto, Y. et al. (2010). The F-actin bundling has been inhibited by phosphatidyl glycerol, IP₃ and PIP₂ but not with phosphatidyl choline. These observations are consistent with our previous results of the binding abilities to the myosin I, presumably to the short tail (Hasegawa Y.et al (1996). We have done further characterization of F-actin bundling activity of the myosin I with respect to the contributions of calcium ion, calmodulin and a structure of actin. The bundling has been inhibited with increase of calcium ion concentration. We are screening the essential site on actin for the bundling by myosin I. A chemical modification of actin also caused a remarkable inhibition of the bundling with myosin I. The F-actin bundling with smooth muscle myosin I appears to be a process depending upon the lipid binding to the short tail and also calcium ion binding to the calmodulin at the junction between the head and tail of myosin I.

3N1522 蛍光顕微鏡によるコフィリンのアクチンフィラメントへの協同的結合の観察

Cooperative binding of cofilin to the actin filament examined by fluorescence microscopy

Kimihide Hayakawa^{1,3}, Hitoshi Tatsumi², Kazuki Nishida², Masahiro Sokabe^{1,2,3} (¹Cell Mechanosensing Project, SORST, JST, ²Dept. of Physiol., Nagoya Univ. Graduate School of Medicine, ³Res. Center for Innovative Nanobiodevice, Nagoya Univ.)

ADF/cofilin proteins bind to F-actin and modulate the structure and dynamics of the F-actin. Detailed examinations by electron microscopy (EM) have revealed two types of filaments in the same EM-micrograph, bare filaments and cofilin-decorated filaments under a low cofilin to actin ratio condition, suggesting that cofilin binds to the F-actin in a cooperative manner. However, bare filaments and cofilin-decorated filaments in solution have not been imaged in detail. We examined the fluorescent cofilin-decorated F-actin in solution by fluorescence microscopy. The specimen was prepared based on a newly developed method as follows; rhodamine labeled actin (250 μM) was polymerized in F-buffer and mixed with an equal volume of F-buffer containing iodoacetamide-fluorescein labeled cofilin (125 μM), and incubated for 30 sec. The cofilin to actin ratio was 1 to 2. Actin filaments (0.5 μm to 2 μm) were observed with a fluorescence microscope immediately after (~5 sec) fixation by acetone (95 %). Both bare filaments and cofilin-decorated filaments were detected. In addition to these two types, we found intermittently decorated filaments, along which one or two clusters of cofilin were observed. The size of cofilin cluster was ranged from 0.5 μm to 1 μm in majority of the observations. By contrast, we did not detect filaments decorated with cofilin sparsely and uniformly over the filament. These results consistently suggest that cofilin binding to F-actin is cooperative in solution, which conforms to the results in the past EM studies.

3N1534 電子線クライオトモグラフィー法による神経細胞 NG108-15 の超微細構造観察

The 3D ultrastructure of the intact nerve cells, NG108-15 observed by electron cryo-tomography

Shinji Aramaki¹, Kazuhiro Aoyama², Kouta Mayanagi³, Takuo Yasunaga¹ (¹Dept. of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Inst. of Tech., ²FEL Company Japan Ltd., Application Lab., ³Medical Inst. of Bioregulation, Kyushu Univ.)

Actin filaments, microtubules, and intermediate filaments, called cytoskeleton, play important roles in cells such as cell adhesion, cell motility, and intracellular transportation. Recently, live cell imaging techniques by light microscopy revealed many dynamic mechanisms of cell motility. However, these methods cannot explain the relationship between molecular structures and function of cells at the molecular levels. Thus we need to elucidate ultrastructure of cells by electron-cryo microscopy. In this study we focus on nerve cell elongation system by cytoskeleton.

First, we succeeded in culturing cell on a carbon holey grid for an electron microscope in order to observe cells. Next, for reconstructing 3D ultrastructure we acquired tilt-series of elongation regions of cells by cryo- TEM (Transmission Electron Microscopy) and STEM (Scanning Transmission Electron Microscopy): After the cells on a grid were rapidly frozen, the electron cryo-microscopy supplied observation of intact cells. Their fourier transform suggested that filament should include actin in the dendrite like structure.

We are going to develop transgenic cells which have two kinds of tags for light microscopy and electron microscopy such as GFP (Green Fluorescent Protein) and methallothionein, respectively, to combine electron microscopy and optical microscopy, refereed CLEM (Correlative Light and Electron Microscope). These revolutionary methods will provide us new insights.

3N1546 リアルタイムビデオナノ計測とコンピュータ制御されたレーザー照射により明らかになる、大腸菌の走化性誘引物質への応答時間

Real-time video nanometry and controlled laser irradiation reveals a cellular response time of *E. coli* cell for chemoattractant

Takashi Sagawa¹, Yu Kikuchi¹, Hajime Fukuoka^{1,2}, Yuichi Inoue^{1,2}, Hiroto Takahashi², Takahiro Muraoka², Kazushi Kinbara², Akihiko Ishijima^{1,2} (¹Grad. Sch. Life Sci., Tohoku Univ., ²IMRAM, Tohoku Univ.)

Chemotaxis is an essential process of signal transduction in bacterial cells. The rotational direction of the bacterial flagellar motor is regulated by chemotactic system. We reported last year that serine photo-released from caged-serine by laser irradiation induced continuous CCW rotation of motors as a response to chemotactic signal. Using the system, we tried to estimate cellular response time, which is the time required for the switching from CW to CCW after photolysis. However, the measured time included diffusion of serine from laser irradiation area to cell in addition to cellular response time.

In this study, to eliminate the time for diffusion of serine, laser was irradiated on the cell directly and the laser power is regulated to decrease effect of the laser irradiation for cell. Also we developed a measurement system to measure the rotational direction in real time (every 0.8 ms) and to be able to irradiate laser beam after CW rotation within 60 ms after the switching from CCW to CW rotation by using this system. The measured response time was about 220 ms. This would mean that about 200ms are needed from the recognition of the serine by the receptor to the response of the cell.

We will measure the response time by multiple motors on a cell to understand process of the propagation of intracellular signal by the recognition of attractant signal. We would like to discuss about this result in annual meeting.

3N1558 細胞内シグナル伝達における CheZ の役割についての考察

Consideration of the role of CheZ in the intracellular signaling of *Escherichia coli* cell

Hajime Fukuoka, Yuichi Inoue, Hiroto Takahashi, Akihiko Ishijima (IMRAM, Tohoku Univ.)

E. coli cell senses extracellular stimuli by receptors to transduce to intracellular signal. Phosphorylated CheY (CheY-P) is an intracellular signal protein and regulates the switching of rotational direction of flagellar motors from counterclockwise (CCW) to clockwise (CW). We recently showed the coordinated switching with delay time of two different motors on a cell, therefore we proposed both a transient increase and decrease in the CheY-P concentration are propagated from the receptor patch to motors with a delay time. However we do not understand how such signal propagation is generated. Especially, the decrease of signal is important because motors need to response coordinately to each signal generated at intervals of sub-seconds order. In this

study, to understand the propagation of the decrease of signal, we investigated via coordinated switching whether dephosphorylation of CheY-P by CheZ and its polar localization is required for the intracellular signaling. In *cheZ*-deleted cell, the switching of two motors did not coordinate. In the cell expressing localization-less mutant of CheZ, the coordinated switching was observed. These results indicate dephosphorylation of CheY-P is required for the signal propagation, while polar localization of CheZ would not be essential. However, to understand the role of CheZ in signal propagation, we should investigate the detail of the coordinated switching, for example the measurement of delay time of CCW-to-CW and CW-to-CCW switching individually. We want to discuss this at annual meeting.

9:00~12:00 Q会場 / Room Q

光生物・視覚・光受容 3

Photobiology: Vision & Photoreception 3

3Q0912 センサリーロドプシンの吸収波長制御機構

Spectral tuning in sensory rhodopsin

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Organisms utilize light as energy sources and as signals. Rhodopsins, which have 7 transmembrane alpha-helices with retinal covalently linked to a conserved Lys residue, are found in various organisms as distant in evolution as bacteria, archaea and eukarya. One of the most notable properties of rhodopsins is the large variation in their absorption spectrum. Sensory rhodopsin I (SRI) and sensory rhodopsin II (SRII) function as photo-sensors, and have similar properties except for their $\lambda_{\max}$ (SRI, ~560 nm; SRII, ~500 nm). An expression system utilizing *E. coli* and the high protein stability of a newly found SRI-like protein, SrSRI, enables studies of mutant proteins. To determine the residue contributing to the spectral shift from SRI to SRII, we constructed various SRI mutants, in which individual residues were substituted with the corresponding residues of SRII. Three such mutants of SrSRI showed a large spectral blue-shift (>14 nm) without a large alteration of their retinal composition. Two of them, A136Y and A200T, are newly discovered color tuning residues. In the triple mutant, the $\lambda_{\max}$ was 525 nm. The inverse mutation of SRII (F134H/Y139A/T204A) generated a spectral shifted SRII towards longer wavelengths, although the effect is smaller than in the case of SRI, which is probably due to the lack of anion-binding in the SRII mutant. Thus, half of the spectral shift from SRI to SRII could be explained by only those three residues [1].

[1] Sudo et al. (2011) J. Biol. Chem. 286, 11328.

3Q0924 固体 NMR によるバクテリオロドプシン中の Tyr 残基近傍におけるレチナールの異性化に伴った局所構造とダイナミクスの変化

Local structure and dynamics changes at Tyr residues in Bacteriorhodopsin corresponding to two retinal isomers by solid-state NMR

Miyako Horigome¹, Hirohide Nishikawa¹, Izuru Kawamura¹, Satoru Tuzi², Akira Naito¹ (¹Graduate School of Engineering, Yokohama National University, ²Graduate School of Life Science, University of Hyogo)

Bacteriorhodopsin (BR) has a light-driven proton pump activity triggered by photoisomerization of retinal. Retinal chromophore coexists as a mixture of all-trans and 13-cis, 15-syn configurations with the isomeric ratio close to 1 in the dark. In ¹³C CP-MAS NMR spectrum of [1-¹³C]Tyr-BR, it was found that two major peaks appeared at 176.0 (α -helix) and 174.0 (disorder state) ppm corresponding to the proteins with two retinal configurations in the dark by REDOR filter.

Here, we examined temperature and pressure variation of ¹³C CP MAS NMR spectral pattern of [1-¹³C]Tyr-, [15,20-¹³C]Ret-BR. Overall peak intensity of [1-¹³C]Tyr-BR is higher at a lower temperature and the plot of signal intensity shows a discontinuity at 220 and 260K. It indicates that dynamical transition of protein occurred with freezing of water or change of protein-lipid interaction. The relative signal intensity ratio of two peaks at 213K slightly increased compared with that at 293K. It was attributed to either structural change from disorder to α -helix or configuration change from 13-cis to all-trans in retinal at lower temperature. Next, we applied pressure on BR using a fast MAS in NMR. The 20-C signal intensity of 13-cis retinal at 22.0 ppm and ¹³C NMR signal of [1-¹³C]Tyr185 corresponding to 13-cis state were increased under the fast MAS condition. It was noted that the isomerization from all-trans to 13-cis state was

caused by the application of pressure. We will discuss the correlation between retinal and protein structure induced by temperature and pressure.

3Q0936 Photo-induced dynamics change of phoborhodopsin with transducer protein as studied by in-situ photo irradiated solid-state NMR

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Pharaonis phoborhodopsin (ppR or sensory rhodopsin II) is a negative phototaxis receptor of *Natronomonas pharaonis* and forms a complex with its cognate transducer protein (pHtrII). It is important to investigate the change of protein conformation and dynamics with retinal photoisomerization for understanding signal transduction mechanism. We previously observed Ala C β ¹³C NMR signal at 16.8 ppm of ppR C-terminal region and its dynamics change with pHtrII using by D75N mutant as a mimic of M-intermediate[1]. In this study, we prepared M-intermediate of [3-¹³C]Ala, [20-¹³C]retinal-labeled ppR and simultaneously detected both dynamics change of C-terminal region in the cytoplasmic side and retinal photoisomerization of ppR with pHtrII(1-159) in the lipid environment by in-situ photo-irradiation solid-state NMR. In ¹³C CP-MAS NMR spectra, the signal intensity of C-terminal region in the M-intermediate was decreased compared with that in the dark. This means that dynamics of C-terminal region of ppR change to highly mobile state in the M-intermediate with activated state of pHtrII. Therefore, it is suggested that the dynamics of C-terminal region as well as transmembrane part of Helix F and G correlates closely with photo-signaling transduction.

I. Kawamura et al. (2008) *Photochem. Photobiol.* 84 921-930.

3Q0948 光照射固体 NMR を用いた ppR/pHtrII 複合体の光活性化に伴う構造変化の解析

The conformational change of ppR/pHtrII complex during photo activation as revealed by in-situ photo-irradiated solid-state NMR

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Pharaonis phoborhodopsin (ppR) is a photo-receptor showing negative phototaxis in *Natronomonas pharaonis*. ppR forms a 2:2 complex with its transducer protein, pHtrII, in the membrane. Photoisomerization of retinal chromophore from all-trans to 13-cis, 15-anti trigger the signal transduction. To understand molecular mechanism of negative phototaxis, it is important to detect the conformational change of retinal as well as protein in the photo-activated state. In this study, we focused on hydrogen-bond between Tyr174 and Thr204, which is the most important element of signal transduction in the initial process, and assigned the chemical shifts of [1-¹³C] Tyr174 to observe conformational change during photo activation by using in-situ photo-irradiated solid-state NMR. In the ground state of ppR/pHtrII complex, the ¹³C NMR signals of [1-¹³C] Tyr174 appeared at 174.3 ppm and in the M-state, they appeared at 173.4, 174.0, 175.1 ppm. In our earlier study, the signal of [20-¹³C] retinal in the M-state ppR/pHtrII complex split into three peaks under the same condition. These results indicate that the states of splitting peaks are not attributed to difference of local electron density around retinal, but difference of protein-conformations. Since the peaks of Tyr and ret in the M-state of T204A^{ppR}, which have no photo activity, don't show splitting, it is revealed that only in the M-state of wt-ppR, multi-conformations consist. It is, therefore, stressed that multiple M-states is very important factor for signal transduction.

3Q1012 in-situ 光照射固体 NMR を用いた D96N-bR における M 中間体の捕捉

Trapping M-intermediate of D96N-bR as studied by in-situ photo-irradiated solid-state NMR

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Bacteriorhodopsin (bR) is the membrane protein in the purple membrane. When bR is photo irradiated, it transfers proton from cytoplasmic side into