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

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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

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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

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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

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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

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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