1P-127 ショウジョウバエの胚発生におけるBicoidとHunchbackの空間パターンの制御

Precise spatial patterns of Bicoid and Hunchback in the early *Drosophila* embryo
Yurie Okabe (1), Hiroki Murakami (1) and Masaki Sasai (1). (1: Department of Computational Science and Engineering, Nagoya University)

In the early development of dipteran species such as *Drosophila*, spatial patterns of maternal proteins provide positional information to downstream regulatory genes, leading to normal segmentation. Preciseness is therefore required in forming spatial patterns of proteins, but little is known about the mechanisms which control fluctuations of gene expression and protein diffusion in embryo development.

In this study, we focus attention on regulations of two morphogens, Bicoid (Bcd) and Hunchback (Hb). By simulating our 1-dimensional reaction-diffusion model, in which Bcd is produced at the anterior tip to diffuse and activate hb expression, we clarify the following questions:

- (1) How is scaling of Bicoid profile achieved?
- (2) How is fluctuation of hb expression reduced by several fold compared to the theoretical estimation of Bialek and colleagues?
- (3) How is sharpness of Hb profile achieved around the midpoint of the anterior-posterior axis?

1. Gregor, T., McGregor, A. P. & Wieschaus, E. F. Shape and function of the Bicoid morphogen gradient in dipteran species. *Dev. Biol.* 316, 350-358 (2008).

2. Gregor, T., Tank, D. W., Wieschaus, E. F. & Bialek, W. Probing the limits to positional information. *Cell* 130, 153-164 (2007).

1P-128 発生過程におけるエピジェネティックランドスケープの物理モデル

Physical model for epigenetic landscape in a developmental process

Motoki Nakagawa (1) and Osamu Narikiyo (1). (1: Dept of Physics, Faculty of Sciences, Kyushu Univ)

The scenario of epigenetic landscape is a powerful concept for describing developmental phenomena. It is applied to wide class of phenomena from molecular to sociological levels. However, the physical process generating the landscape has not been explicitly demonstrated so far. In this study we propose a physical model for epigenetic landscape in a developmental process at cellular level. In our simplified model the developmental process of cells is mapped onto the Ising-like model where the spin variable represents the state of each cell and the time-dependent energy landscape is identified with the epigenetic landscape. Here the interaction among cells is the driving force to generate the epigenetic landscape and is altered according as the cell development. We explicitly construct the environment for the cells and its development reflects the precedent state of the cells. The effect of the regulation by gene expression which is a key ingredient in development is renormalized into the interaction and the environment. At earlier stage of the development the energy landscape is rugged with small amplitude. The state of cells in such a landscape is susceptible against fluctuations and not uniquely determined. These cells are regarded as stem cells. At later stage of the development the energy landscape has a funnel-like structure corresponding to the canalization in differentiation. The rewinding or stability of the differentiation is also demonstrated by substituting a test cell into the time sequence of the model development.

1P-129 管周囲が一箇の上皮細胞からなる細い管の形成

Epithelial tube morphogenesis - Formation of a fine tube of single cell-size circumference

Hisao Honda (1) and Tatsuzo Nagai. (1: Faculty of Health Science, Hyogo University; 2: Kyushu Kyoritsu Univ. RC)

Tracheal branches of *Drosophila* are simple tubes consisting of an epithelial monolayer wrapped around a lumen. A process of tube formation is observed from a large, multi-cellular tube with intercellular adherence junctions (AJs) to a fine tube consisting of single cells with auto-cellular AJs¹.

On the other hand, we have been constructing a cell model of vertex dynamics², by which we elucidated the mechanism of active cell intercalation leading to polarized tissue morphogenesis³. We modified the cell model so to be applied to the formation of fine tube with auto-cellular AJs.

Here we investigated from mechanical and dynamical aspects the formation of fine tubes with cell rearrangement through cell intercalation. Perturbation of cell shape (polarized extension of cell bodies and contraction of AJs) caused the cell intercalation and then led to the formation of the fine tubes with auto-cellular AJs.

1. Ribeiro et al., *Curr. Biol* 14: 2197-2207, 2004.
2. Nagai & Honda, *Philosophical Magazine B* 81: 699-719, 2001.
3. Honda, Nagai & Tanemura, *Devel. Dynamics* 237 (No. 7): in press.

1P-130 枯葉に隠蔽擬態した蛾の翅模様に見られる合理的なモジュールデザイン

Sophisticated Modular Design of Moth Wing Pattern Cryptically Mimicking a 'Dead Leaf'

Takao Suzuki (1), Shigeru Kuratani (1). (1: Laboratory for Evolutionary Morphology, Center

for Developmental Biology(CDB), RIKEN)

How animal body designs acquire sophisticated patterns through changes in developmental program is a fundamental question of design principles of living systems. Here, we report morphological organization in the moth wing cryptically mimicking a dead leaf.

As a model organism, we picked up a noctuid moth, *Oraesia excavata*, whose forewing is thought to mimic a dead leaf by assuming a detailed leafy-patterning with one main nerve and two lateral veins. Based on the moths collected in Mt. Rokko, Japan, we established a breeding method of this species in the laboratory. To analyze the leafy-patterning, we used statistical morphometrics to measure phenotypic variation and correlation. We found the elements for the dead leaf showed variance several hundreds times lower than that of the others. These results suggested this leafy-patterning had established an invariant developmental program resistant to individual genetic variation. It was also indicated that these function-related elements (i.e. one main nerve and two lateral veins) had gained a dense correlation among themselves, enough to form functional modules to maintain the shape of a dead leaf and to be indeed rationalized. In addition, we found functional modules of the wing pattern are not only produced by historically conserved relationships, but also by novel relationship acquired by integration/percellation of pattern elements. These results suggested underlying developmental program have flexible characteristics to reconstruct module architecture for ecological adaptation.

1P-131 粘菌の増殖キネティクスと細胞分化

Growth kinetics and differentiation in Dictyostelium

Noritaka Masaki(1) and Satoshi Sawai(1,2). (1: ERATO Complex Systems Biology Project, JST; 2: Graduate School of Arts and Sciences, University of Tokyo)

Feeding behavior of *Dictyostelium* cells ceases as cells enter multicellular development upon starvation. To investigate the kinetics of its transition from growth to development, we studied the time course of phagocytic activities. From flow-cytometric analyses of starved cells incubated together with fluorescently labeled bacteria, we discovered that the *Dictyostelium* cells consist of two distinct populations during the first 7 hr of development; one that remains in the feeding state and the other that switches to a non-feeding state. The expression level of cAMP receptor (carA) gene in the non-feeding population increases rapidly and peaks at 3hr. In contrast, carA expression in the feeding state cells is low even after 4hr into starvation. Consistent with earlier studies, we found that strains deficient in cAMP signaling do not lose phagocytic ability even after 7hr into starvation. These results clearly demonstrate that the transition is dependent on cAMP signaling and that its asynchrony is related to the heterogeneity in the expression of the cAMP receptor. In a batch co-culture, we show that the growth rate of *Dictyostelium* cells is determined by the bacteria densities and that there exists a critical density below which genes required for the cAMP signaling such as PDE are expressed. Based on these data and a dynamical model, we discuss how an asynchronous transition from a single-cell to a multicellular life-cycle may be viewed as an optimization problem in a fluctuating environment.

1P-132 ミオシンVIの1方向運動のための張力依存的な前方アクチンへの結合機構

Strain-dependent search and capture mechanism for directional motion of myosin-VI

Mitsuhiro Iwaki (1), Atsuko H. Iwane (2) and Toshio Yanagida (1,2). (1: Graduate School of Medicine, Osaka Univ.; 2: Graduate School of Frontier Biosciences, Osaka Univ.)

Myosin-VI can constitute a two-headed form and processively moves along actin helical pitches in a hand-over-hand fashion. When the two heads span the actin helical pitch, the front and rear heads are exposed to backward and forward strains, respectively. It is well established that the movement is started by binding of ATP to the rear head in a strain-dependent manner to release it from actin. However, how the released head searches for the forward actin target and captures it has remained unsolved. The detached head with ATP or ADP and Pi complex undergoes Brownian motion back and forth along an actin filament, repeating rapid association and dissociation with it (weak binding) and should be exposed to backward strain near the forward target region at a moment when the head attached to actin.

In the last annual meeting, we examined the effect of strain on the weak binding head. We applied forward and backward strain to a single-headed myosin-VI at weak binding state tethered to an optically-trapped bead by moving the bead back and forth at various speeds. The result showed that the transition from weak to strong binding state was greatly accelerated when the backward strain was applied at sufficiently large speed for the head to be exposed to strain in the weak binding. Thus, we proposed a strain-dependent capture mechanism for directional motion of myosin-VI. In this meeting, we will also discuss the result of myosin-V, a processive motor that moves in the opposite direction compared with myosin-VI, to confirm our model.

1P-133 ミオシン線維上アクチン滑り双方向運動それぞれのエネルギー障壁

Energy barriers for bi-directional movement of F-actin along myosin thick filament

Shigeru Chaen (1), Kotaro Morioka (1). (Dept of Integrated Sciences in Physics and Biology, College of Humanities and Sciences, Nihon University)

In vitro motility assays have shown that along myosin thick filament, actin filaments moved at a fast speed towards the central bare zone and at a slower

speed away from the bare zone (Yamada et al., 1990; Sellers & Kachar, 1990; Yamada & Wakabayashi, 1993). The force and displacement of myosin crossbridges interacting with actin filament in the backward direction was smaller than that in the forward direction (Ishijima et al., 1996; Tanaka et al., 1998). However, it remains to be answered why the velocity and force are smaller in the backward direction. In the present study, we examined the actin filament movement along a thick filament from purified rabbit skeletal muscle myosin in the both directions at various temperatures. The apparent activation energy was estimated by an Arrhenius plot. The values of activation energy of the movement in the forward and backward directions were estimated to be 32 and 58 kJ/mol, respectively. Similar results were obtained from an experiment using molluscan thick filaments. It has been observed that a less flexible enzyme has a higher energy barrier for the reaction processes (Varley & Pain, 1991; Chaen et al., 1997). We discuss the cause for the slower speed in relation to the flexibility of the myosin molecule.

neck bending occurs between the 2nd and 3rd IQ motifs in the lever arm, as predicted previously (M. Terrak et al, 2005). Interestingly, the bending is often reversed and thus the bending and unbending motions are frequently repeated. Most of the leading-head hopping motion (~83 %) occurs on the same actin subunit, and the rest strongly depends on the separation of the two heads (i.e., in the shorter separation, the hopping is biased by ~5 or ~10 nm toward the plus end, while in the longer separation it is oppositely biased by ~5 or ~10 nm). These results imply that myosin V produces the multiple mechanical events responding to the chemical transition between the strong and weak M-ADP states which repeatedly occurs during one ATP-hydrolysis cycle

1P-137 ATP合成酵素の結晶化と結晶解析

Crystallization and crystal analysis of ATPsynthase

Yasuo Shirakihara(1), Hiromi Tanikawa(1), Satoshi Murakami(2), Toshiharu Suzuki(3) and Masasuke Yoshida(2). (1: National Inst of Genetics; 2: Tokyo Inst of Technology)

ATP synthase is responsible for ATP production in living cells, and is a membrane protein located in the energy conversion membrane. We have crystallized ATPsynthase from a thermophilic bacterium PS3, and now improving crystals and examining their diffraction patterns currently extending to a 7 Å resolution.

Our diffraction analysis had been hampered by low resolution of data and particularly a high mosaicity of crystals. After intensive examination of the problem, we have an impression that the problems can be overcome if we could get accurate cell parameters since other possible problems have been ruled out. Efforts along this line are being made.

Recently we have not been able to reproduce crystals once produced with our best detergent dodecyl-maltoside. The problem was cured only partially in spite of lots of efforts. We therefore have searched for better bacterial sources, and got bacterial strains that show interesting features on growth temperature. While usual thermophilic bacteria like PS3 grow only at high temperatures like 70 degree but do not at usual temperatures like 37 degree, some of our bacteria grow at both temperatures. Considering a limited temperature range for stability of proteins and that we crystallize proteins at much lower temperatures, our new strains may be generally suited to structural study of proteins and ATP synthase as well. We have identified some strains whose membranes put off ATP synthase by dodecyl-maltoside treatment.

1P-138 Zipping model for kinesin neck-linker docking

Zipping model for kinesin neck-linker docking

Neil Thomas (1) (2) and Yasuhiro Imafuku (1). (1: Biology Dept., Kyushu University, Fukuoka, Japan., 2: Physics Dept., Birmingham University, UK.)

Neck-linker docking is essential for the processive motion of kinesin along a microtubule. When ATP binds to the kinesin head attached to a microtubule, it promotes a conformational change that allows successive residues in the neck-linker to bind weakly to the head. This process has been likened to closing a molecular zip fastener. However, the weakly bound neck-linker can easily unzip from the kinesin head. Docking therefore entails the continual zipping and unzipping of the neck linker. It is only completed when the second kinesin head attaches to the microtubule. We present here a physical model for the dynamic zipping process. Since the unzipped neck linker is a protein β -chain that can fold and unfold extremely rapidly, it exhibits entropic elasticity and behaves as a nonlinear spring. Our model therefore takes account of the free energy of both the zipped and unzipped portions of the neck linker. This zipping model can account for the load dependence of the neck-linker docking rate. We show that it can be integrated into the complete cycle for the processive motion of kinesin. Moreover, a similar model may also apply to the zipping and unzipping of an RNA hairpin and of double-stranded DNA.

1P-139 ミオシンVの1分子ATPase計測

Simultaneous observation of individual mechanochemical events of myosin V during processive 36 nm steps

Mitsuhiro Sugawa (1), So Nishikawa (1), Atsuko H. Iwane (1) and Toshio Yanagida (1). (1: Graduate School of Frontier Biosciences, Osaka Univ)

Myosin V is a dimeric molecular motor that moves processively on an actin filament. A coordination of mechanochemical cycles between front and rear heads of Myosin V has been widely believed to be important for the processive movement. However, the coordinated ATPase activity during the processive movement has not been observed directly. Here we attached a quantum dot to the tail domain of myosin V and observed the 36 nm step movements of the tail. Simultaneously, the ATP turnover events by head were visualized using Cy3-labeled ATP. We found that the lifetime of Cy3-nucleotide bound to the head was much longer than that expected from the biochemical kinetic study of single-headed myosin V ATPase activity. The timing between the mechanical and Cy3-nucleotide release events indicates that ADP release from the front head is inhibited during processive movement on an actin filament, and no back-step occur even though ATP binds to the front head. Our results favor a model in which ADP release followed by ATP binding predominantly occur at the rear head. The prohibition of back step supports the one-directional movement. Thus, myosin V achieves the efficient processive movement without dissociating from

1P-134 F_1 -ATPase をモデルとした 1 分子構造変化検出による酵素機能発現の理解

F_1 -ATPase as a model for understanding mechanisms of enzyme functions through detection of conformational changes in single molecules

Tomoko Masaike (1), Fumie Koyama-Horibe (1), Kazuhiro Oiwa (2), Masauke Yoshida (3) and Takayuki Nishizaka (1). (1: Dept. Physics, Gakushuin Univ.; 2: National Institute of Information and Communications Technology; 3: Chemical Resources Lab., Tokyo Institute of Technology)

Conformational changes are the key to solve mechanisms of enzyme functions as was postulated in the induced-fit model by Koshland. Our ultimate goal is to establish approaches to visualize conformational changes of enzymes which lead to functions. The enzyme adopted as a model is F_1 -ATPase whose $\alpha_3\beta_3\gamma$ catalytic core is the smallest rotary molecular motor. Its central γ subunit rotates against the surrounding $\alpha_3\beta_3$ cylinder during ATPase reactions at the catalytic site located in the β subunit. Conformational changes of β are our focus as they are directly responsible for catalysis and also expected to drive rotation of γ . We first detected conformational changes of β by biochemical approaches. Next, we observed domain motions of β and rotation of γ in the same single molecules simultaneously under the microscope; Orientation of a fluorophore linked to the C-terminal helix of β in $\alpha_3\beta_3\gamma$ immobilized on a glass surface was measured by TIRF microscopy with polarization modulation while stepping rotation of beads attached to γ was monitored. As a result, polarization dipole of the fluorophore showed three distinct angles, 0° , $\sim 40^\circ$ and $\sim 20^\circ$. It indicated cooperative three-step bending of β subunits between Open, Closed, and partially-Closed conformations upon chemical steps that drive rotational substeps of γ . Consequently, the novel conformation set of three β subunits which has never been found in crystal structures has been revealed. The present approach has a potential to be applied to diverse proteins to reveal their mechanisms.

1P-135 量子ドットで標識したモータータンパク質の細胞内イメージング

Intracellular imaging of targeted proteins labeled with Quantum Dots.
Jungwoo Yoo (1), Taketoshi Kambara (2), Kohsuke Gonda (3), and Hideo Higuchi (2). (1: Inst. of Multidisciplinary Res. for Adv. Materials, Tohoku Univ.; 2: Dept of Phys., Grad. Sch. of Science, Univ. of Tokyo; 3: Grad. Sch. of Med., Tohoku Univ.)

Imaging single molecules of proteins within cells is very important to understand dynamic cellular processes. Here, we present a new method for imaging the movement of targeted proteins in living cancer cells with photostable and bright quantum dots (QD). QDs were conjugated with various molecules and proteins, such as phalloidin, anti-tubulin antibody and kinesin. These bio-conjugated QDs were mixed with a transfection reagent and successfully internalized into living cells. The movements of individual QDs were monitored for long periods of time. Phalloidin conjugated QDs bound to actin filaments in living cells and showed almost no movement. On the other hand, anti-tubulin antibody conjugated QDs bound to microtubules and showed dynamic movement of microtubules. Kinesin showed an interesting behavior whereby kinesin came to an almost complete halt, paused briefly for a few seconds and then moved once again. This is in direct contrast to the smoothly continuous movement of kinesin observed in an in vitro assay. Maximum velocity of kinesin in cells was faster than that in the in vitro assay. These results suggest that intracellular movement of kinesin is different from that in the in vitro assay. This newly described method will be a powerful tool for investigating the functions of other proteins in living cells.

1P-136 高速 AFM で可視化されたアクトミオシンVの運動の詳細解析

Detailed analysis of actomyosin V motion visualized by high-speed atomic force microscopy

Noriyuki Kodera (1),(2), Daisuke Yamamoto (1),(2) and Toshio Ando (1),(2). (1: School of Mathematics & Physics, College of Science & Engineering, Kanazawa Univ.; 2: CREST, JST)

Myosin V moves along actin filaments and thereby transports cargos to specific destinations in the cell. Although numerous studies support the hand-over-hand model, the details of the motion are not elucidated yet. We directly visualized the motor behavior using a high-speed AFM developed by us (T. Ando et al, 2001). Last year, we reported the characteristic manners of myosin V binding to an actin filament in the stationary chemical states and the dynamic behaviors of myosin V movement in a hand over hand fashion in the presence of ATP. Therein, we have clearly established the occurrence of bending motion of the leading neck and hopping motion of the leading head on an actin protofilament. We have been analyzing these behaviors and thus far obtained following results. The leading