2nd JOINT SYMPOSIUM

BETWEEN

MECHANOBIOLOGY INSTITUTE
National University of Singapore

AND

UNIVERSAL BIOLOGY INSTITUTE
The University of Tokyo, Japan

September 19-20,2019

Venue:
3F #1320, Faculty of Science Bldg.4, Hongo-Campus, Univ. Tokyo, Tokyo,Japan

Organizers:
G.V. Shivashankar, Mechanobiology Institute, Singapore
Hideo Higuchi , Universal Biology Institute, Japan

Thursday　September 19,2019

9:30-10:00	**Nen Saito and Satoshi Sawai**(UBI) Phase-field modeling for 3D morphodynamics in macropinocytosis
10:00-10:30	**Wu Min**(MBI) Opportunities and Challenges in Understanding Cortical Pattern Formation
10:30-11:00	**Tony Kanchanawong**(MBI) Probing the Actin Cortex in Embryonic Stem Cells by Super-resolution Microscopy
11:00-11:30	Coffee Break
11:30-12:00	**Chikara Furusawa**(UBI) Analysis of Evolutionary Constraints and Plasticity by Microbial Laboratory Evolution and Computational Models.
12:00-12:30	**Paul Matsudaira, Jun Zhong, Dipan Bhattacharya, Sahar Tavakoli, Alexandre Kabla**(MBI) Imaging the mechanics of early zebrafish development
12:30-13:30	Lunch
13:30-14:00	**Sosuke Ito**(UBI) Thermodynamic interpretation of information geometry and thermodynamic uncertainty relationships
14:00-14:30	**Tetsuhiro S. Hatakeyama** and **Jumpei F. Yamagishi**(UBI) Microeconomics of metabolism: Overflow metabolism as Giffen behavior
14:30-15:00	**Chwee Teck Lim**(MBI) Modes of Collective Cell Migration on 2- and 3-D Substrata
15:00-15:30	**Tetsuya Hiraiwa**(MBI) Dynamical ordering of migrating eukaryotic cells
15:30-16:00	Coffee Break
16:00-16:30	**Masashi K. Kajita, Kazuyuki Aihara** and **Tetsuya J. Kobayashi**(UBI) Stochastic mechanism of cellular ligand discrimination
16:30-17:00	**Gen Honda, Akihiko Nakajima, Hirofumi Yoshida, Toshihisa Osaki, Shoji Takeuchi, Satoshi Sawai** (UBI) Micro-topographical guidance of feeding cups in Dictyostelium discoideum.
17:00-17:30	**Michael Sheetz**(MBI) Out of Touch: Depletion of Mechanosensors Drives Wound-Healing and Cancer
18:00-20:00	Dinner

Friday September 20,2019

9:30-10:00	**Taketoshi Kambara**(RIKEN) and **Yasushi Okada**(UBI) Does giraffe kinesin move faster than mouse?
10:00-10:30	**Alexander D. Bershadsky**(MBI) Global self-organization of the actomyosin cytoskeleton and emerging left-right asymmetry
10:30-11:00	**Motoshi Kaya, Yongtae Hwang** and **Hideo Higuchi**(UBI) Reverse stroke of cardiac myosin revealed by single molecule microscopy is essential for heart function.
11:00-11:30	Coffee Break
11:30-12:00	**Kazunari Mouri**(RIKEN) and **Yasushi Okada**(UBI) Directional diffusion of axonal proteins captured by a confocal laser scanning microscopy
12:00-12:30	**Fumio Motegi**(MBI) Symmetry breaking in C. elegans zygotes
12:30-14:30	Lunch
14:30-15:00	**Toshinori Namba** and **Shuji Ishihara**(UBI) Essential role of cytoskeleton polarity to determine the direction of basal bodies in multi-ciliated cells
15:00-15:30	**Yusuke Toyama**(MBI) Mechanical impact of apoptosis in tissue homeostasis
15:30-16:00	Coffee Break
16:00-16:30	**Tetsuya J. Kobayashi**, **Kazumasa B. Kaneko,** and **Taishin Akiyama**(UBI) Quantitative Approaches for understanding homeostatic regulation of population size and diversity of T cells in thymus
16:30-17:00	**Kunihiko Kaneko**(UBI) Homeorhesis revisited
17:15-18:15	Lab Tour & Individual Discussion
18:30-20:30	Dinner

Phase-field modeling for 3D morphodynamics in macropinocytosis

Nen Saito# and Satoshi Sawai*

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Ameboid cells, such as neutrophils, macrophages and Dictyostelium discoideum, show drastic deformation in cell shape, where sometimes topology of shape can change via endocytosis, exocytosis, fission and so on. Such deformation plays fundamental roles in many biological processes.

A remarkable example of such drastic deformation in morphology is macropinocytosis, which is actin-dependent endocytosis and defined as the non-specific uptake of the extracellular fluid droplet by internalization of plasma membrane. A variety of cell types shows this process to perform specific and distinct functions: Dictyostelium discoideum and tumor cells show constitutive macropinocytosis for uptake of nutrients from extracellular fluid, and immune cells use it to survey their external environment and capture antigens. Although many molecules that regulate macropinocytosis have been identified, how this large internalization is spatially and temporally regulated remains still unclear.

We introduce a mathematical model based on 3D phase-field method, which enables to simulate reaction-diffusion process on the membrane and large membrane deformation simultaneously. Using this method, we perform simulation for macropinocytosis. Simulation results indicate that simple chemical reactions lead to drastic membrane deformation, which results in an engulfment of extracellular fluid. This study provides a new insight for macropinocytosis as a self-organization phenomenon via feedback between drastic deformation of membrane and reaction-diffusion on it.

Opportunities and Challenges in Understanding Cortical Pattern Formation

Wu Min
Mechanobiology Institute, National University of Singapore, Singapore

Periodic wave patterns are widely observed in oscillatory or excitable chemical systems and in multicellular systems such as cardiac tissue and slime molds. More recently, waves of cortical activity, linked to actin dynamics in many cases, have been documented in a variety of single-cell systems, including various immune cell types. The mechanisms of pattern formation and their biological significance remain largely unknown. We will discuss our recent results characterizing cortical waves of active Cdc42 and curvature-generating F-BAR proteins in mast cells. We are particularly interested in the interconversions between patterns and the possibility that patterns might encode spatial information in a context-dependent manner.

Probing the Actin Cortex in Embryonic Stem Cells by Super-resolution Microscopy

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Although the cytomechanical properties of pluripotent stem cells are known to be drastically different from specialized tissue cells, the underlying structural basis has not been fully understood. Here we investigated the integrin-based focal adhesions (FAs) and cortical actin cytoskeleton in mESCs using super-resolution microscopy. We observed that mESC FAs exhibited a multi-layer nanoscale architecture comparable to FAs of differentiated cells, but that the mESC cortex adopted a remarkably sparse architecture that largely exclude myosin II. Combining structural and mechanical measurements with molecular perturbation, our results suggested that mutual competition between formins, Arp2/3, and actin capping protein, CapZ, governed cortical structure and mechanics, in part through transient aster-like intermediate structures. This generated low network density that physically excluded myosin II from the cortex. Our results suggest that the distinctive actin cytoskeletal organization in mESCs may significantly contribute to their unusual cell mechanical properties.

Analysis of Evolutionary Constraints and Plasticity by Microbial Laboratory Evolution and Computational Models

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Biological systems change their state to evolve and adapt to changes in environmental conditions. Despite the recognized importance of characterizing the biological capacity to adapt and evolve, studies on biological evolvability and plasticity have remained at a qualitative level. To unveil how the course of evolution is constrained in high-dimensional phenotype and genotype spaces, we performed laboratory evolution under various (more than 100) stress environments, and changes in phenotypes and genome sequence were analyzed [1,2]. The results of these comprehensive analyses demonstrated that the expression changes were restricted to low-dimensional dynamics, while diverse genomic changes can contribute to similar phenotypic changes. Furthermore, to analyze the nature of evolutionary constraint, we performed computer simulations of adaptive evolution using simple cell models. Again, we found that cellular state changes in adaptation and evolution are generally restricted to low-dimensional dynamics. In this simulated dynamics of adaptive evolution, logarithmic changes in expression are shown to be proportional across all genes, with the proportionality coefficient given by the change in the growth rate of the cell, which was consistent with the experimental data [3,4]. Based on these results, we will discuss the nature of phenotypic plasticity and constraint in bacterial evolution, and possible strategies to predict and control the evolutionary dynamics.

References:

[1] S. Suzuki, T. Horinouchi, and C. Furusawa, ***Nature Comm.***, 5:5792 (2014)

[2] T. Maeda et al, in preparation

[3] K. Kaneko, C. Furusawa, and T. Yomo, ***Phys. Rev. X***, 5(1), 011014 (2015)

[4] C. Furusawa and K. Kaneko, ***Phys. Rev. E***, 97(4-1):042410 (2018)

Imaging the mechanics of early zebrafish development

Paul Matsudaira[1,] Jun Zhong[1], Dipan Bhattacharya[1,3], Sahar Tavakoli [1,4], Alexandre Kabla [2]

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The major morphogenetic movement during gastrulation is convergence and extension when the spherical symmetry of the blastula is transformed into the bilateral symmetry of the adult body plan. Gastrulation is powered by the motility, reordering, and shape changes of individual embryonic cells under the guidance of chemical morphogen gradients. The motility of individual cells is mechanically translated into motions of the embryonic tissues over the entire dorsal and ventral hemispheres. We have mapped the mechanical coupling over the surface of the zebrafish embryo during gastrulation from changes in cell density. The resulting strain maps identify the mechanical signatures of compression when cells converge to the dorsal midline, expansion when cells extend to form the head and tail structures, and compression during somite formation. Interestingly, the two step linear motions of convergence and extension can be more simply represented by strain as curl or rotation of the dorsal and ventral hemispheres. What else can be learned from mechanical maps? The strain maps also indicate stationary points and saddle points which are coincident with key morphological locations on the embryo surface such as the dorsal organizer and morphogen gradient source and sinks. This correspondence with the positions of developmentally important regions of the embryo highlights a possible significance between developmental, mechanical, and mathematical features.

Thermodynamic interpretation of information geometry and thermodynamic uncertainty relationships

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Biochemical reaction in a cell is stochastic, and it requires the thermodynamic cost. To understand information transmission of biological systems, the thermodynamic cost of a non-stationary transition from one chemical distribution to another is a fundamental topic. For stochastic dynamics described by the master equation, we consider stochastic thermodynamics to discuss the thermodynamic cost. However the thermodynamic cost can be calculated if we have the model of biochemical reaction, the universal law of information transmission and the thermodynamic cost has been elusive.

In quantum mechanics, the universal law of information transmission has been discussed in the context of the uncertainty relationship. For example, the quantum speed limit, that is the uncertainty relationship between time and energy, has been widely discussed as an energetic bound of a transition time from one quantum state to another. In our study, we obtain a kind of thermodynamic speed limits for classical biochemical reaction described by the master equation [1,2]. To consider the set of probability distribution well known as the statistical manifold in information geometry, we derive a thermodynamic speed limit geometrically. This derivation is based on an analogy of the quantum speed limit for the quantum information geometry, then our result is a classical counterpart of the uncertainty relationship for the master equation. Our result implies a trade-off relationship between speed and the thermodynamic cost; The faster speed of a transition is, the more thermodynamic cost is needed.

We numerically illustrate our speed limits for a model of enzyme reaction.

[1] Sosuke Ito, Stochastic Thermodynamic Interpretation of Information Geometry, Physical review letters, **121**, 030605 (2018).
[2] Sosuke Ito, Andreas Dechant, Stochastic time-evolution, information geometry and the Cramer-Rao Bound, arXiv preprint arXiv:1810.06832 (2018).

Microeconomics of metabolism: Overflow metabolism as Giffen behavior

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Evolution optimizes the fitness of living beings through natural selection. In particular, intracellular metabolic systems are rationally regulated to maximize the cellular growth rate. Correspondingly, the field of microeconomics investigates the behavior of individuals assumed to act rationally to maximize their utility. Since both are based on optimization, microeconomics can be applied to analyze the metabolic strategies of cells. Towards this end, we developed a microeconomics-based theory of cellular metabolism by precisely mapping the regulation of metabolic systems onto the theory of consumer choice in microeconomics. As a representative example, we focus on overflow metabolism, a seemingly wasteful strategy in which cells utilize fermentation instead of the more energetically efficient respiration (so-called Warburg effect in cancer). We formulate overflow metabolism as an optimization problem of the allocation of carbon fluxes under the guidance of microeconomic theory. Accordingly, we demonstrate that overflow metabolism corresponds to Giffen behavior in economics, the strange consumer behavior by which greater amounts of goods are consumed as their price increases. We reveal the general conditions required for both overflow metabolism and Giffen goods: trade-off and complementarity, i.e., the impossibility of substitution for different goods, among multiple objectives. Based on correspondence with Giffen behavior, a counterintuitive response of metabolism against the leakage and degradation of intermediate metabolites, which corresponds to the change in the price of a consumer good, is predicted. Overall, this demonstration highlights that application of microeconomics to metabolic systems will offer new predictions and potentially new paradigms for both biology and economics.

Modes of Collective Cell Migration on 2- and 3-D Substrata

Chwee Teck Lim
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Cells migrating in sheets or large cohorts tend to behave very differently from cells migrating individually, especially under geometrical or physical constraints and on 2 and 3D substrata. Such distinctive behavior of cells migrating in a collective manner underlies several important biological processes such as wound closure, maintenance of intestinal epithelium, developmental processes and even cancer metastasis. As such, they can also provide important insights towards better tissue repair and regenerative medicine. Here, we characterized the kinematic behavior of epithelial cell cohorts migrating under well defined geometrical constraints and physical confinements such as on a 2D narrow strips and within 3D microtubes and domes.

Dynamical ordering of migrating eukaryotic cells

Tetsuya Hiraiwa
Mechanobiology Institute, National University of Singapore, Singapore

Migration is a ubiquitous kind of eukaryotic cell motility. Some cells migrate around on the substrate or extracellular matrix according to intracellular signals that localize at their front or back. Such localization occurs even without extracellular cues. In light of this, we established a theoretical model for single eukaryotic cell migration with such intrinsic polarity [1], and are recently also trying to apply the model to the multicellular behavior when the cells are communicating with each other [2].

In this presentation, I will share the results of our numerical model simulations for the multicellular case with various types of cell-cell communications. I would like to explain firstly various dynamical ordering found in numerical simulation in the presence of contact following of locomotion, where the backside cell follows the forward but not the other way around. I will compare some of the results with observation of the social cellular slime mold, Dictyosteloum discoideum [3]. I also plan to explain our finding that, incorporating the contact inhibition of locomotion into the model with volume exclusion, collective directional migration occurs without any explicit alignment interaction [2]. If time permits, the results for the mutual attacking case, where two colliding cells try to reorient their polarities to each other, which we found leads to cell clusters, will be also explained.

Reference:

[1] T. Hiraiwa, A. Nagamatsu, N. Akuzawa, M. Nishikawa, T. Shibata, Physical Biology 11, 056002 (2014). [2] T. Hiraiwa, Phys. Rev. E 99, 012614 (2019).
[3] M. Hayakawa, T. Hiraiwa, Y. Wada, H. Kuwayama and T. Shibata, In preparation.

Stochastic mechanism of cellular ligand discrimination

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Intercellular reactions are inherently stochastic. Nevertheless, cells can precisely respond to environmental signals by sensing their target signaling molecules among the other non-target ones. In the environment, structurally similar non-target molecules are ubiquitous, and the non-targets may also hamper appropriate reactions to transmit environmental signal by non-specific interactions with receptors. The problem still remains unsolved is how cells can mitigate the crosstalk by distinguishing the targets from structurally similar non-targets even using such a stochastic system. In this talk, we introduce a stochastic chemical reaction network motivated by a recently observed receptor clustering with single-cell imaging techniques. Through this model, we provide an explanation about a possible function of the biological system from the viewpoint of a biophysical error correction mechanism.

Micro-topographical guidance of feeding cups in *Dictyostelium discoideum*.

○Gen Honda[1], Akihiko Nakajima[1], Hirofumi Yoshida[2], Toshihisa Osaki[2], Shoji Takeuchi[2], Satoshi Sawai[1, 3]

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[2]Institute of Industrial Science, University of Tokyo
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Large-scale membrane evagination of micrometer scale takes place during particle and fluid uptake. Patches of dendritic F-actin with locally activated Ras, PIP3 and SCAR/WAVE complex appear at the initial stage of the feeding cup formation. In case of phagocytosis, these membrane patches must be positioned and extended along the particle surface to support accurate engulfment. Since efficiency of the engulfment is known to depend on the particle size and shape, the cup formation and extension must be subject to surface geometry. Due to experimental limitation in controlling particle shape variation and the alignment between the cell and the particle orientation, exact parameters that guide membrane protrusion in this process remains unclear. Here, by taking advantage of the actin patches that appear on the ventral side of *Dictyostelium* which permits live-imaging approach combined with substrate micropatterning experimentation, we demonstrate that *de novo* nucleation of ventral patches is topography-dependent and occurs predominantly at the micrometer-scale ridge. Once initiated, the patches were attracted to positively curved surface and conversely excluded from negatively curved surface. In accordance with these properties, we observed that patches propagated along the edge of microstructures, and often gave rise to directed cell migration. The occurrence of patches and the accompanying cell displacement required appropriate strength of cell-substrate adhesion and was PI3K-dependent. Moreover, we found that the topography-directed process and chemotaxis to extracellular cAMP was mutually exclusive. These results suggest that the direction to which the membrane is protruded during the initial stage of phagocytic/macropinocytic cup formation is dictated by micrometer-scale topography of the substrate surface and that it can drive contact-guided cell migration, independent of chemotaxis.

Out of Touch: Depletion of Mechanosensors Drives Wound-Healing and Cancer

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Since repeated tissue damage correlates with increased risk of cancer, there could be a correlation between tissue regeneration and cancer in that both involve growth in adult tissues. Indeed microRNA-21 levels are upregulated in both tissue regeneration and cancer. miRNA-21 causes depletion of several proteins but particularly, tropomyosin (Tpm) 2.1 depletion blocks rigidity sensing and causes growth on soft surfaces. In over forty cancer cell lines tested, at least 75% were missing major components of the rigidity sensing complex (about 60% had low Tpm 2.1). The rigidity sensing complex (about 2 m in length) contracts matrix adhesions by ~100nm; and if the force generated is greater than ~25 pN, then adhesions are reinforced and cells can grow (Wolfenson et al., 2016. Nat Cell Bio. 18:33). However, if the surface is soft and matrix force low, then the rigidity sensor in normal cells causes apoptosis by DAPK1 activation (Qin et al., 2018 BioRxiv. 320739). Transformed cancer cells lack rigidity-sensing contractions and grow on soft surfaces. Restoration of rigidity sensing in cancer cells by normalizing cytoskeletal protein levels (most often by restoring Tpm 2.1 levels) restores rigidity-dependent growth (Yang, B. et al., 2018 Nature Mat. In Press). Surprisingly, we find that cyclic mechanical stretch of transformed cancer cells activates apoptosis through calpain-dependent apoptosis. Restoring rigidity sensing in transformed cancer cells blocked stretch-induced apoptosis and caused rigidity-dependent growth (Tijore et al., 2018 BioRxiv. 491746). Conversely, normal cells become stretch-sensitive for apoptosis after transformation by depleting rigidity sensors through Tpm2.1 kockdown or knockdown of other tumor suppressor proteins needed for rigidity sensing . Thus, it seems that stretch sensitivity is a weakness of many cancer cell lines and this is related to the transformed cell state and not to the tissue type or other factors. Depletion of the rigidity sensor to allow regenerative growth is found in the great majority of cancer cells and results in transformed growth. Tumor growth involves many different aspects such as telomere elongation or changes in metabolism but transformation appears necessary.

Does giraffe kinesin move faster than mouse?

Taketoshi Kambara#, and Yasushi Okada#*

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Axonal transport has been demonstrated to be essential for various neuronal functions including neurite formation and extension, synaptic functions and survival. Many neurodegenerative diseases are known to be caused by the mutations in the genes related to this transport system. For example, various point mutations in the motor domain of KIF5A, a vertebrate specific neuronal isoform of kinesin-1, is known to be causative for an autosomal dominant form (SCG10) of hereditary spastic paraplegia (HSP), which mainly affects the distal part of the long motor tracts in the spinal cord. Most of the HSP mutations partially impairs the motor activity of KIF5A. Its velocity is reduced by 25-75% of the wild type. Considering that the mutated proteins are expressed at the similar amount to the wild type proteins, the transport would be only slightly slower in the heterozygous patients' neurons. That would explain why neurons with longest axons are affected. If fast velocity is important for the survival of neurons with long axons, large animals with longer axons than human would require faster kinesin. Here, we addressed a simple question. Does kinesin of giraffe move faster than that of small animals such as mice? The motility of recombinant giraffe KIF5A (GcKIF5A) was compared with mouse KIF5A (MmKIF5A) both in vitro and in cellulo. GcKIF5A moved about 25 % faster than MmKIF5A on purified neuronal microtubules in vitro and in axon, but similar velocity with MmKIF5A in dendrites and non-neuronal cells. Interestingly, specific amino acid substitutions found in the motor domain of GcKIF5a were conserved only on burmese python KIF5a (PbKIF5a) among 150 species analyzed, and PbKIF5a moved significantly faster than MmKIF5a on purified neuronal microtubules in vitro. These results suggest that KIF5a of large animals with longer axons might have adapted for the longer axonal transport.

Global self-organization of the actomyosin cytoskeleton and emerging left-right asymmetry

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Processes of self-organization of the actin filament bundles (actin fibers) in cells are not sufficiently understood. Here, we discuss two group of mechanisms underlying such self-organization: (i) assembly and periodic ordering of myosin IIA filaments, determining formation of actin fiber arrays, cell contractility and adhesion, and (ii) chiral actin cytoskeleton swirling responsible for emerging left- right asymmetry.

Super-resolution imaging of myosin IIA filaments using structured illumination microscopy (SIM) revealed a number of local and global factors (often operating via Rho-ROCK-signaling axis), which regulate filament assembly and mutual organization. Degree of the myosin IIA filament ordering is also regulated by other actin-associated proteins, such as tropomyosins and alpha-actinins. Traction forces developed by cells inversely correlate with the myosin IIA filament ordering. Myosin IIA -driven remodeling of the actin cytoskeleton affects the integrin-mediated cell matrix interaction, promoting assembly of focal adhesions and eliminating the podosome-type adhesions.

Chiral swirling of the actin cytoskeleton, observed in individual cells confined to isotropic circular adhesive islands, is found to be driven by interactions between two types of actin fibers: radial, growing from focal adhesions, and transverse, enriched in myosin IIA and moving centripetally through the lamella. While myosin IIA-dependent centripetal flow provides a driving force for swirling, the chirality is determined by asymmetric tilting of the radial fibers. Such tilting critically depends on formin mDia1 and is regulated by other proteins controlling actin polymerization (profilin-1) and cross- linking (alpha-actinins 1 and 4). We showed that genetic and pharmacological perturbations that reverse or inhibit the actin cytoskeleton chirality in individual cells similarly affect the chiral alignment in groups of ~100 cells confined to rectangular adhesive substrate. Thus, actin cytoskeleton chirality can potentially underlie the asymmetric morphogenesis in cell groups and therefore could determine the left-right asymmetry of tissues and embryos.

Reverse stroke of cardiac myosin revealed by single molecule microscopy is essential for heart function.

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In order to elucidate the molecular mechanism of how dynamics of cardiac myosins contribute to heart function, we measured forces of synthetic β-cardiac myosin filaments using optical tweezers and revealed stepwise displacements of actin filaments driven by myosins under a wide range of loads. The stepping ratio, which is the ratio of the numbers of forward steps relative to backward steps, under unloaded conditions decreased with increasing ATP concentrations. Compared with skeletal myosin, the stepping ratio of cardiac myosin is much lower than that of skeletal myosin, indicating cardiac myosin shows frequent backward steps. Meanwhile, the peak forces generated by cardiac myofilaments with ~15 interacting molecules were 1.5-2 times higher than those observed in skeletal myofilaments with nearly the same number of interacting molecules. Based on these findings, we developed a simulation model to understand which molecular properties critically affect on stepping behaviors and force outputs in cardiac myofilaments. The simulation suggested that reverse stroke in ADP states is a key feature to cause frequent backward steps at higher ATP concentrations, resulting lower stepping ratio. Moreover, switching between two ADP states associated with the alternate execution of power and reverse strokes keeps many myosins populated in force-generating states, enhancing the duty ratio and force outputs. Therefore, we further investigated whether single cardiac myosin can execute the power and reverse strokes in ADP state under a variety of loading conditions. When single cardiac myosins interacting with single actin filaments were stretched by optical tweezers, beads' positions were occasionally switched between two discrete levels for high loads, implying the load-dependent execution of power and reverse strokes. To know physiological meaning of reverse stroke, we simulated dynamics of myosins in sarcomere and found that the reverse stroke plays a crucial role in reducing the rate of ATP consumption during isometric contraction. Also, we implemented such molecular properties into a whole heart simulator and found that the reverse stroke is a unique future of cardiac myosin and essential for maintaining high systolic blood pressure and a rapid relaxation of diastolic blood pressure.

Directional diffusion of axonal proteins captured by a confocal laser scanning microscopy

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Some neuronal axons extend over several meters long. Motor proteins, such as kinesin and dynein, enable to transport synaptic vesicles and other soluble proteins, but characteristics of motion have not been captured clearly. We developed a method which can extract the speed and direction of flow based on fluorescent correlation spectroscopy (FCS) with image processing algorithm. In the reconstructed image sequences captured by this FCS method, we find trajectories of single particles. Applying single particle tracking (SPT) analyses for these trajectories, we observed biased Brownian motions, where the molecules diffuse, but are gradually transported to one direction. We apply these methods to several axonal proteins, and discuss quantitative results of them.

Symmetry breaking in C. elegans zygotes

Fumio Motegi
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Cell polarity is facilitated by a rearrangement of the actin cytoskeleton at the cell cortex. The program triggering the asymmetric remodeling of contractile actomyosin networks remains poorly understood. We show that polarization of Caenorhabditis elegans zygotes is established through sequential downregulation of cortical actomyosin networks by the mitotic kinase, Aurora-A. Aurora-A accumulates around centrosomes to locally disrupt the actomyosin contractile activity at the proximal cortex, thereby promoting cortical flows during symmetry breaking. Translocation of Aurora-A from the cytoplasm to the cortex is sufficient to interfere with the cortical actomyosin networks independently of its roles in centrosome maturation and cell-cycle progression. We propose that Aurora-A activity is the long sought-after centrosome-mediated symmetry-breaking cue that breaks symmetry in actomyosin contractile activity. We will also discuss how this Aurora-A cascade downregulates actomyosin contractility at the cortex.

Essential role of cytoskeleton polarity to determine the direction of basal bodies in multi-ciliated cells

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Synchronous and directed ciliary beating in trachea plays an important role for transport and ejection of virus and dust from the body, known as “mucociliary transport.” This ciliary function depends on the coordinated configuration of basal bodies (BBs, root of cilia) in apical cell membrane, where BBs are regularly aligned and are oriented in the same direction. It has been experimentally suggested that microtubules (MT) and apical cytoskeleton (CSK) such as planar cell polarity (PCP) are involved in the formation of the BBs pattern, however, we still lack coherent explanation how these factors are acting for coordinating BBs, particularly for directing of BBs. By considering symmetry of the pattern to be formed, here we first show the necessity of the polarity in the MT bundles. Distribution in relative angles between the MTs and BBs is shown to be biased, supporting that the polarity is maintained in the bundle of MTs. Next, we derive a mathematical model for BB patterning by combining the polarity and self-organizational ability of CSKs. The effect of PCP is incorporated as inhomogeneous boundary condition. The model reproduces various experimental observations including normal and drug-treated phenotypes. We will discuss mechanism of the ordering of BBs by investigating the interaction between BBs and CSK. Implication of our study on cell chirality will be also mentioned.

Mechanical impact of apoptosis in tissue homeostasis

Yusuke Toyama[1,2]
[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore

Apoptosis, or programmed cell death, is the most common mechanism of eliminating damaged or unnecessary cells during embryonic development, tissue homeostasis, and certain pathological conditions. When a cell undergoes apoptosis within a tissue, the apoptotic cell is expelled from its neighboring non- dying cells. It has been shown by many labs, including ours, that this mechanical process is driven by the formation and contraction of the actomyosin cables in the dying and the neighboring cells, and/or by the lamellipodial crawling of the neighboring cells. However, how cell mechanics arises upon apoptotic cell extrusion and feedbacks to cellular and molecular function especially in the neighboring non-dying cells is largely illusive. In this presentation, I will present our current understandings of how mechanical tension and biochemical natures are altered in the neighboring cells as a consequence of apoptosis, and how these two factors are related to each other.

Quantitative Approaches for understanding homeostatic regulation of population size and diversity of T cells in thymus

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The immune system is essential for maintaining the homeostasis of our bodies, and the immune system itself is highly homeostatatic. In particular, for the function of the adaptive immune system, it is essential to appropriately control and maintain the quantity (population size) and quality (diversity) of T cells, which are central to the operation of the adaptive immune system. Recent advances in quantitative imaging and single cell sequencing technologies have enabled us to quantitatively measure the quantity and quality of cells by combining mathematical and bioinformatic techniques.

In this talk, we focus on the development of T cells in the thymus and it maintenance and recovery of homeostasis in response to environmental perturbations.

First, we investigate how the population size of the developing T cells in a thymus is regulated by the interactions between T thymocytes and thymic epithelial cells. We estimated the interactions by applying mathematical modeling to quantitative data on the recovery dynamics of the number of T cells. Our analysis has revealed that the population sizes of the thymic T and epithelial cells are reciprocally regulated in a quite complex manner.

Next, we investigate the diversity of the thymic T cell population and its dynamics by analyzing sequence data of T cell receptors (TCRs). By quantifying diversity measures of the population, we identified subset of T cells that are lost upon perturbation but eventually recovered. We also show that the composition of different T cells changes over time by reflecting recovery kinetics.

Finally, I would like to describe the current state of our efforts to combine these two approaches to determine how receptor diversity is maintained during T-cell development in the thymus.

Homeorhesis revisited

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Homeostasis or robustness of cellular states has gathered much attention over decades. To study the evolution and development, however, we must consider not only the robustness of the final state, but also the stability of the process/pathway leading to it. In fact, Waddington, with his celebrated epigenetic landscape, coined the term homeorhesis to discuss the latter issue. Indeed, the homeostasis is represented by the motion of a ball falling on along the valley in the landscape, now understood as the approach to an attractor, whereas the motion along the slow change in the landscape can lead to the homeorhesis. Then, what does this slow landscape-change mean? First, we discuss how slow modes emerge through evolution. Next, we discuss the homeorhetic cell-differentiation process through the epigenetic modification process and cell-cell interaction**.**

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