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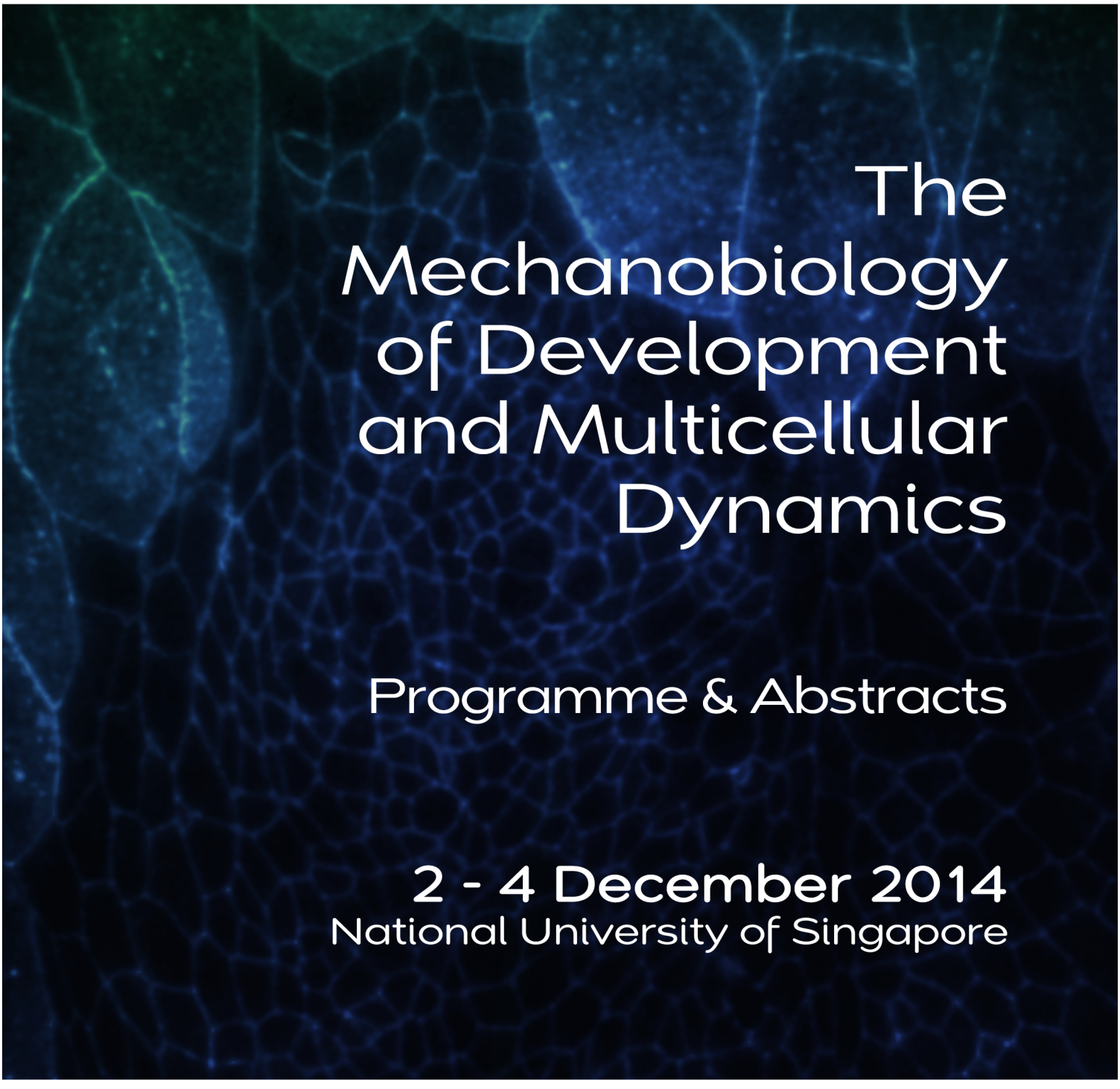
Joint Symposium 2014



The Mechanobiology of Development and Multicellular Dynamics

Programme & Abstracts

2 - 4 December 2014
National University of Singapore

MBI-Japan Joint Symposium on

Mechanobiology of Development and Multicellular Dynamics

2 - 4 December, 2014

lecture theatre 1
national university of singapore

Organized by the
Mechanobiology Institute, National University of Singapore

MBI-Japan Joint Symposium on The Mechanobiology of Development and Multicellular Dynamics

Mechanobiology Institute, National University of Singapore
T-Lab, #05-01
5A Engineering Drive 1
Singapore 117411
Website: http://mbi.nus.edu.sg
Fax: (65) 6872 6123
Email: mbi@nus.edu.sg

Acknowledgements

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Contents

Welcome to Conference Participants

We extend our warmest welcome to you to the MBI-Japan Joint Symposium, entitled “Mechanobiology of Development and Multicellular Dynamics”.

During the development of multicellular organisms, cells undergo cell proliferation, shape change, and migration, which are the basis of tissue morphogenesis. Elucidating the forces that form and reshape multicellular structures and elicit a myriad of physiological responses is essential for understanding development. However, only recently the molecular and cellular mechanisms that control the physical properties of cells and tissues, and their environments have begun to be investigated. This symposium aims to bring together experts from different fields to pursue an integral and mechanical view of development. The symposium will feature 27 invited talks and 12 short talks selected from the abstracts, given by scientists from Japan, Singapore and 4 additional countries. In addition, we will have over 20 poster presentations, presented mainly by young scientists.

The success of this symposium lies not only in the quality of the scientific programme, but also to a large extent, on the dedicated team efforts of the many volunteers including the staff. In particular, we thank Ms Siti Haryanti and Ms Sue Ping Kho, who have given their best to ensure the smooth organization and running of the symposium. Last but not least, we would like to acknowledge with sincere thanks our sponsors, Nikon and Grant-in-Aid for Scientific Research on Innovative Areas “From molecules and cells to organs: trans-hierarchical logic for higher-order pattern and structures” from Ministry of Education, Culture, Sport, Science and Technology, Japan for their generous sponsorship.

To all participants, we hope this symposium will be fruitful not only from the scientific perspective, but also in the joy of meeting and making new friends.

Best regards,

Organizers:
Fumio Motegi
Timothy Saunders
Masahiro Sokabe
Hiroyuki Takeda
Yusuke Toyama
Naoto Ueno
Ronen Zaidel-Bar

Programme

MBI-Japan Joint Symposium on
The Mechanobiology of Development
and Multicellular Dynamics

2 - 4 December 2014

Lecture Theatre 1
National University of Singapore

DAY 1 - Tuesday, 2 December 2014

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Mechanochemical coupling between tissue deformation and gene expression based on cell shape

10:30 - 11:00 Tea break

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Multilevel dynamics in embryonic morphogenesis

11:30 - 12:00 Lecture 4 - Timothy Saunders, Mechanobiology Institute, NUS
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Self-stabilization of plant shoots: resilience of biological structures through mechano-responses

15:00 - 15:30 Lecture 7 - Isao Matsuo, Osaka Medical Centre for Maternal and Child Health
External mechanical forces trigger the establishment of the anterior-posterior axis in the early mouse embryo

15:30 - 16:00 Lecture 8 - Masahiro Sokabe, Nagoya University / Mechanobiology Inst, NUS
Mechanosensitive ATP release and following Ca^{2+} waves are critical for collective cell migration during wound closure in keratinocytes

16:00 - 16:30 Tea break

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Left-right asymmetric planar cell polarity controls directional cell migration during epithelial morphogenesis in *Drosophila*

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Shaping epithelial gap closure

17:45 - 18:00 Short Talk 2 – Lei Qin, Mechanobiology Institute, NUS
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09:30 - 10:00 Lecture 12 – Ronen Zaidel-Bar, Mechanobiology Institute, NUS
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Cell competition that regulates epithelial homeostasis in *Drosophila*

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The novel membrane structure capturing centrosome determines the orientation of cell division

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PLST-1 is essential for cortical contractility during early *C. elegans* embryogenesis

15:30 - 16:00 Tea break

16:00 - 16:30 Lecture 27 – Yanlan Mao, University College, London
Getting in shape: *in vivo* and *in silico* studies of tissue mechanics in growth control

16:30 - 16:45 Short talk 11 – Nanami Akai, Kyoto University
Tissue growth regulation by "cell turnover" in *Drosophila*

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Dynein light intermediate chains maintain spindle bipolarity by functioning in centriole cohesion

17:00 - 17:15 Concluding remarks

18:30 Social sightseeing at Gardens by the Bay

Speaker Abstracts

Session I
Multiscale Dynamics

Mechanical and signaling controls of 3D tissue organization

PAUL MATSUDAIRA[1,2,3]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Centre for Bioimaging Sciences (CBIS), National University of Singapore, Singapore

dbsmpt@nus.edu.sg

The mechanical properties of the microenvironment play a large role in influencing cellular behaviour. In particular, the effect of substrate viscosity on mechanosignalling in adherent cells is highly physiologically relevant, but remains poorly understood. We studied the signaling pathways that control the reorganization of epithelial cell monolayers on substrates with varying viscosities and elasticities. Through quantitative image analysis of monolayer disruption and subcellular protein redistribution, we identified the mechanosensor protein vinculin is necessary and sufficient for the reorganization response and that vinculin acts as a diffusible switch that couples cell-matrix with cell-cell signaling pathways.

Mechanochemical coupling between tissue deformation and gene expression based on cell shape

YASUHIRO INOUE
Taiji Adachi

Institute for Frontier Medical Sciences, Kyoto University, Japan

inoue@frontier.kyoto-u.ac.jp

Dynamic tissue deformation during tissue morphogenesis can be regulated by coupling between mechanical and chemical signals. However, the mechanism of this coupling remains elusive. Here, based on a theoretical concept, we suggest a mechanism wherein a spatiotemporal gene expression pattern formed at the tissue level can be modified by cell shapes. We focused on the spatiotemporal oscillatory gene expression pattern of *Hes1*. Assuming that the oscillatory gene triggers cell proliferation and the oscillation of the gene expression level in a cell is propagated to neighbour cells by ligand–receptor kinetics on an intercellular interface such as that by the Delta–Notch signaling pathway, we constructed a mathematical model of a dynamic system of oscillatory gene expression level, ligand and receptor kinetics, and tissue deformation. The model captured emergence of a multicellular wave, where some cell populations synchronized their oscillating gene expression. Further simulation results indicated that the number of established ligand–receptor complexes varies in each cell because the intercellular interface area determines the extent of ligand–receptor binding and varies with cell shape. This inhomogeneity causes the generation and propagation of the wave sources of gene expression, which triggers cell proliferation, changing cell shape at the tissue level.

Multi-level dynamics in embryonic morphogenesis

NADINE PEYRIÉRAS

CNRS Gif-sur-Yvette, France

peyrieras@inaf.cnrs-gif.fr

We approach the understanding of model organisms' embryonic morphogenesis through the quantitative analysis of multiscale *in vivo* imaging data. The automated reconstruction of the cell lineage tree, annotated with nucleus and membrane segmentation, provides measurements for cell behavior: displacement, division, shape and contact changes, as well as cell fate and identity. This data is sufficient to determine statistical models for the spatial and temporal evolution of characteristic parameters. Combining numerical simulation derived from a multi-agent based biomechanical model with empirical measurements extracted from the reconstructed digital specimens provides the basis for the predictive understanding of morphogenetic processes. Further correlation of cell behavior, tissue biomechanics and biochemical activities by comparing the patterns revealed by cell fate, cell velocity, tissue deformation or gene expression studies, is a step toward the integration of multi-level dynamics. This overall framework lays the ground for a trans-disciplinary approach to investigate morphogenesis of living systems.

Quantitative lightsheet microscopy

TIMOTHY SAUNDERS
L. Durrieu[1, 2], M. Knop[2] and L. Hufnagel[1]

[1]European Molecular Biology Laboratory, Heidelberg, Germany
[2]Heidelberg University, Heidelberg, Germany
[3]Mechanobiology Institute, National University of Singapore, Singapore
[4]IMCB A*Star, Singapore

dbsste@nus.edu.sg

Light sheet fluorescent microscopy (LSFM) enables *in toto* live imaging of developing embryos at subcellular resolution[1]. Cell tracking and cell morphology measurements can be accurately performed using such LSFM data[1, 2]. However, lightsheet microscopy has not been used to perform quantitative measurements of, for example, gene expression levels across an entire developing organism. Here, we develop techniques to enable quantitative measurements *in vivo* using LSFM, focusing on the Bicoid morphogen gradient—a morphogen in the early *Drosophila* embryo important for precise anterior-posterior patterning. We demonstrate that by using multiple imaging views combined with improved microscope sensitivity, LSFM can detect the Bicoid-Venus signal across the entire embryo, even in the posterior pole. This enables the morphogen signal in every single nucleus in the embryo (up to around 6000 nuclei in cycle 14) to be analyzed. We find that the observed noise in the gradient is comparable to measurements from two-photon confocal microscopy. By taking advantage of the rapid imaging speed of LSFM, we (near) simultaneously image histone-mCherry and Bicoid-eGFP for over two hours during early *Drosophila* development. We are able to track 95% of all nuclei over the imaging period, from cycle 9 to gastrulation. By focusing on cells that form the cephalic furrow, we interrogate when and where cells interpret the Bicoid morphogen gradient and what role time averaging may play in improving signal readout. Overall, such work demonstrates that LSFM can be used in developmental biology to probe quantitatively spatiotemporal concentration variations across an entire specimen.

References

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2. F. Amat et al., (2014) Nature Methods. 11, 951

Session II
Mechanosensing

Hemodynamic forces as a regulator of cardiogenesis and circulatory homeostasis

TOSHIHIKO OGURA
Toshihiro Banjo, Yusuke Watanabe, Atsushi Kubo and Kota Y. Miyasaka

Department of Developmental Neurobiology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

ogura@idac.tohoku.ac.jp

A heartbeat is required for the normal development of the heart, and perturbation of intracardiac blood flow leads to morphological defects that resemble congenital heart diseases. These observations indicate that intracardiac haemodynamics play an essential role in cardiogenesis. However, signaling cascades connecting physical forces, gene expression and morphogenesis are largely unknown.

Using zebrafish as a model, we reported that the microRNA ‘miR-21’ is crucial for regulating valve formation. Expression of miR-21 is rapidly switched on and off by blood flow. Vasoconstriction and increases in shear stress induce ectopic expression of miR-21 in both heart and head vasculatures. Flow-dependent expression of mir-21 governs valvulogenesis by regulating the expression of its targets (sprouty2, pdcd4 and ptenb), thereby inducing cell proliferation in the valve-forming endocardium where the shear stress is highest. From these observations, we conclude that miR-21 is an essential component of the mechanotransduction system in a physic-genetic regulatory loop.

We will discuss how the physical forces regulate gene expression, what is controlled by force-dependent miR-21 in mice and how physical parameters regulate homeostasis of the circulatory system and energy metabolism.

In addition, we will present our new technique, by which we can introduce solid objects up to 2µm in diameter directly into the cytoplasm, without affecting the viability of cells. This safe and efficient method enables us to introduce, for example, iron nanoparticles into living cells and move them arbitrarily by a neodymium magnet. We would like to discuss the application of this technique to the mechanical manipulation of cells, intracellular organelles and chromosomes, and even to artificial symbiogenesis.

Self-stabilization of plant shoots: resilience of biological structures through mechano-responses

NAOMI NAKAYAMA

Institute of Molecular Plant Sciences and SynthSys Centre for Systems and Synthetic Biology, University of Edinburgh, UK

naomi.nakayama@ed.ac.uk

The shapes of living organisms are determined not only by their physiological function, but also by the engineering stability of their structures. Recently mechanical parameters of tissues are emerging as important regulators of multicellular development; both intrinsic and external mechanical stimuli can impact cell division, growth, and differentiation. This is particularly the case in plants, and plants effectively adjust their morphology and anatomy according to the mechanical demands. How plants sense and respond to mechanical challenge to sustain their engineering stability is an exciting yet largely unexplored question. My group aims to comprehend mechanically induced developmental plasticity in the model plant *Arabidopsis*, through a highly integrative program spanning from biochemistry and systems biology to computer simulation and material science analyses. Self-stabilization of plant shoot engineering is mediated by the accumulation dynamics of the hormone auxin, a major morphogen in plants that is sensitive to mechanical strain and stress. We are currently developing a microfluidics platform to characterize mechano-sensing and immediate responses at the cellular level. Since engineering stability of plants is also crucial to agriculture, we are also exploring improvement of cereal grain production via mechanical stimulation. Specifically, we are investigating the molecular and mechanical mechanisms behind a longstanding agricultural practice in Japan called mugi-fumi.

External mechanical forces trigger the establishment of the anterior-posterior axis in the early mouse embryo

ISAO MATSUO
Chiharu Kimura-Yoshida and Ryuji Hiramatsu

Department of Molecular Embryology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka Prefectural Hospital Organization, Osaka, Japan

imatsuo@mch.pref.osaka.jp

Mouse anterior-posterior axis polarization is preceded by formation of the distal visceral endoderm (DVE) by unknown mechanisms. Here we show, by *in vitro* culturing of mouse embryos immediately after implantation in microfabricated cavities, that the external mechanical cues exerted on the embryo are crucial for DVE formation as well as the elongated egg cylinder shape. This implies that these developmental events, which occur immediately after implantation, are not simply embryo-autonomous processes, but require extrinsic factors from maternal tissues. Notably, external mechanical forces appear to induce DVE formation and change the embryonic shape without affecting embryo-intrinsic transcriptional programs, except those involving DVE-specific genes. Strikingly, the external mechanical forces induce a local breach of the basement membrane barrier at the distal portion. This allowed the transmigrated epiblast cells to emerge as the DVE cells and the subsequent AVE cells. Thus, we propose that external mechanical forces exerted by the interaction between embryo and maternal uterine tissues at the peri-implantation stage directly control the location of DVE formation at the distal tip and consequently establish the mammalian primary body axis.

Session II - Lecture 8
2 December 2014, 15:30 - 16:00

Mechanosensitive ATP release and following Ca^{2+} waves are critical for collective cell migration during wound closure in keratinocytes

MASAHIRO SOKABE[1, 3]
Hiroya Takada[2], Kishio Furuya[1]

[1]Mechanobiology Laboratory, Nagoya University Graduate School of Medicine, Nagoya, Japan
[2]Department of Physiology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[3]Mechanobiology Institute, National University of Singapore, Singapore

msokabe@med.nagoya-u.ac.jp

We have found that wound healing in cultured HaCaT keratinocytes was accelerated by exogenous mechanical forces and was impaired by extracellular deprivation of ATP or Ca^{2+}. The present study was designed to determine how these factors contribute to this phenomenon. Three hours after scratching a confluent cell layer, monolayer cells were live-imaged and an increase in ATP release and intracellular Ca^{2+} observed in response to stretching (20%, 1 sec). ATP release was observed only from lead cells facing the scar. The diffusion of released ATP caused intercellular Ca^{2+} waves to propagate towards the rear cells in a P2Y receptor dependent way. The Ca^{2+} response and wound closure were inhibited by ATP diphosphohydrolase apyrase, P2Y antagonist suramin, hemichannel blocker CBX and the TRPC6 inhibitor diC8-PIP2. The hemichannel permeable dye calcein was taken up only by ATP-releasing cells. These results suggest that stretch-accelerated wound closure is due to the release of ATP through mechanosensitive hemichannels from the lead cells, and the subsequent Ca^{2+} waves mediated by P2Y and TRPC6 activation. Finally, wound closure devoid of external force showed essentially the same pharmacology as that of the stretch accelerated closure. We suspect that even normal wound closure is mediated by the same signaling mechanism activated by intrinsic forces generated in migrating cells.

Session III
Collective cell migration

Mechanobiology of collective cell migration

CHWEE TECK LIM[1,2]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biomedical Engineering, National University of Singapore, Singapore

ctlim@nus.edu.sg

The mechanics of cells migrating in sheets or large cohorts tend to behave very differently from cells migrating individually. In fact, the distinctive behavior of collective cell migration underlies several important biological processes such as in wound healing and developmental biology. Though the factors determining the distinctive migratory characteristics of cells in a cohort is still not well understood; *in vitro* (e.g. traditional wound healing) and *in vivo* (e.g. imaging embryos) experiments suggest that intercellular adhesion, guidance from chemical cues and mechanical constraints of the extracellular matrix (ECM) do play important roles. However, in such experiments, it is difficult both to independently assess the contribution of physical constraints vis-à-vis guidance from chemical cues as well as map the distribution of traction forces exerted by cells on the substrate. Here, we employed microfabricated techniques to study epithelial cell cohorts migrating under well-defined geometrical constraints as well as mapped the distribution of cellular traction forces. We also examined situations of wound injury that may result in the heterogeneous distribution of ECM. We demonstrated how monolayers of skin keratinocytes were able to migrate along the ECM surfaces and pull on the other cells to form suspended cell sheets over the non-ECM areas to enable wound healing. Our studies provide a new framework to systematically control and study the formation of epithelial cell sheets and determine their biomechanical properties and integrity. This will have applications not only in understanding tissue formation, but also the pathophysiology of tissue related diseases.

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Left-right asymmetric planar cell polarity controls directional cell migration during epithelial morphogenesis in *Drosophila*

ERINA KURANAGA

Laboratory for Histogenetic Dynamics, RIKEN CDB, Kobe, Japan

kuranaga@cdb.riken.jp

The collective migration of cohesive cells is a key process involved in epithelial morphogenesis, wound healing, and cancer metastasis. Collective cell migration is thought to respond to cell-extrinsic chemoattractants sensed by the leading edge of a moving cell cluster. Here, we describe an alternative mechanism occurring during *Drosophila* development that requires the generation of local left-right (LR) asymmetric mechanical force within the apical plane of epithelial cells. During the morphogenesis of *Drosophila* male terminalia, the genitalia undergo a 360˚ clockwise rotation, inducing dextral spermiduct looping. While genitalia rotation involves the rotation of surrounding epithelial tissue during metamorphosis, the underlying mechanistic details have remained unclear. We found that individual epithelial cells surrounding the genitalia adopt LR asymmetric polarity within their apical plane, termed planar cell-shape chirality (PCC), which was discovered as an intrinsic cellular process. An intact PCC can determine the clockwise genitalia rotation because MyosinID (MyoID) mutant, which shows counter-clockwise genitalia rotation, exhibited reversed PCC in surrounding epithelial cells. Moreover, using live imaging analysis we found that the epithelial cells migrated collectively, resulting in epithelial tissue rotation. The migrating cells exhibited cell junction remodelling, while remaining attached to their neighbours through adherence junctions. Most of the remodelled cell boundaries formed a right oblique angle with the anterior-posterior axis, and were associated with Myosin-II accumulation at right oblique cell boundaries during genitalia rotation. The LR asymmetric distribution of Myosin-II was reversed in flies expressing MyoID dsRNA. Numerical simulations showed that LR asymmetric contractility and junction remodelling were sufficient to induce the collective cell migration in virtual 2D tissues. These findings provide new mechanistic insights into the collective cell migration that induces the LR asymmetric morphogenesis of epithelial tissue.

Shaping epithelial gap closure

ANDREA RAVASIO[1]
Ibrahim Cheddadi[2], Chen Tianchi[1], Telmo Pereira[3], Hui Ting Ong[1], Cristina Bertocchi[1], Agusti Brugues[4], Antonio Jacinto[4], Alexandre J. Kabla[5], Yusuke Toyama[1], Xavi Trepat[3], Nir Gov[6], Luis Almeida[2], Benoit Ladoux[1,7]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Laboratoire Jacques-Louis Lions, Univ. P. et M. Curie and CNRS, Paris, France
[3]Instituto Gulbenkian de Ciência, Oeiras, Portugal
[4]Institute for Bioengineering of Catalonia, Barcelona, Spain
[5]Engineering Department, University of Cambridge, Cambridge CB2 1PZ, United Kingdom
[6]Weizmann Institute of Science, Rehovot, Israel
[7]Institut Jacques Monod, Paris, France

mbiar@nus.edu.sg

Effective closure of wounds and gaps in tissues is fundamental to the correct development and physiology of multicellular organisms. When closure is misregulated, open ulcers can have a deleterious impact with potential for inflammation, immune response and tumorigenesis. To re-establish tissue integrity, epithelial cells exhibit coordinated and collective motion into the voided area by active crawling on the substrate and by constricting a supracellular actomyosin cable – i.e. purse string mechanism. These mechanisms coexist and possibly interact to different extents depending on the context. However, the nature of this interaction is elusive due to the complexity of the overall process. Here, by employing microfabrication techniques, we created damage-free gaps of well-defined shapes to specifically investigate the mechanical interaction between cell crawling and purse string mechanism. Our results show a clear curvature dependence for selecting the migratory mechanism of wound closure, with preferential cell crawling observed for the edges with positive curvature i.e. protruding into the gap. Conversely, purse-string mechanism dominates the movement of edges with negative curvature. Despite this local regulation, mechanical feedback loops between purse-string and cell crawling mechanisms are observed in large scale multicellular gaps and regulate the overall dynamics of gap closure. Myosin and Arp2/3 inhibition experiments demonstrate that these feedback loops are force dependent and, additionally, they are subject to the physical status of the ECM. Furthermore, we employed traction force microscopy, laser ablation and computational modelling to quantitatively determine the signature mechanical interactions that occur during closure of gaps in an epithelium.

Collective cell migration during *Drosophila* mid-oogenesis

LEI QIN [1]
Yusuke Toyama[1, 2, 3]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Temasek Life Sciences Laboratory, National University of Singapore, Singapore

qin_lei@nus.edu.sg

Collective cell migration is a widely observed multi-cellular dynamic in developmental morphogenesis and tissue metastasis. The current working model for collective cell migration includes active contributions from front leader cells and passive or little contributions from back follower cells. However, this model cannot explain how tissue deforms under spatial constraints. To understand the mechanism of collective cell migration *in vivo*, we conduced time-lapse live imaging on follicle cells during *Drosophila* mid-oogenesis. We observed a global and gradual migration of follicle cells during stage 9. This migration involves active cellular morphology changes of anterior follicle cells, which locate at the rear side of migrating epithelium. Furthermore, we identified 2~3 cell-rows of follicle cells at the anterior side, called centripetal follicle cells (CFCs), which had large apical protrusions and highly dynamic morphology. Preliminary data also indicated that migration of CFCs is Rac-dependent. Experimental interruption of CFCs migration influenced the movement of the entire follicle cell epithelium. In summary, our data display an important role of CFCs in controlling global follicle cell epithelium movement during mid-oogenesis. Our results also suggest a new model for collective cell migration, involving an active contribution of back follower cells over front leader cells during collective cell migration.

Session IV
Cell adhesion regulation and tissue integrity

Anillin maintains cell-cell junction integrity by controlling the distribution of junctional RhoA-GTP and stabilizing the apical actomyosin belt

ANN L. MILLER
Ciara C. Reyes[1], Torey R. Arnold[2], Rachel E. Stephenson[2] and Tomohito Higashi[2]

[1]Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI 48109, USA
[2]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA
annlm@umich.edu

Anillin is a scaffolding protein that organizes and stabilizes actomyosin contractile rings. Using Xenopus laevis embryos as a model system to examine Anillin's role in the intact vertebrate epithelium, we recently found that a population of Anillin localizes to epithelial cell-cell junctions and plays a critical role in regulating cell-cell junction integrity. Both tight junctions and adherens junctions were disrupted when Anillin was knocked down, leading to increased intercellular spaces between cells. Moreover, Anillin perturbation affected the dynamics of the tight junction protein ZO-1. FRAP analysis revealed that ZO-1 recovered faster when Anillin was knocked down and slower when Anillin was overexpressed, indicating that Anillin stabilizes ZO-1 at junctions. To determine how Anillin regulates cell-cell junction maintenance, we examined the effects of perturbing Anillin upon its binding partners F-actin, and Myosin II, and RhoA. Junctional F-actin and phospho-Myosin II accumulation were reduced when Anillin was depleted and increased around hypercontractile cells when Anillin was overexpressed. Perturbation of Anillin also led to changes in cell shape including apical doming upon Anillin KD and apical constriction upon Anillin overexpression. Notably, we observed increased dynamic ''flares'' of active RhoA (RhoA-GTP) at cell-cell junctions when Anillin was knocked down. These RhoA-GTP flares occurred after local breaks in tight junctions and, therefore, could be involved in reestablishment of the junction. Together, these results reveal a novel role for Anillin in maintaining epithelial cell-cell junctions and suggest that Anillin may be required for orchestrating proper tension distribution at cell-cell junctions. To that end, we are currently developing approaches to directly investigate Anillin's role in regulating junctional tension.

Non-junctional E-cadherin clusters regulate cytokinesis by modulating the actomyosin cortex in *C. elegans*

RONEN ZAIDEL-BAR[1,2]
Anup Padmanabhan[1]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biomedical Engineering, National University of Singapore, Singapore

zaidelbar@gmail.com

Cadherins are best known for their essential role in mediating adhesion between animal cells. Nevertheless, a significant amount of cadherin on the cell surface is found outside of cell-cell junctions. The cellular function of these non-junctional cadherin clusters has not been addressed before. Here we show in early *C. elegans* embryos that E-cadherin/HMR-1 formed non-junctional puncta whose size and number depended on Arp2/3 and myosin II/NMY-2, respectively. Strikingly, RNAi-mediated depletion of E-cadherin/hmr-1 accelerated the formation of a cytokinetic furrow and resulted in faster furrow ingression, even in the first cell division, where no cell-cell junction exists. Conversely, overexpression of E-cadherin/HMR-1 led to adhesion-independent slowing down of cytokinesis. At the molecular level we observed E-cadherin/HMR-1 and myosinII/NMY-2 to negatively regulate each other and localize to distinct regions both at the cortex and along the ingression furrow. Moreover, we show that depletion of E-cadherin/HMR-1 can partially rescue a myosinII/NMY-2 loss of function mutant. Finally, we discovered the spatial segregation of E-cadherin/HMR-1 and myosinII/NMY-2 to be dependent on actin filaments polymerized by the formin/CYK-1 and profilin/PFN-1. Our findings suggest a non-canonical mechanism by which cadherins resist cortical deformations, such as furrow ingression, by inhibiting myosin activity and are therefore excluded from the sites of myosin activity by a formin-mediated process.

The cellular dynamics in mosaic pattern formation of the sensory organs

HIDERU TOGASHI

Department of Biochemistry and Molecular Biology, Graduate School of Medicine, Kobe University, Kobe 650-0017, Japan

htogashi@med.kobe-u.ac.jp

The olfactory epithelium is a specialized epithelial tissue inside the nasal cavity that is involved in odor perception. Within the olfactory epithelium, olfactory cells and supporting cells form characteristic mosaic patterns depending on developmental stages, but the mechanism underlying this cellular patterning is unclear. We previously showed that heterophilic interactions of nectins, a small sub-family of immunoglobulin-like cell adhesion molecules, regulate the checkerboard-like cellular pattern of the auditory epithelia of the cochlea in the inner ear. Nectins, which comprise a family with four members, interact with other nectins in either a homophilic or heterophilic way. Heterophilic interactions of nectins are much stronger than homophilic interactions, in contrast with the homophilic nature of cadherin interactions. For example, when cells expressing nectin-1 and -3 are mixed, these cells are arranged in a mosaic pattern due to their heterophilic interactions. Nectins have been shown to form adherens junctions in epithelial cells cooperatively with cadherins. The association between nectin and cadherin molecules is physically mediated by afadin and α-catenin. These properties suggest that nectins and cadherins also work cooperatively to form the mosaic cellular pattern in the developing olfactory epithelium. We recently found that cell movements and rearrangements were required for the formation of the characteristic mosaic pattern of the olfactory epithelium. In the developing mouse olfactory epithelium, nectin-2 and N-cadherin were expressed in the olfactory cells, whereas nectin-2, nectin-3, N-cadherin, and E-cadherin were expressed in the supporting cells. Genetic removal of nectin-2 or -3 affected the mosaic cellular pattern formation. Furthermore, in N-catenin deficient mice, olfactory cells were aberrantly attached to each other and the mosaic cellular pattern was perturbed. *In vitro* studies revealed that cells lacking cadherin activity failed to form the mosaic cellular pattern. These results indicate that a cooperative action of nectins and cadherins is required for the mosaic cellular patterning. In this meeting, we will describe the molecular mechanism behind how nectins and cadherins cooperatively regulate mosaic cellular patterning in the olfactory epithelium.

Adhesion disengagement and maintenance of tissue integrity during apoptosis in epithelial tissue

YUSUKE TOYAMA[1,2,3]
Xiang Teng[2]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Temasek Life Sciences Laboratory, National University of Singapore, Singapore

dbsty@nus.edu.sg

How tissue cohesion is preserved during apoptosis in epithelia is largely unknown. We used a tissue replacement process during *Drosophila* metamorphosis, histoblast expansion, as a model system to study the maintenance of tissue integrity during apoptotic cell extrusion. Histoblast nests (precursor of adult abdominal epidermis) are originally embedded in the larval epidermis. During the expansion, histoblasts undergo rapid proliferation and the preexisting larval epidermal cells undergo caspase-mediated apoptosis. Eventually, the tissue replacement from larval tissue to adult tissue completes.

We find that cell-cell adhesion and mechanical forces are progressively regulated in a coordinated process during apoptotic cell extrusion. Following commitment to apoptosis, adherens junction (AJ) components, including E-Cadherin and α- and β-catenins, dissociate from junctional membranes in the middle of the apical constriction which is powered by an actomyosin cable in apoptotic cell. This dissociation of AJ components provokes AJ disengagement and tension release between apoptotic and neighboring cells. Concurrently, a supra-cellular actomyosin cable forms in the neighboring non-dying cells and contracts to complete the cell extrusion and to recover the tissue integrity after AJ disengagement. Upon the completion of apical constriction, de novo AJs between the non-apoptotic neighboring cells are restored and E-Cadherin is re-recruited to AJs. The extrusion-associated contraction deforms not only the nearest-neighbor cells but also the surrounding tissue and contributes to large-scale tissue dynamics. Here, we present an intriguing mechanical coordination between AJ disassembly and tissue remodeling to preserve tissue integrity during apoptosis.

Afadin and ZO proteins regulate actomyosin-driven tension at the zonula adherens and maintain epithelial tissue integrity

WANGSUN CHOI[1]
Mark Peifer[1], Alan S. Fanning[2]

[1]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[2]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, USA

wchoi@email.unc.edu

Morphogenesis and epithelial homeostasis require dynamic coordination between cell-cell adhesion and cytoskeletal tension at cell-cell junctions. We used SIM superresolution and electron microscopy to explore how junction architecture is modified in response to tension, and to define how cells respond to tension without losing epithelial integrity. We focused on the junction-cytoskeletal linkers ZO-1 and Afadin. We found that depleting ZO family proteins in MDCK cells induces a highly organized contractile actomyosin array at the zonula adherens (ZA) and dramatically enhances tissue tension, cell shape uniformity and leads to decreased cell motility. Using tension-sensitive probes for the cytoskeletal linkers α-catenin and vinculin, we found that tension is particularly focused at tricellular contacts, where our data suggests actomyosin bundles are anchored. Cells respond to elevated tension by increasing junctional Afadin. While Afadin depletion did not affect assembly of contractile arrays at cell junctions, it prevented cells from balancing tension, leading to dramatic changes in cell shape, reorganization of epithelial structure and barrier failure. We propose that ZO proteins normally suppress actomyosin contractility at the ZA, whereas Afadin regulates the uniform distribution of tension at cell-cell contacts in response to increased tension.

Session IV - Short talk 4
3 December 2014, 11:45 - 12:00

Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions

YAO WU[1]
Pakorn Kanchanawong[1, 2], and Ronen Zaidel-Bar[1, 2]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biomedical Engineering, National University of Singapore, Singapore

wuyao198831@hotmail.com

E-cadherin is the major adhesion receptor in epithelial adherens junctions (AJs), which connect cells to form tissues and are essential for morphogenesis and homeostasis. The mechanism by which E-cadherin monomers cluster and become organized in AJs remains poorly understood. Here, using superresolution microscopy techniques in combination with structure-informed functional mutations, we found that loosely organized clusters of 3-15 E-cadherin molecules, which are delimited by the cortical F-actin meshwork and form independently of cis or trans interactions, are the precursors of trans-ligated adhesive clusters that make up the adherens junction. The density of E-cadherin clusters was wide ranged, and importantly, we could detect densities consistent with the crystal structure lattice at the core of adhesive clusters. Thus, our results elucidate the nanoscale architecture of adherens junctions, as well as the molecular mechanisms driving its assembly.

Session V
Emergent properties of patterns

Dynamic anisotropies in cytoskeletal organisation induced by chemical and mechanical cues underlie multicellular sensing and spatial patterning in a *Drosophila* epithelium

MAITHREYI NARASIMHA
Sonia Muliyil and Surat Saravanan

Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India
maithreyi@tifr.res.in

The morphogenesis and maintenance of tissues relies on dynamic and heterogeneous cell behaviours. The origin of these heterogeneities and their coordination remain poorly understood. We investigate how heterogeneities and transitions in cell behavior are patterned using, as our model, the amnioserosa, which is an active participant during *Drosophila* dorsal closure. We also seek to understand the cellular, molecular and physical bases of individual behaviours (both stochastic and collective) and the coordination that ensures the stereotypical dynamics of this tissue. Using a combination of approaches, including targeted (single cell) genetic and nanoscale laser perturbations, cell biology, 4D live confocal microscopy and quantitative morphological analysis, we find that differences in cell behavior result from differences in the spatial organization the actomyosin cytoskeleton. These anisotropies are in turn influenced by stochasticities and asymmetries in physical (geometry and tension) and chemical (metabolic and oxidative stress signaling) cues that we find act both autonomously and non-autonomously. We have identified pathways operating downstream of oxidative stress signaling that generate asymmetries in cytoskeletal organisation. We are currently investigating how mechanical cues are transduced to generate anisotropies in adhesion and cytoskeletal organisation. Our findings are beginning to provide insights into the local control of cell behavior and their influence on the spatial patterning of tissues during morphogenesis. They also provide an explanation for compromised tissue integrity in metabolic and oncological pathologies.

Mechanism of skin pattern formation in living organisms

SHIGERU KONDO

Osaka University

shigerukondo@gmail.com

The beauty of animal skin patterns fascinates people who are interested in the mystery of nature. For what purpose does each marking exist? Why are the patterns so rich in diversity? What are the mechanisms behind the generation of these patterns?

A mathematical theory which solves these mysteries was first presented by the legendary mathematician Alan Turing 60 years ago, and has since become known as the Turing mechanism. According to this theory, the interaction and the diffusion of molecules can give rise to a kind of “wave” in the animal body, which generates various periodic and stable patterns in a field that was originally pattern-less.

For the past 15 years, we have been engaging in experimental studies that seek to clarify the mysteries described by the Turing mechanism, using zebrafish, a small fish with beautiful horizontal stripes, as a model. In the talk, I will present recent experimental results showing that the “Turing wave” indeed occurs in zebrafish, and is made by the interaction of pigment cells.

Evolution of color in butterfly wings via changes in scale nano-morphology

ANTÓNIA MONTEIRO[1,2,3]
Bethany Wasik, Seng Fatt Liew, David Lillien, April Dinwindie, Heeso Noh and Hui Cao

[1]Ecology and Evolutionary Biology, Yale University, USA
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Yale-NUS College, Singapore

antonia.monteiro@nus.edu.sg

Brilliant animal colors are often produced from light interacting with intricate nano-morphologies present in biological materials such as butterfly wing scales. Surveys across widely divergent butterfly species have identified multiple mechanisms of structural color production, however, little is known about how these colors evolved. I'll detail how closely-related species and populations of *Bicyclus* butterflies have evolved violet structural color from brown-pigmented ancestors with UV-structural color. We used artificial selection on a lab model butterfly, *B. anynana*, to evolve violet scales from ultra-violet brown scales, and compared the mechanism of violet color production with that of two other *Bicyclus* species: *B. sambulos* and *B. medontias*, who have independently evolved violet/blue scales via natural selection. The UV reflectance peak of *B. anynana* brown scales shifted to violet over six generations of artificial selection (i.e. less than a year) due to an increase in the thickness of the lower lamina in ground scales. Similar scale structures and the same mechanism for producing violet/blue structural colors were found in the other *Bicyclus* species. This work shows that populations harbor large amounts of standing genetic variation that can lead to rapid evolution of scales' structural color via slight modifications to their physical dimensions. The genetic and developmental basis of such scale modifications are currently being investigated.

Directional sensing and cell polarity during collective cell migration in *Dictyostelium*

SATOSHI SAWAI

Graduate School of Arts and Sciences, The University of Tokyo, Japan

cssawai@mail.ecc.u-tokyo.ac.jp

In the social amoebae *Dictyostelium discoideum*, stimulation by chemoattractant cAMP invokes a transient rise in the level of cytosolic cAMP that is secreted to excite other cells in the neighborhood. Here, by precise microfluidics emulation of the chemoattractant waves, we demonstrate that, in *Dictyostelium*, small guanosine triphosphatase (GTPase) Ras activation at the leading edge is suppressed when the chemoattractant concentration is decreasing over time. This 'rectification' of directional sensing is optimal for wave passage time of ~6min and likely occurs at or upstream of Ras signaling. However, simultaneous live-cell observations of propagating cAMP waves and the leading edge response show that during the later cell aggregation stage, there is a transition in the oscillation frequency from about 6 to 3 minutes, which should be less than optimal for directional sensing. The timing of this shift in periodicity coincides with the onset of "cell streaming" – a distinct mode of collective cell migration where cells appear tightly connected between head to tail. Our observations suggest that while the 3min period of waves are less optimal for directional sensing, it maintains directionality by not allowing enough time for lateral pseudopods to form during the wave interphase. The distinct head-to-tail cell contact and the resulting cell polarity may serves as a means to compensate for the relative insensitivity to the diffusive chemoattractant signal.

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Curvature-generating proteins and subcellular pattern formation

MIN WU[1, 2, 3]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Centre for Bioimaging Sciences (CBIS), National University of Singapore, Singapore

dbswum@nus.edu.sg

Ordered assembly of cellular processes in the form of oscillations and waves is an emerging theme in living cells. Occurrences of such patterns indicate the presence of local and global coupling mechanisms. However, the nature of the coupling remains to be determined. In our recent work, we discovered a striking appearance of FBP17, one of the Bin1/amphiphysin/Rvs167 (BAR) domain proteins, in actin waves of stimulated mast cells. In addition to being a reporter, FBP17 is essential for wave formation. BAR domain proteins are widely known for their curvature sensing and inducing capabilities, motivating us to directly visualize membrane shape and test its function. We will discuss our findings on the role of physical parameters such as membrane curvature and plasma membrane tension in the propagation of waves. Collectively, our work suggests a mechanochemical basis for pattern formation, which regulates the dynamic reorganization of the cell cortex in response to external stimulation, the first and essential step in cellular activation.

Cell extrusion as a mechanical instability

HORACIO LOPEZ-MENENDEZ[1]
Leyla Kocgozlu[2], Thuan Beng Saw[2], Chwee Teck Lim[2, 3] and Benoit Ladoux[1, 2]

[1]Institut Jaques Monod, University Paris Diderot, France
[2]Mechanobiology Institute, National University of Singapore, Singapore
[3]Department of Biomedical Engineering, National University of Singapore, Singapore

horacio.lopez.menendez@gmail.com

Apoptotic cell extrusion is a process guided by the dying cell itself, which activates an actomyosin contraction from neighboring cells to drive its own extrusion in epithelia (Rosenblatt et al., 2001; Slattum et al., 2009). However, the mechanical regulation of cell extrusion during tissue growth is poorly understood. In this work we propose a simple model of extrusion considering that when the apoptotic cell is sufficiently compressed by the adjacent cells, via the purse string mechanism, it will always develop shape instability at critical compression. The origin of this instability can be linked with the reported rupture of talins and the consequent detachment of the membrane. After the critical compression, the cell structure transits from a symmetric to an asymmetric shape. The force required to deform the cell peaks at the critical point then progressively decreases the indicative catastrophic instability (Elfring et al., 2012). This model is validated with experimental results measured with micro force sensing arrays. Our results shows a contractile force during the shape instability characterized by a increase of local forces applied on the substrate, and a strong relaxation suggesting a release of tension most probably due to the disorganization of stress fibers.

Carrying skeletal elements by novel type of cells enables the self-organizing construction of 4D skeleton of sponges

NORIKO FUNAYAMA[1]
Sohei Nakayama[1], Kazushi Arima[1], Kotoe Kawai[1], Kurato Mohri[1], Chihiro Inui[1], Wakana Sugano[1], Kouji Kishimoto[1], Takeo Matsumoto[2], Toshihiko Fujimori[3], Kiyokazu Agata[1]

[1]Department of Biophysics, Graduate School of Science, Kyoto University, Kitashirakawa-oiwake, Sakyo-ku, Kyoto 606-8502, Japan
[2]Department of Mechanical Engineering, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, Aichi, 466-8555, Japan
[3]Division of Embryology, National Institute of Basic Biology, 5-1 Higashiyama, Myodaiji, Okazaki, Aichi, 444-8787, Japan
[4]Precursory Research for Embryonic Science and Technology (PRESTO) Program, Japan Science and Technology Agency (JST), 4-1-8 Honcho Kawaguchi Saitama 332-0012, Japan

funayama@mdb.biophys.kyoto-u.ac.jp

How organisms develop complex, beautiful structures has long been an intriguing question. Despite advances in developmental biology, the mechanisms underlying the assembly of some biological structures remain virtually unexplored. The skeletons of sponges, seen, for example, in Haeckel's famous drawings of glass sponges (hexactinellids), show great morphological diversity and beautiful macroscopic patterns. The question naturally arises of how sponges – one of the evolutionarily oldest extant animals – construct such elaborate skeletons, but this remains unanswered.

We succeeded in clarifying that the spiculous skeleton construction is a dynamic process using a self-devised live-imaging system. This allowed us to discover and identify the key type of cells, designated here "transport cells" that carry the spicules and act like carpenters in skeleton construction. These "transport cells" not only carry the spicules but also play important role(s) in building the spicules up step by step into the pole-and-beam architecture of the spiculous skeleton. Based on the steps we have identified during the spiculous skeleton construction, the possible mechanisms underlying the adjustment of the spiculous skeleton-construction to the indeterminate and variable growth of sponges will be discussed.

Session VI
Symmetry breaking and tissue mechanics

Cell chirality derives the left-right directional torsions of *Drosophila* gut

KENJI MATSUNO

Department of Biological Sciences, Osaka University Toyonaka, Osaka, Japan

kmatsuno@bio.sci.osaka-u.ac.jp

Internal organs of animals often show directional Left-Right (LR) asymmetry. Although recent studies have provided knowledge about the mechanisms of LR axis formation, the mechanisms of LR asymmetrical morphogenesis remain largely unknown. In *Drosophila*, many internal organs, including the embryonic hindgut, show directional LR asymmetry. The LR asymmetric structure of the embryonic hindgut is formed through a 90 degree counterclockwise rotation by the mechanical force generated by the hindgut itself. Before this rotation, the shape of hindgut epithelial cells at the apical plane is LR asymmetric *in vivo*. Given that their three dimensional structure does not overlap with that of their mirror image, this LR asymmetric property is referred to as “planar cell-shape chirality” (PCC). Our computer modeling suggests that PCC is responsible for this left-handed rotation. We also found that PCC is intrinsically formed in each individual cell.

To explore the genetic pathway that generates the mechanical force that induces the rotation of the hindgut, we first need to quantify the force. We developed a novel method to measure the torque of the hindgut counterclockwise rotation, which involves the application of magnetic beads. Magnetic beads were injected into the lumen of the hindgut anterior end, which is LR symmetric and curved towards the ventral side of the embryos, before its rotation. Using neodymium magnets, we induced counterbalancing magnetic force against the twisting of the hindgut. As a reference of how much force this counterbalancing created, we measure the magnetic force between the magnetic beads and the neodymium magnet in viscous fluid using Stokes’ law. We successfully quantified the magnetic force required to stop the contortion of the hindgut, which is equivalent to the force generated by the hindgut for the twisting.

We have genetically identified several genes that affect the hindgut rotation. Thus, the measured values of the force are crucial for performing quantitative genetic analysis of mechanical force inducing the LR asymmetric morphogenesis.

Partitioning of cell cortex in *C. elegans* zygote

FUMIO MOTEGI[1,2,3]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Temasek Life Sciences Laboratory, National University of Singapore, Singapore

fmotegi@tll.org.sg

The complex journey from an embryo to adult relies on the establishment of cellular asymmetry. A hallmark of polarized animal cells is the establishment of a graded distribution of PAR (partition defective) kinases, atypical protein kinase C (aPKC), and PAR-1/MARK kinase, into asymmetric domains at the cell cortex. These kinases are engaged with antagonistic relationship and segregate into mutually exclusive domains at cell cortex, but the mechanisms that controls their spatial asymmetry remains elusive. Here we describe that the PAR-1 asymmetric gradient is a consequence of dual mechanisms, by which the PAR-1 adopter protein PAR-2 “protects” PAR-1 from cortical exclusion by aPKC and “stabilizes” PAR-1 on the plasma membrane. The PAR-1 gradient arises after PAR-2 accesses the cortex, and the cortical level of PAR-1 shows PAR-2 dose-dependency. aPKC phosphorylation interferes with PAR-1 binding to membrane phospholipids. PAR-2 acts as a competitive inhibitor for PAR-1 phosphorylation by aPKC, thus protecting PAR-1 from cortical exclusion. PAR-2, in parallel, stabilizes the dynamic state of PAR-1 at the cortex in a manner independent of aPKC. These two mechanisms act in parallel to ensure the establishment of a proper gradient of PAR-1 complementary to aPKC. Our findings illustrate the principles underlying cellular gradients which rely on a spatial modification of the balance between two antagonizing kinases.

A molecular mechanism underlying planar cell polarity orientation in *Drosophila*

MASAKAZU YAMAZAKI[1, 2]
Tomonori Ayukawa[1, 2], Masakazu Akiyama[3], Jennifer L. Mummery-Widmer[4], Thomas Stoeger[4], Junko Sasaki[5], Juergen A. Knoblich[4], Haruki Senoo[1] and Takehiko Sasaki[2, 5]

[1]Department of Cell Biology and Morphology, Akita University Graduate School of Medicine, Japan
[2]Research Center for Biosignal, Akita University, Japan
[3]Research Institute for Electronic Science, Hokkaido University, Japan
[4]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Austria
[5]Department of Medical Biology, Akita University Graduate School of Medicine, Japan

yamazaki@med.akita-u.ac.jp

How the polarity of individual cells becomes aligned along a global axis within a tissue is a central question in developmental biology. In Drosophila, planar cell polarity (PCP) molecules such as Dachsous (Ds) and Four-jointed (Fj) may function as global directional cues orienting cellular asymmetry, which is manifested in the polarized localization of PCP core proteins such as Frizzled (Fz). However, the relationship between the Ds/Fj gradients and Fz asymmetry in the eye is opposite to that in the wing, thereby causing controversy about how these two systems are connected.

Here, we show that this relationship is determined by the ratio of two Prickle (Pk) isoforms, Pk and Spiny-legs (Sple). Pk and Sple have antagonistic functions and form different complexes with distinct subcellular localizations. In wings where a Pk:Sple ratio representative of the eye was artificially created, Sple-Ds cooperation polarized Sple at the cell edge exhibiting the highest Ds level, leading to a reversal of PCP orientation. A mathematical model was used to demonstrate that Sple is the key regulator connecting the Ds/Fj gradients and the PCP core proteins. Our model may explain the previously noted discrepancies in terms of variation in the relative amounts of Sple in the eye and wing.

Periodic actomyosin contractility contributes to convergence movements in zebrafish neurulation

MAKOTO SUZUKI
Naoto Ueno

Division of Morphogenesis, National Institute for Basic Biology, Japan

msuzuki@nibb.ac.jp

Convergence is a mode of tissue/cellular movements during embryogenesis that narrow body/organ shapes along specific directions, yet the dynamics of how individual cells behave during this process is not fully understood. We analyzed zebrafish neurulation at a single cell resolution using cell transplantation and live-cell imaging techniques. We found that progenitor cells of the neural tube showed non-muscle myosin II-dependent asynchronous and periodic movements, with a period of approximately 60 sec. We also found that F-actin underwent an isotropic, periodic remodeling in cell cortex, whereas F-actin in peripheral cellular protrusions showed directional constant movements. Similarly, the non-muscle myosin II partially colocalized with F-actin during periodic accumulation along the cell cortex. Careful examination revealed that the F-actin remodeling was temporarily correlated with a deformation of lateral end of the cell, suggesting that the periodic actomyosin contractility contributes to the convergence movements via transient tail retraction. We further found that planar cell polarity (PCP) proteins Vang-like 2 and RhoA were required for the periodic F-actin remodeling, suggesting that a PCP/noncanonical Wnt pathway controls the periodicity of the actomyosin. Our findings unveil a repertoire of the cellular movements based on the periodic actomyosin contractility that contributes to the convergence movements of multicellular organisms.

Matrix metalloproteinase regulation by Hox protein controlling sexually dimorphic morphogenesis in *Drosophila*

EMI MAEKAWA[1]
Aimi Tsukioka[1], Ayako Isomura[1], Kazuyo Misaki[2], Shigenobu Yonemura[2], Erina Kuranaga[1]

[1]Laboratory for Histogenetic Dynamics, RIKEN CDB, Japan
[2]Electron Microscope Laboratory, RIKEN CDB, Japan

maekawa@cdb.riken.jp

During *Drosophila* development, male-specific looping morphogenesis, in which genitalia performs a 360° clockwise rotation, contributes to the looping of the dextral ejaculatory duct. Our previous image analysis studies indicated that the collective movement of epithelial cells in the A8 segment that surrounded male genitalia was involved in genitalia rotation. To elucidate the mechanisms of genitalia rotation, we performed RNAi screening in the A8 segment, which revealed that matrix metalloproteinases (MMPs) were required for genitalia rotation. Inhibition of MMPs in the male A8 segment resulted in a high-density extracellular matrix (ECM), while ECM became sparse in the A8 of wild-type flies. These results suggest that MMPs may contribute to genitalia rotation through ECM regulation. We focused on the Hox protein, Abdominal-B (Abd-B) as a candidate A8-specific regulator of MMPs. Knock down of Abd-B in the A8 segment led to the ECM remaining intact, reduction of *Mmp1* expression, which resulted in a rotational defect. Taken together, these data demonstrate that Abd-B contributes to MMP regulation of the ECM in A8, which is required for genitalia rotation in development. Furthermore, we found that *Mmp1* gene expression in male A8 was higher than in female A8. We thus propose that sexually dimorphic regulation of the ECM by Hox protein induces looping morphogenesis.

Engineering approaches toward estimation of stress and strain distributions in *Xenopus laevis* embryos

TAKEO MATSUMOTO

Biomechanics Laboratory, Nagoya Institute of Technology, Japan

takeo@nitech.ac.jp

The effect of mechanical factors, such as stress and strain, during morphogenesis is attracting rising attention. To reveal stress distribution in an embryo, we need to know its stress-free configuration, i.e., its shape when all external and internal loads are removed. We also need to know its mechanical properties such as the distribution of Young's modulus. The stress distribution required to restore a stress-free configuration similar to that in the embryo is regarded as in situ stress distribution. Fresh embryonic tissue at the gastrula stage was cut in half, and the surface topography of the cut surface was observed to obtain the stress-free configuration. To reduce artifacts caused by gradual deformation of fresh embryos after they were cut, measurements were completed within 45 s. Young's modulus was measured on the cut surface at multiple sites simultaneously by an indentation test with particles sprayed on the surface. We found that the ectoderm was in tension whereas the meso-endoderm was in compression with the height difference of several 100 µm. Young's modulus was in the range of 10-40 Pa. Strain in the fresh embryo was estimated to be in the order of 0.1, and thus stress was estimated to be several Pa. Stress and strain distributions will be obtained by combining these data with finite element analyses.

Session VI - Lecture 24
4 December 2014, 12:00 - 12:30

Quantifying and modeling forces and stress in a developing tissue

SHUJI ISHIHARA[1]
Kaoru Sugimura[2]

[1]Department of Physics, Meiji University, Japan
[2]iCeMS, Kyoto University, Japan

shuji@meiji.ac.jp

During morphogenesis, the shape of a tissue emerges from collective cellular behaviors, which are in part regulated by mechanical and biochemical interactions between cells. Quantification of force and stress is therefore necessary to identify the underlying physical principles required to regulate morphogenesis. However, performing direct and non-invasive force-measurement has been technically challenging. In this study, by using the framework of Bayesian statistics, we developed a novel method to infer unobservable forces and stress from observed patterns of epithelial cell shapes and their connectivity [1]. Bayesian force inference provides us with space-time maps of forces and stress in a whole tissue in a non-invasive manner, unlike existing methods. Comparative tests of the inference method with artificial data, laser ablation experiments, and other methods proved that our Bayesian force inference performs well in terms of accuracy and robustness[2, 3].

We used our newly developed force inference method to understand regulation of epithelial development. As the first step in this research direction, we studied a physical mechanism that controls the packing of cells into a honeycomb pattern. By combining mechanical and genetic perturbations along with live imaging and Bayesian force inference, we identified the mechanism through which the mechanical anisotropy in a tissue promotes ordering in cell packing geometry[4].

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Session VII
Cell division and growth control

Mechanical control of cell division orientation in epithelial tissue

SARAH WOOLNER

Wellcome Trust Centre for Cell Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Sarah.Woolner@manchester.ac.uk

The orientation of cell division, determined by the mitotic spindle, must be carefully controlled in epithelia in order to generate shape, maintain tissue organisation and regulate cell fate. Defective spindle orientation leads to failures in organogenesis and morphogenesis and has been associated with cancer. We are investigating the intra- and extracellular forces that position the mitotic spindle in epithelia during embryonic development. We have found that antagonistic microtubule and actomyosin forces function to hold the spindle in place during symmetric divisions. We speculate that this mechanism provides more flexibility than a model based on tethering the spindle to a specific cortical landmark, allowing spindle orientation to be quickly adapted to changes in the tissue environment. We are now investigating how these intracellular mechanisms are linked to the external environment of the tissue. In particular, we are testing how tissue tension provides a cue for spindle orientation.

Cell competition that regulates epithelial homeostasis in *Drosophila*

SHIZUE OHSAWA[1]
Kei Kunimasa[1], Masatoshi Yamamoto[1], and Tatsushi Igaki[1, 2]

[1]Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Japan
[2]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Japan

ohsawa@lif.kyoto-u.ac.jp

Cell competition is a form of cell-cell interaction in which cells with higher fitness (“winners”) eliminate cells with lower fitness (“losers”) in multicellular communities. It has been suggested that cell competition could function as a buffering system against genetic or environmental perturbation, thereby contributing to organ size control and tissue homeostasis. In *Drosophila* developing imaginal epithelia, clones of cells mutant for evolutionally conserved apico-basal polarity genes such as *scribble (scrib)* and *discs large (dlg)* are eliminated from the tissue by cell competition. To dissect the mechanism of cell competition that eliminates polarity-deficient cells, we conducted a “non-cell autonomous” genetic screen in the eye imaginal disc. Wild-type “winner” cells surrounding polarity-deficient “loser” cells were mutagenized by EMS and mutations that modify the efficiency of cell competition were screened. We isolated a mutant *eld-4 (elimination-defective-4)*, which fails to eliminate polarity-deficient cells from the tissue. Our genetic analysis revealed that *eld-4* encodes a transmembrane protein that can act as a cell surface receptor. The mechanism by which *eld-4* regulates cell competition between normal cells and polarity-deficient cells will be presented.

The novel membrane structure capturing centrosome determines the orientation of cell division

TAKEFUMI NEGISHI[1,4]
Naoyuki Miyazaki[2], Kazuyoshi Murata[2], Hitoyoshi Yasuo[3] and Naoto Ueno[1]

[1]National Institute for Basic Biology, Division of Morphogenesis, Okazaki, Japan
[2]National Institute for Physiological Science, Electron microscopy group, Okazaki, Japan
[3]l'Observatoire Océanologique de Villefranche-sur-Mer, Villefranche-sur-mer, France
[4]Japan Society for the promotion of science, Tokyo, Japan

negishi@nibb.ac.jp

The orientation of the mitotic spindle has been proposed to control cell fate choices and tissue morphogenesis and architecture, thus playing an important role in shaping embryonic forms. It is already reported that in the ascidian epidermis almost all cells divide along the anterior posterior (A-P) axis at the last division.

Using live cell imaging, we found a novel membrane structure invaginating with A-P polarity directed towards the centrosome in the last cell division cycle of the epidermis. Several observations indicated that this membrane invagination occurred due to mechanical tension, which was generated between the centrosome and plasma membrane. During cell cycle progression, the length of invagination became shorter and the centrosome was pulled to the posterior side. Thus, I hypothesize that these membrane invaginations are involved in orientation of the mitotic spindle along the A-P axis. We would like to propose a novel template of spindle orientation with membrane invagination.

PLST-1 is essential for cortical contractility during early *C. elegans* embryogenesis

DING WEI YUNG[1]
Ronen Zaidel-Bar[1, 2]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biomedical Engineering, National University of Singapore, Singapore

dwy@nus.edu.sg

Cortical contractility, driven by an actomyosin network underlying the plasma membrane, is responsible for the control of cell shape and the generation of contractile forces that drive polarization and cell division. Contractility depends on myosin activity as well as the organization of filamentous actin, which is determined by the concerted action of a multitude of actin binding proteins. Plastin/fimbrin is an evolutionary-conserved actin-bundling protein with two tandem repeats of calponin homology (CH) domains. To examine the role of *C. elegans* plastin, PLST-1, in early embryogenesis, we characterized the *plst-1(tm4255)* allele, a deletion that abrogates the third and fourth CH domains.

We found in early *plst-1(tm4255)* embryos a severe attenuation of cortical ruffling and complete absence of pseudocleavage. During polarization, the anterior cortical flow speed was more than two-fold slower in the mutant, resulting in only partial establishment of polarity. Subsequent maintenance of polarity was also defective, as manifested by a distinctively slanted anterior cap. We also found that PLST-1 is required for two highly asymmetric cell division events: the second polar body extrusion and the first cytokinesis. 85% of mutant embryos exhibited a delay in cytokinesis initiation, while 15% failed to initiate or failed to complete cytokinesis. Interestingly, following the delay in initiation, the subsequent furrowing speed was similar to that of wild-type. A careful evaluation of cortical NMY-2 at anaphase onset revealed that PLST-1 is essential for the proper cortical arrangement of NMY-2 into a furrowing band. On the other hand, partially compromising NMY-2 function in a *plst-1(tm4255);nmy-2(ne3409)* double mutant (at 20˚C) lead to complete failure in initiation of furrowing, implying that PLST-1 and NMY-2 function in parallel to modulate the cortical cytoskeleton to initiate cytokinesis. Current work, employing high-resolution imaging along with genetic and optical perturbations, is aimed at elucidating the molecular mechanism by which PLST-1 modulates the actomyosin network.

PLST-1 is unique among eukaryotes as it contains only three repeats of CH domain, challenging the notion that tandem CH domains are required to form a fully functional F-actin binding domain. We will employ biochemical assays as well as electron microscopy in order to characterize the interaction between PLST-1 and actin and elucidate its capability to bundle F-actin.

Getting in shape: *in vivo* and *in silico* studies of tissue mechanics in growth control

YANLAN MAO

MRC Laboratory for Molecular Cell Biology, University College London, United Kingdom

y.mao@ucl.ac.uk

How tissue size and shape are controlled is a fundamental biological question that remains remarkably ill understood. Tissues can grow in a particular direction by controlling the orientation of cell divisions. Using a combination of genetics, live imaging, experimental biophysics and computational modeling, we show that tissue mechanical forces can have an instrumental role in controlling cell shape patterns and cell division orientations. The first part of the presentation will discuss how planar polarization of an atypical myosin, Dachs, can polarize junctional tension to orient cell divisions, while the second part will focus on how differential proliferation rates can generate global patterns of mechanical tension to orient tissue growth in a self-perpetuating and self-organizing manner. Novel techniques for automated image analysis, and for mechanically manipulating tissues, will be discussed.

Tissue growth regulation by “cell turnover” in *Drosophila*

NANAMI AKAI[1,2]
Shizue Ohsawa[1], Tatsushi Igaki[1, 3]

[1]Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Japan
[2]Division of Genetics, Kobe University Graduate School of Medicine, Japan
[3]PRESTO, JST, Japan

nanami_akai@stu.kobe-u.ac.jp

Development of multicellular organisms typically results in a stereotypical, normal phenotype, even in the presence of various perturbations such as genetic mutations and environmental fluctuations. However, the mechanism of how such a buffering system works is poorly understood. We have carefully analyzed the developmental processes of the *Drosophila Minute* mutants, which exhibit a developmental delay during their larval period but finally produce normal flies without any significant defects in tissue/organ patterning. We found that both cell death and cell proliferation (“cell turnover”) are significantly increased in *Minute* wing imaginal discs. Suppression of cell death in *Minute* wing imaginal discs causes morphological defects in adult wings, indicating that increased “cell turnover” is essential for normal organogenesis in *Minute* wing. Furthermore, we found that the developmental delay in the larval period triggers “cell turnover” in wing imaginal discs. The molecular mechanism of “cell turnover” in *Minute* wing discs will be discussed.

Dynein light intermediate chains maintain spindle bipolarity by functioning in centriole cohesion

VIKI ALLAN
Laura A. Jones, Cécile Villemant, Toby Starborg, Anna Salter, Georgina Goddard, Peter Ruane, Philip G. Woodman, Nancy Papalopulu, Sarah Woolner and Victoria J. Allan

Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom

viki.allan@manchester.ac.uk

Cytoplasmic Dynein-1 (dynein) is a minus-end-directed microtubule motor protein with many cellular functions, including during cell division. The role of the light intermediate chains (LICs; DYNC1LI1 and 2) within the complex is poorly understood. Here, we have used siRNAs or morpholino oligonucleotides to deplete the LICs in human cell lines and Xenopus laevis early embryos to dissect the LICs' role in cell division. We show that although dynein lacking LICs drives microtubule gliding at normal rates, the LICs are required for the formation and maintenance of a bipolar spindle. Multipolar spindles with poles that contain single centrioles were formed in cells lacking LICs, indicating that they are needed for maintaining centrosome integrity. The formation of multipolar spindles via centrosome splitting following LIC depletion could be rescued by inhibiting Eg5. This suggests a novel role for the dynein complex, counteracted by Eg5, in the maintenance of centriole cohesion during mitosis.

Poster Presentation Abstracts

Nanoscale integrin clusters stabilized by Talin function are the fundamental units of cell-matrix adhesions

RISHITA CHANGEDE[1]
Felix Margadant[1, 2] and Michael P Sheetz[1, 2]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, Columbia University, New York, USA

rishitac@nus.edu.sg

Clustering of matrix-activated integrins is a key step in the formation of matrix adhesions but the molecular basis of this cluster formation is poorly understood. To analyze the cellular but not the matrix factors controlling clustering, we used supported lipid bilayers (SBL) with fluid, lipid-linked RGD ligands. Fibroblasts bound to the fluid RGD surfaces and formed integrin clusters that were motile. These clusters were analyzed at the nanometer scale using photoactivated light microscopy (PALM). Clusters of ~100nm in diameter were observed, with ~40 integrin molecules per cluster. Surprisingly, similar clusters formed on RGD-glass surfaces, indicating that integrins cluster around activated integrins as a key first step to formation of adhesions. Actin plays an important role in organization of these clusters. Furthermore, depletion of Talin1 and 2 function dramatically inhibited cluster formation; but expression of either Talin head or rod domains restored nearly normal clusters. Upon analysis, talin rod clusters depended upon actin filament organization whereas talin head clusters displayed active movements. With PALM microscopy, we find that the early integrin adhesions form from 100 nm clusters of activated integrins; and clusters depend upon talin head and/or rod but are independent of ligand mobility or external traction forces.

Regulating cell spreading through a signaling scaffold BNIP-2

TI WENG CHEW[2]
Lim Gim Keat, Kenny[1, 2,] Leong Man Chun[2, 3,] Soh Hui Ming, Gary[2,] Vedula Sri Ram Krishna[2, 3,] Anon Ester[2, 4,] Lim Chwee Teck[2, 3,] Ladoux Benoit[2, 4] and Low Boon Chuan[1, 2]

[1]Department of Biological Sciences, National University of Singapore, Singapore
[2]Mechanobiology Institute, National University of Singapore, Singapore
[3]Division of Bioengineering and Department of Mechanical Engineering, National University of Singapore, Singapore
[4]Laboratoire Matière et Systèmes Complexes (MSC), UMR CNRS 7057 and Université Paris Diderot, Paris, France

mbichew@nus.edu.sg

Cell attachment to extracellular matrix and the spreading of cell membrane are key steps leading to cell migration. Rho GTPases, molecular switches, tightly control these processes via their regulation on the cytoskeletal networks in a precise spatio-temporal manner. BNIP-2, a scaffold protein, has been identified as a regulator of Rho GTPase activity during cell protrusion and muscle differentiation. However, its function in regulating cell spreading remains elusive. Using bioimaging, force-measurement on micropillars, pharmacological inhibition and biochemical assays, we demonstrate that the ectopic expression of BNIP-2 in MDCK cells exerts greater traction force and impairs cell spreading in a substrate dependent manner. Interestingly, collagen coating abates BNIP-2 mediated retarded cell spreading, but fibronectin does not. This suggests that during the process of cell spreading, BNIP-2's scaffold function is modulated by the integrin/FAK/Src signalling cascade. The significance of this will be discussed.

F-actin dependent self-generation of chemoattractant gradient in *Dictyostelium*

FUMIHITO FUKUJIN[1, 4]
Satoshi Sawai[1, 3, 4]

[1]Graduate School of Arts and Science, University of Tokyo, Japan
[2]Research Fellow of Japan Society for the Promotion of Science, Japan
[3]Research Center for Complex Systems Biology, University of Tokyo, Japan
[4]PRESTO, Japan Science and Technology Agency, Japan

ffukujin@physbio.c.u-tokyo.ac.jp

In *Dictyostelium discoideum* amoebae, cAMP binding induces a signal transduction pathway that leads to further synthesis and secretion of cAMP. This cAMP relay response becomes synchronized and gives rise to propagating waves of extracellular cAMP that direct chemotactic cell aggregation. Both the cAMP relay and the chemotactic response are under the control of G-protein coupled receptor mediated Ras/PI3K signaling, thus these two events are expected to be finely coordinated with possible crosstalk. Here, by combining microfluidics and quantitative live-cell imaging of cytosolic cAMP, we show that in cells treated with the actin polymerization inhibitor Latrunculin A, the key features of cAMP-induced elevation of cytosolic cAMP at the single cell level, the amplitude and duration were severely impaired. The amplitude was most severely affected at nanomolar cAMP stimulus consistent with the observed increase in the period of cAMP waves in the LatA treated cell populations. Moreover, the persistent elevation of cytosolic cAMP observed under high cAMP stimulus became more attenuated in the presence of LatA. Simultaneous visualization of intracellular cAMP and F-actin revealed that changes in the amount of cortical F-actin under some conditions were closely correlated with cytosolic cAMP changes. From these results we suggest that in *Dictyostelium* cell aggregation, the timing of the cAMP gradient formation is dictated strictly by the state of actin polymerization.

Protrusive waves guide 3D cell migration along nanofibers

CHARLOTTE GUETTA-TERRIER[1]
Hongyan Long[2], Pascale Monzo[1], Chew Sing Yian[2], Alexander Mogilner[3], Benoit Ladoux[1, 4,] Nils C. Gauthier[1]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Division of Chemical and Biomolecular Engineering, Nanyang Technological University (NTU), Singapore
[3]Department of Neurobiology, Physiology and Behavior and Department of Mathematics, University of California, Davis, Davis, California, United States of America
[4]Institut Jacques Monod, CNRS UMR 7592 & Université Paris Diderot, Paris, France

terrier.cha@gmail.com

While the mechanisms governing cell migration on two-dimensional flat surfaces have been extensively studied, our knowledge of cell protrusion in fiber-rich three-dimensional environments in which cells operate *in vivo* remains poor. By adapting the technique of 3D electrospun fibers to the study of single cell dynamics, we report that during cell migration along nanofibers, cells exhibit unexpected modes of migration, with highly persistent movements characterized by laterally diffusing waves, switching occasionally to non-persistent movements driven by lobopodia formation. Furthermore, we show that the highly persistent mode of migration relies on protrusive activities that depend on Rac1 and ARP2/3 activation. Computational models confirm our experimental data and show that the formation of lateral propagating waves relies on competition between actin polymerization and local plasma membrane tension explaining the high persistence observed on the fibrillar system. Based on our experiments and the numerical simulations, we propose that motility modules based on actin polymerization mechanisms distinct from the ones usually described for 2D migration lead to different modes of cell migration in fibrillar 3D environments.

Probing the cell-cell junctional tension at different states of cell oscillation

YUSUKE HARA[1, 2]
Su Ee Tan[1], Murat Shagarov[1], and Yusuke Toyama[1, 2, 3]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Temasek Life Sciences Laboratory, National University of Singapore, Singapore
[3]Department of Biological Sciences, National University of Singapore, Singapore

mbiyhara@nus.edu.sg

Understanding collective tissue level dynamics requires knowledge of the cellular level mechanics that evolve in time and in space. We aim to address how the robust patterns of tissue dynamics emerge from stochastic cell oscillation and heterogeneous cell behaviors during development. In this study, we use dorsal closure during *Drosophila* embryogenesis as a model system. During dorsal closure, two cell sheets of the lateral epidermis demarcate an eye shaped opening on the dorsal side of the embryo, exposing the amnioserosa. The amnioserosa exhibit reversible and uncoordinated cell shape oscillations (1 cycle = 2-4 mins) in early stage of dorsal closure. It has been shown that the area reduction of the amnioserosa cells generates mechanical tension to enforce the closure of lateral epidermis, indicating that tissue tension of amnioserosa is one of the key mechanical factors supporting dorsal closure. However, it remains an open question as to how stochastic cell oscillation emerges as a tissue level tension that promotes robust tissue dynamics during dorsal closure. The state of the mechanical tension at the cell-cell junction during cell oscillation needs to be probed in detail.

We probed tension at the cell-cell junction in the amnioserosa cell at the specific timing of cell oscillation. More specifically, we severed the cell-cell junctions by UV laser when the junction was contracting, expanding, or in-between (i.e., stable) and measured the recoil velocity of intercellular vertices.

Our results indicated that the cell boundary tension highly depends on the dynamics and morphology of the cell-cell junction. Deformation speed of cell boundary correlates with the junctional tension; the contracting boundary associated with high tension, on the other hand, stable or expanding boundary showed lower tension. Moreover, cell boundaries with non-straight (i.e., winding edge) boundary exhibited slower recoil velocity than straight boundaries, indicating the straightness of boundary is important factor for force transmission. We also found good correlations between the cell boundary tension, the cell behavior, and the spatial distribution of non-muscle myosin II. Based on these actual measurements and correlations, we are trying to estimate the tension at cell boundaries in a tissue without destructive measurement. We will discuss how stochastic cell oscillations contribute to a robust pattern of tissue dynamics.

Tension-dependent ERK activation on actin stress fibers

HIROAKI HIRATA[1]
Mukund Gupta[1], Sri Ram Krishna Vedula[1], Chwee Teck Lim[1, 2], Benoit Ladoux[1, 3] and Masahiro Sokabe[1, 4]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biomedical Engineering and Department of Mechanical Engineering, National University of Singapore, Singapore
[3]Institut Jacques Monod (IJM), CNRS UMR 7592 and Université Paris Diderot, Paris, France
[4]Mechanobiology Laboratory, Nagoya University Graduate School of Medicine, Nagoya, Japan

mbihh@nus.edu.sg

Tensile force generation by stress fibers drives signal transduction events at focal adhesions. Here, we report that stress fibers *per se* act as a platform for tension-induced activation of biochemical signal. The MAP kinase, ERK, is activated on stress fibers in a myosin II-dependent manner. In myosin II-inhibited cells, uniaxial stretching of cell adhesion substrates restores ERK activation on stress fibers. By quantifying myosin II- or mechanical stretch-mediated tensile forces in individual stress fibers, we show that ERK activation on stress fibers correlates positively with tensile forces acting on the fibers, indicating stress fibers act as a tension sensor in ERK activation. Myosin II-dependent ERK activation is also observed on actomyosin bundles connecting E-cadherin clusters, suggesting that actomyosin bundles in general work as a platform for tension-dependent ERK activation.

Transcriptome analysis to identify genes responding to mechanical force in developing *Drosophila* embryos

TOMOKI ISHIBASHI[1]
Shuji Shigenobu[2], Kenji Matsuno[1]

[1]Department of Biological Science, Osaka University, Japan
[2]National Institute for Basic Biology, Japan

ishibaki@bio.sci.osaka-u.ac.jp

Mechanical forces are involved in various biological processes. Especially during development, cells are subjected to dynamic mechanical force. Cells growing in a limited area push and pull their neighboring cells, which may change the shape of the tissues that these cells develop into. Recently, it has been reported that mechanical force also modulates cell behaviors such as polarization, cell migration, cell growth, and differentiation. However, most of these studies rely on cultured cells. Therefore, it is largely unknown what molecules are involved in generation and reception of mechanical force in developmental events.

In order to reveal these issues, we performed RNA-seq transcriptome analysis to identify genes that were induced by mechanical force in *D. melanogaster* embryos. First, we constructed the mechanical force application system. We confirmed that the compression during the whole embryogenesis perturbed development, however the compression for 30 minutes did not affect the hatching rate. Next, we checked the global stress response to the compression. We confirmed that ER-stress response was not affected by the compression for 30 minutes.

Here, we will describe our experimental system and discuss our future directions.

Physical characterization of collective rotational dynamics in epithelial tissues

SHREYANSH JAIN[1]
Philippe Marcq[2], Chwee Teck Lim[1, 3] and Benoit Ladoux[1, 4]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Physico-Chimie Curie, Institut Curie, CNRS, Universite Pierre et Marie Curie, France
[3]Division of Bioengineering and Department of Mechanical Engineering, National University of Singapore, Singapore
[4]Institut Jacques Monod (IJM), CNRS UMR 7592 and Université Paris Diderot, Paris 75013, France

jain.shreyansh@nus.edu.sg

Cell migration plays a pivotal role in regulating numerous biological processes under both physiological as well as pathological conditions. Some cells are known to exhibit collective behaviour. This refers to the emergence of complex migration patterns over scales larger than those of the individual elements constituting a system. Collective behaviour plays a major role in biological systems in regulating various processes such as gastrulation, morphogenesis, wound closures, cancer metastasis and tissue organization. Collective cell behaviours depend upon different geometrical shapes and confinements. Thus the use of various geometries may help us to understand the emergent properties of collective cell dynamics.

Here we used microfabricated and micromechanical tools to study the behaviour of epithelial MDCK cells within fibronectin-micropatterned rings whose dimensions can be varied. These 'racetracks' allow for periodic boundary conditions. By confining cells in racetracks with periodic boundaries, we observed large scale coordinated cell movements. After reaching confluency, cells rotated in a particular direction over tens of hours. Taking into account the viscoelastic, active and polar properties of cells, we aim to compare our experimental data with theoretical modeling based on active gels.

Mechanical regulation in epithelia growth to control tissue overcrowding

LEYLA KOCGOZLU
Thuan Beng Saw, Murat Shagirov, Eunice Law, Yusuke Toyama and Benoit Ladoux

Mechanobiology Institute, National University of Singapore, Singapore

mbilk@nus.edu.sg

Control of tissue growth is a major key in maintaining the protective barrier provide by the epithelium. The formation of adhesive contacts supported mainly by E-cadherin molecules plays a critical role for tissue cohesion. Keeping a balance between cell division and cell extrusion rates is critical to preserve tissue integrity. When this balance is unregulated it can lead to uncontrolled proliferation and tumorigenesis (Farhadifar et al., 2007; Reinsch and Karsenti, 1994). Apoptotic cell extrusion is a process guided by the dying cell itself, which activates an actomyosin contraction from neighboring cells to drive its own extrusion in epithelia (Rosenblatt et al., 2001; Slattum et al., 2009). However, the mechanical regulation of cell extrusion during tissue growth is poorly understood. Here, we show that the tissue dynamics reside in the ability to remodel cell junctions. This remodeling is a key player during the cell extrusion process, which is characterized with a mechanical signature provided by a large scale contraction, or local contraction of the tissue according to the compaction level of the tissue. The crowding step induces both apoptotic and live cell extrusion whereas during the overcrowding step we observe an increase of live cell extrusion. Our results demonstrate that the tissue dynamics leads to different mechanics of extrusion due to the compaction of the cells and the pressure inside the tissue. During tissue growth lamellipodia protrusion is involved during cell extrusion whereas at high cell density the extrusion process requires only the purse-string mechanism.

Patterns of calcium activities in rat osteoblast-like cells under fluidic shear stress

IMSHIK LEE[1, 2]
Guixian Meng[1], Leiting Pan[2]

[1]School of Physics, Nankai University, Tianjin 300071, China
[2]The Key Laboratory of Weak-Light Nonlinear Photonics, Ministry of Education, TEDA Applied Physics Institute, Nankai University, Tianjin 300457, China

ilee@nankai.edu.cn

It is known that bone can adapt to mechanical environments by remodeling. Osteoblasts, which play critical role in bone remodeling, are believed to be sensitive to physical stimulation. Calcium signaling, one of the early responses to mechanical forces, is a diverse and colorful regulator of various physiological activities. In our work, we found multiple and diverse patterns of calcium responses induced by fluidic shear stress in rat osteoblast-like cells. The homogeneous and synchronous calcium responses generally display two distinct phases: (i) an early peak phase and (ii) a following plateau phase. Multiple and heterogeneous calcium oscillations with different frequencies also existed. Here, we summarized the characteristics of homogeneous calcium responses and heterogeneous calcium oscillations under repetitive stimulations, with a modified theoretical model for the hypothesis of FSS-induced diversity of calcium activities.

Exploring the limit: the robustness landscape of embryonic PAR polarity

YEN WEI LIM[1]
Fumio Motegi[1, 2, 3]

[1]Temasek Lifesciences Laboratory, National University of Singapore, Singapore
[2]Mechanobiology Institute, National University of Singapore, Singapore
[3]Department of Biological Sciences, National University of Singapore, Singapore

yenwei@tll.org.sg

Cell polarization is critical for proper cell function and architectures. A hallmark of cell polarity in animal cells is the asymmetric distribution of partition defective (PAR) proteins into two distinct domains on the cell cortex. Segregation of PARs relies on self-regulation through reciprocal cortical exclusion between two groups of PAR proteins, anterior PARs (aPAR) and posterior PARs (pPAR). The self-organizing PAR system dynamically shapes the cellular asymmetry with high accuracy in space and time, but the general principles that secures the robustness and adaptability of PAR polarity in diverse cell types remains elusive.

Here, we explore to map the robustness landscape of PAR polarization topologies, by identifying parameter variations in PAR protein levels capable of driving functional cellular asymmetry in one-cell stage *C. elegans* zygote. We systematically modified levels of aPAR (PAR-6) and pPAR (PAR-2) by a combination of tuned expression of transgenes, a mutation in the endogenous par gene, and a second mutation that increases and decreases PAR-6 level (lgl-1 and nos-3, respectively). Asymmetric PAR segregation is highly stable under a wide range of changes in relative PAR-6/PAR-2 levels: 1) mutual segregation of aPAR/pPAR was extensively robust over wide ranges of higher PAR-6/lower PAR-2 conditions with non-linear changes in aPAR/pPAR boundary position. 2) In contrast, lower PAR-6/higher PAR-2 conditions caused sharp loss of mutual exclusion but remained to keep asymmetric aPAR domain with homogeneous pPAR distribution. Quantitative measurement of PAR behaviors in zygotes where aPAR/pPAR ratio was off-balance showed remarkable modification of cortical PAR-6 concentration in response to different PAR-2 levels, suggesting a dynamic balance of cortical PAR levels that compensate aPAR/pPAR unbalance. We will further discuss potential mechanisms that allow PAR system to produce robust polarization with tolerance to relative PAR level variations.

Mechanical stress induced muscle cell differentiation and adapts lipid metabolism to energy demand

KOTA MIYASAKA
Eriko Sato, Toshihiko Ogura

Department of Developmental Neurobiology, IDAC, Tohoku University, Japan

k.miyasako@idac.tohoku.ac.jp

Exercise is an effective remedy for obesity because it enhances the muscle mass and promotes lipolysis. However, it is not fully understood why exercises that mechanically stress muscles can lead to an improvement in muscle mass and function. We hypothesize that mechanical stress affects muscle differentiation and lipid metabolism. To investigate this, we applied stretching stimuli to mouse myoblast C2C12 cells. In stretched cells, the expression of muscle differentiation markers was increased compared to non-stretched cells. This result shows that physical stimuli can induce muscle differentiation. To explore the role of transcription factors related to mechano-dependent muscle differentiation, we focused on the function of MRTF-B. Although MRTF-B localized to the cytoplasm in static conditions, we found MRTF-B translocated to nucleus after stretching. Therefore, we hypothesized that MRTF-B functions as the mechano-sensor. As expected, when we knocked down MRTF-B in C2C12 cells, mechano-dependent muscle cell differentiation was inhibited.

Interestingly, using RNA-sequencing, we identified that expression of lipid metabolism-related genes was also increased in the stretched condition. Furthermore, we showed MRTF-B elevates ERR promoter activity *in vitro*. From ChIP assay, we discovered that MRTF-B was recruited to the promoter of ERR target genes in a mechano-dependent manner. These lines of evidence indicate that MRTF-B controls muscle cell differentiation and metabolic adaptation in response to stretching stimuli.

Mechanobiology for membrane potential-dependent neuronal axon guidance

YUICHI SAKUMURA[1, 2]
Kazushi Ikeda[3]

[1]School of Information Science and Technology, Aichi Prefectural University, Japan
[2]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan
[3]Graduate School of Information Science, Nara Institute of Science and Technology, Japan

sakumura@ist.aichi-pu.ac.jp

The neuronal growth cone guides an axon to an appropriate synaptic target, an essential process during nervous system development. The membrane potential is a downstream effector of the growth cone guidance cue; it modulates the growth cone turning direction in response to diffusible guidance molecules, such as Sema3A. For instance, a growth cone in a control condition exhibits repulsive response to a Sema3A gradient, but the repulsion is inverted to attraction if the membrane potential is depolarized. In this study we propose a mathematical model which describes how an electrical signal of membrane potential in a neuronal growth cone membrane controls the direction of growth cone turning. The model is composed of mechanical processes of actin retrograde flow, and focal adhesion between actin filaments and substratum, as well as calcium signaling. By incorporating random protrusion of the growth cone into our model, we show bidirectional growth cone guidance depending on the membrane potential.

Mechanism of cardiomyocytes differentiation induced by mechanical stretching

ERIKO SATO
Kota Miyaska and Toshihiko Ogura

Department of Developmental Neurobiology, IDAC, Tohoku University, Japan

e.sato@idac.tohoku.ac.jp

Recently, it has been reported that mechanical stress regulates homeostasis and cell differentiation. However, it is still unclear how conversion of physical stimuli into biochemical signaling leads to cellular reactions.

Previously, we focused on the mechanism of mechanical stress response in cardiomyocytes and hypothesized that mechanical stretching can induce cardiomyocyte differentiation from cardiomyoblasts. To investigate this, we applied mechanical stretching to cardiac fibroblasts. Surprisingly, stretched cardiac fibroblasts induced the expression of cardiomyocyte differentiation markers. To clarify what factor was responsible for mechanotransduction, we explored a factor that regulates mechano-dependent cellular differentiation by several screening assays. As a result we identified one transcriptional cofactor. Interestingly, this cofactor is normally located in the cytoplasm, but translocates into the nucleus and interacts with the transcription factors that regulate cardiomyocyte differentiation in mechanical stress loaded condition.

Usually, undifferentiated cardiomyocytes produce energy from glucose. However, mature cardiomyocytes use lipids as an energy source. This energetic shift is essential for cardiomyocyte maturation. We also revealed that muscle differentiation induced by mechanical stress caused such metabolic change. Stretched stimuli upregulated the expression of lipid oxidation pathway genes and downregulated that of glucose metabolic pathway genes. In this poster presentation, we are going to discuss the molecular mechanism of these cellular responses.

Primary septum synthesis in *S. pombe*: A search for new players

KRITI SETHI
Mayalagu Sevugan, Naweed I Naqvi and Mohan Balasubramanian

Temasek Life Sciences Laboratory, National University of Singapore, Singapore

kriti@tll.org.sg

Schizosaccharomyces pombe undergoes cytokinesis by constriction of a contractile ring and concomitant centripetal deposition of the division septum. The catalytic subunit of linear 1, 3-β-glucan synthase, *cps1*, is responsible for synthesis of the primary septum component of the division septum. The mutant, *cps1-191* is defective in primary septum synthesis at restrictive temperature. A screen for multi-copy suppression was performed to identify potential genes whose overexpression could rescue this mutant. The screen identified two essential genes, *smi1* and *SPBP22H7.03* (named *sbg1* – suppressor of beta glucan synthase 1). This study aims at characterizing these two gene products, their roles and the mechanism of rescue by these two genes.

Non-uniform Cell Junctional Tension Governs Anisotropic Apical Constriction of Delaminating *Drosophila* Neuroblasts

SUN ZIJUN[1]
Yusuke Toyama[1, 2, 3]

[1]Mechanobiology Institute, National University of Singapore, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore
[3]Temasek Life Sciences Laboratory, National University of Singapore, Singapore

zijun.sun89@nus.edu.sg

During embryogenesis, cell delamination is a conservative mechanism in tissue specification from vertebrates to invertebrates. It describes the physical separation wherein individual cells or groups of cells detach from epithelium to adopt distinct cell fates. In early *Drosophila* embryogenesis (stage 9-11), neural stem cells (neuroblasts) delamination from ectoderm is the first step to generate precursor neurons of central nervous system. Here, we studied the early event of neuroblast delamination process, the apical constriction.

We first quantify the apical constriction dynamics, including decline of surface area, degree of asymmetry in cell shape, and number of neighboring cells as a function of time. We found that the delaminating neuroblasts undergo anisotropic apical constriction as the cells decrease the apical area more significantly along anterior/posterior (A/P) axis compared with dorsal/ventral (D/V) axis. The cell junctions along D/V axis preferentially shrink instead of junctions along A/P axis, resulting in asymmetric apical constriction. Furthermore, our data show that the apical constriction has two distinct modes: uni- directional and bi-directional apical constriction, depending on the physical location of the neuroblast. In the first mode, delaminating neuroblasts at the interfaces between ectoderm and the mesoectoderm (midline cells) constrict towards the boundary adjacent with midline, showing uni-directional constriction. In the second mode, the delaminating neuroblasts that undergo bi-directional constriction are located away from the midline and constrict in all directions but more significantly along D/V axis. Based on the observation that the cell junctions at higher tension are less likely to be deformed, we hypothesized that the uneven local junctional tension distribution could mechanically constrain the cell-cell junction remodeling and leads to anisotropic apical constriction. To test this hypothesis, we measured the non-muscle myosin II (MyoII) accumulation and quantified the junctional tension at different locations by laser ablation. As expected, the tension at cell junctions correlates with MyoII accumulation, and the junctions that exhibit less deformation (i.e., D/V junctions and midline cell junctions) are at high tension and with higher accumulation of MyoII than A/P junctions. We will also discuss how non-uniform cell junctional tension governs anisotropic apical constriction of delaminating neuroblasts.

Dynamic cell movement during somite morphogenesis in zebrafish

HATSUMI SUZUKI
Kyohei Kunifuji, Atsuko Shimada and Hiroyuki Takeda

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan

hsuzuki@bs.s.u-tokyo.ac.jp

Most skeletal muscle and trunk bones are derived from the somite. Somites are round in shape when generated from the presomitic mesoderm, but soon elongate dorso-ventrally and finally form a V-shaped structure in fish, called the “chevron”. This change in morphology is dynamic and is complete within a few hours. What cellular mechanism underlies this process? To address this question, we have observed cell migration in detail in zebrafish somites. First, we took the time-lapse image of somites by confocal microscopy and recorded the movement of all somitic cells by tracking nuclear fluorescence. We then built a detailed map of surface data at certain time points.

Soon after segmentation, each somite elongates by 200-300% and their morphology changes into a chevron within 2-3 hours. The number of cells in a single somite is about 200, and the cell number and somite volume do not significantly change during this process. Cell tracking data demonstrated that somitic cells, especially in surface layers, undergo rotation so that cells near the notochord migrate out towards the lateral surface. We will discuss the importance of this rotational movement in dynamic somite morphogenesis.

Transient membrane localization of SPV-1 drives cyclical actomyosin contractions in the *C. elegans* spermatheca

PEI YI TAN[1]
Ronen Zaidel-Bar[1, 2]

[1]Mechanobiology Institute, National University of Singapore, Singapore, Singapore
[2]Department of Biomedical Engineering, National University of Singapore, Singapore

a0038688@nus.edu.sg

Actomyosin contractility is the major cellular force driving changes in cell and tissue shape. A principal regulator of contractility is the small GTPase RhoA. External mechanical forces have been shown to impact RhoA activity and cellular contractility. However, the mechanotransduction pathway from external forces to actomyosin contractility is poorly understood.

Here we show that in the *C. elegans* spermatheca, the F-BAR and RhoGAP protein SPV-1 functions to transduce physical cues from the plasma membrane into a biochemical signal controlling contractility. SPV-1 localizes through its F-BAR domain to the apical membrane of the relaxed spermatheca, where it inhibits RHO-1/RhoA activity through its RhoGAP domain. Oocyte entry forces the spermatheca cells to stretch and as a consequence SPV-1 detaches from the membrane, permitting RHO-1 activity to increase. The increase in RHO-1 activity facilitates spermatheca contraction and expulsion of the newly fertilized embryo into the uterus, leading to formation of membrane folds in the spermatheca, SPV-1 membrane localization, and initiation of a new cycle.

Our results demonstrate how transient membrane localization of a novel F-BAR domain, likely via specific binding to curved membranes, coupled to a RhoGAP domain, provides feedback between a mechanical signal and actomyosin contractility. We anticipate this to be a widely utilized feedback mechanism used to balance actomyosin forces in the face of externally applied forces, as well as intrinsic processes involving cell deformation, from single cell migration to tissue morphogenesis.

Stretching morphogenesis of the roof plate during late neurulation and mild neural tube defects

KORZH VLADIMIR[1, 2]

[1]Institute of Molecular and Cell Biology, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore

vlad@imcb.a-star.edu.sg

Neurulation is defined as a process of neural tube closure. Recent reports suggested that upon completion of this process the major driving forces of neurulation remain in force at least until the central canal of the neural tube is formed. Importantly, in addition to several well-established factors of neurulation, including an apical constriction, cell proliferation and build-up of axonal pathways, the mechanical role of the roof plate has been defined recently during conversion of the primitive lumen into central canal. Henceforth an idea is put forward to define the two periods of neurulation: early neurulation corresponds to a period of neural tube closure and late neurulation to a period of formation of central canal. These ideas will be discussed in a context of neural tube defects that affect late neurulation resulting in distention of central canal.

Rotating melanophores exhibit an intrinsic chirality

HIROAKI YAMANAKA
Shigeru Kondo

Graduate School of Frontier Biosciences, Osaka University, Japan

hiroyama@fbs.osaka-u.ac.jp

Cellular chirality has attracted attention as the origin of asymmetry in the animal body. In vertebrates, the unidirectional rotation of the cilia on the primitive node cells determines the left-right body axis. Recent studies reported that various types of cells showed left-right asymmetric cell arrangement in a micro-patterned culture system. Another study reported that neutrophil-like differentiated HL60 cells showed a strong tendency to move leftward when chemotaxis was invoked by uniform stimuli. However, because the left-right bias observed to date was subtle or was represented in the stable orientation of cells, it was difficult to identify the molecular mechanism underlying their intrinsic chirality. Here, we report that zebrafish melanophores exhibited counter-clockwise rotation *in vitro*. The direction of the rotation was not affected by the extracellular matrix, which suggested that the unidirectional cellular rotation reflected an inherent cellular chirality. Treatment with cytoskeletal inhibitors showed that the cellular rotation was generated by the actin cytoskeleton and was inhibited by the presence of microtubules. We observed the clockwise rotation of actin fibers by expressing Lifeact-GFP. The results suggested that the counter-clockwise cellular rotation was due to the counter-action to the rotation of the actin fibers. We assumed that similar mechanisms underlie the chirality of other types of cells. Therefore, we believe that zebrafish melanophores are a useful new system for future studies that will elucidate the chirality of other types of cells.

Direct determination of the stability of c-MYC promoter G-quadruplexes by single-molecule manipulation

YOU HUIJUAN[1]
Yan Jie[1, 2, 3]

[12]Department of Physics, National University of Singapore, Singapore
[3]Centre for Bioimaging Sciences, National University of Singapore, Singapore

mbiyouh@nus.edu.sg

DNA G-quadruplexes (G4) in the promoter region of oncogenes have drawn increasing attention for their involvement in transcription regulation and as a potential target for anticancer therapy. The G4 formed at the oncogene c-myc promoter region functions as a gene silencer with high stability. However, this stability has made direct measurement of its unfolding rates a challenge. Here, by using single-molecule manipulation, we measured the unfolding force distribution and folding-unfolding rate of single c-myc G4. From this, we characterized the stability of the wild-type G4 and several truncated or point mutants under physiological KCl buffer conditions. The wild type G4 can fold into multiple species with the major species exhibiting an unfolding force peak at ~50 pN, which corresponds to a slow unfolding rate of 2.7x10-6 s-1. As the c-myc G4 is prototypical for many other intermolecular parallel-stranded G4, our measurements provide important insights on the transcription repression mechanism of promoter G4.

Speaker Directory

YASUHIRO INOUE – LECTURE 2
Kyoto University
Email: inoue@frontierkyoto-u.ac.jp
Website: http://www.frontier.kyoto-u.ac.jp/bf05/membersHP/inoue/index-e.html

SHUJI ISHIHARA – LECTURE 24
Meiji University
Email: shuji@meiji.ac.jp
Website: http://chaos.c.u-tokyo.ac.jp/~shuji/index.html

SHIGERU KONDO – LECTURE 16
Osaka University
Email: skondo@fbs.osaka-u.ac.jp
Website: http://www.fbs.osaka-u.ac.jp/labs/skondo/indexE.html

ERINA KURANAGA – LECTURE 10
RIKEN Center of Developmental Biology
Email: kuranaga@cdb.riken.jp
Website: http://www.cdb.riken.jp/en/02_research/0202_creative27.html

CHWEE TECK LIM – LECTURE 9
Mechanobiology Institute, National University of Singapore
Email: ctlim@nus.edu.sg
Website: http://mbi.nus.edu.sg/lim-chwee-teck/

YANLAN MAO – LECTURE 27
University College, London
Email: y.mao@ucl.ac.uk
Website: http://www.ucl.ac.uk/lmcb/research-group/yanlan-mao-research-group

PAUL MATSUDAIRA – LECTURE 1
Mechanobiology Institute, National University of Singapore
Email: dbsmpt@nus.edu.sg
Website: http://mbi.nus.edu.sg/paul-matsudaira/

TAKEO MATSUMOTO – LECTURE 23
Nagoya Institute of Technology
Email: takeo@nitech.ac.jp
Website: http://researcher.nitech.ac.jp/html/90_en.html

KENJI MATSUNO – LECTURE 20
Osaka University
Email: kmatsuno@bio.sci.osaka-u.ac.jp
Website: http://www.bio.sci.osaka-u.ac.jp/bio_web/lab_page/matsuno/Etop.html

ISAO MATSUO – LECTURE 7
Osaka Medical Center for Maternal and Child Health
Email: imatsuo@mch.pref.osaka.jp
Website:http://www.mch.pref.osaka.jp/english/research/embryology/embryology01.html

ANN MILLER – LECTURE 11
University of Michigan
Email: annlm@umich.edu
Website: http://sites.lsa.umich.edu/miller-lab/

ANTONIA MONTEIRO – LECTURE 17

Yale NUS College, Singapore
National University of Singapore
Email: antonia.monteiro@nus.edu.sg
Website: http://www.yale-nus.edu.sg/about/faculty/antonia-monteiro/

FUMIO MOTEGI – LECTURE 21

Mechanobiology Institute, National University of Singapore
Email: fmotegi@tll.org.sg
Website: http://mbi.nus.edu.sg/fumio-motegi/

NAOMI NAKAYAMA – LECTURE 6

University of Edinburgh
Email: naomi.nakayama@ed.ac.uk
Website: http://www.ed.ac.uk/schools-departments/biology/molecular-plant-sciences/staff-facilities/staff-profiles?id=nnakayam&cw_xml=research_profile.php

MAITHREYI NARASIMHA – LECTURE 15

Tata Institute of Fundamental Research
Email: mnarasimha.tifr@gmail.com
Website: http://www.tifr.res.in/~dbs/faculty/M_Narasimha.html

TOSHIHIKO OGURA – LECTURE 5

Tohoku University
Email: ogura@idac.tohoku.ac.jp
Website: http://www2.idac.tohoku.ac.jp/dep/devn/english/index2.htm

SHIZUE OHSAWA – LECTURE 26

Kyoto University
Email: ohsawa@lif.kyoto-u.ac.jp
Website: http://www.lif.kyoto-u.ac.jp/e/?post_type=labos&p=192

NADINE PEYRIÉRAS – LECTURE 3

Institut de Neurobiologie Alfred Fessard
Email: peyriéras@inaf.cnrs-gif.fr
Website: http://www.inaf.cnrs-gif.fr/ned/equipe07_eng/accueil_07.html

TIMOTHY SAUNDERS – LECTURE 4

Mechanobiology Institute, National University of Singapore
Email: dbsste@nus.edu.sg
Website: http://mbi.nus.edu.sg/timothy-saunders/

SATOSHI SAWAI – LECTURE 18

The University of Tokyo
Email: cssawai@mail.ecc.u-tokyo.ac.jp
Website: http://sawailab.c.u-tokyo.ac.jp/

MASAHIRO SOKABE – LECTURE 8

Nagoya University
Mechanobiology Institute, National University of Singapore
Email: msokabe@med.nagoya-u.ac.jp
Website: http://www.med.nagoyau.ac.jp/physiol2/ming_da_yi_xue_bu_di_er_sheng_li/Home.html

HIDERU TOGASHI – LECTURE 13

Kobe University
Email: htogashi@med.kobe-u.ac.jp
Website: http://www.pubfacts.com/author/Hideru+Togashi

YUSUKE TOYAMA – LECTURE 14

Mechanobiology Institute, National University of Singapore
Email: dbsty@nus.edu.sg
Website: http://mbi.nus.edu.sg/yusuke-toyama/

SARAH WOOLNER – LECTURE 25

The University of Manchester
Email: Sarah.Woolner@manchester.ac.uk
Website: http://www.wellcome-matrix.org/research_groups/sarah-woolner.html

MIN WU – LECTURE 19

Mechanobiology Institute, National University of Singapore
Email: dbswum@nus.edu.sg
Website: http://mbi.nus.edu.sg/wu-min/

MASAKAZU YAMAZAKI – LECTURE 22

Akita University
Email: yamazaki@med.akita-u.ac.jp
Website: http://www.med.akita-u.ac.jp/~kaibo2/CBH.html

RONEN ZAIDEL-BAR – LECTURE 12

Mechanobiology Institute, National University of Singapore
Email: zaidelbar@gmail.com
Website: http://mbi.nus.edu.sg/ronen-zaidel-bar/

Short Talk Speaker Directory

NANAMI AKAI – SHORT TALK 11
Kyoto University
nanami_akai@stu.kobe-u.ac.jp

VIKI ALLAN – SHORT TALK 12
University of Manchester
viki.allan@manchester.ac.uk

WANGSUN CHOI – SHORT TALK 3
University of North Carolina at Chapel Hill
wchoi@email.unc.edu

WEI YUNG DING – SHORT TALK 10
Mechanobiology Institute, National University of Singapore
dwy@nus.edu.sg

NORIKO FUNAYAMA – SHORT TALK 6
Kyoto University
funayama@mdb.biophys.kyoto-u.ac.jp

EMI MAEKAWA – SHORT TALK 8
RIKEN Center of Developmental Biology
maekawa@cdb.riken.jp

HORACIO LOPEZ-MENENDEZ – SHORT TALK 5
Institut Jaques Monod, University Paris Diderot
horacio.lopez.menendez@gmail.com

TAKEFUMI NEGISHI – SHORT TALK 9
National Institute for Basic Biology
negishi@nibb.ac.jp

LEI QIN – SHORT TALK 2
Mechanobiology Institute, National University of Singapore
qin_lei@nus.edu.sg

ANDREA RAVASIO – SHORT TALK 1
Mechanobiology Institute, National University of Singapore
meperme@gmail.com

MAKOTO SUZUKI – SHORT TALK 7
National Institute for Basic Biology
msuzuki@nibb.ac.jp

YAO WU – SHORT TALK 4
Mechanobiology Institute, National University of Singapore
wuyao198831@hotmail.com

Poster Presenter Directory

RISHITA CHANGEDE – POSTER 1
Mechanobiology Institute, National University of Singapore
rishitac@nus.edu.sg

TI WENG CHEW – POSTER 2
Mechanobiology Institute, National University of Singapore
mbichew@nus.edu.sg

FUMIHITO FUKUJIN – POSTER 3
The University of Tokyo
ffukujin@physbio.c.u-tokyo.ac.jp

CHARLOTTE GUETTA-TERRIER – POSTER 4
Mechanobiology Institute, National University of Singapore
terrier.cha@gmail.com

YUSUKE HARA – POSTER 5
Mechanobiology Institute, National University of Singapore
mbiyhara@nus.edu.sg

HIROAKI HIRATA – POSTER 6
Mechanobiology Institute, National University of Singapore
mbihh@nus.edu.sg

TOMOKI ISHIBASHI – POSTER 7
Osaka University
ishibaki@bio.sci.osaka-u.ac.jp

SHREYANSH JAIN – POSTER 8
Mechanobiology Institute, National University of Singapore
jain.shreyansh@nus.edu.sg

LEYLA KOCGOZLU – POSTER 9
Mechanobiology Institute, National University of Singapore
mbilk@nus.edu.sg

IMSHIK LEE – POSTER 10
Nankai University
ilee@nankai.edu.cn

YEN WEI LIM – POSTER 11
Temasek Lifesciences Laboratory, Singapore
National University of Singapore
yenwei@tll.org.sg

KOTA MIYASAKA – POSTER 12
Tohoku University
k.miyasako@idac.tohoku.ac.jp

YUICHI SAKUMURA – POSTER 13
Aichi Prefectural University
sakumura@ist.aichi-pu.ac.jp

ERIKO SATO – POSTER 14
Tohoku University
e.sato@idac.tohoku.ac.jp

KRITI SETHI – POSTER 15
Temasek Lifesciences Laboratory, Singapore
National University of Singapore
kriti@tll.org.sg

ZIJUN SUN – POSTER 16
Mechanobiology Institute, National University of Singapore
zijun.sun89@nus.edu.sg

HATSUMI SUZUKI – POSTER 17
The University of Tokyo
hsuzuki@bs.s.u-tokyo.ac.jp

PEI YI TAN – POSTER 18
Mechanobiology Institute, National University of Singapore
a0038688@nus.edu.sg

KORZH VLADIMIR – POSTER 19
Institute of Molecular and Cell Biology
vlad@imcb.a-star.edu.sg

HIROAKI YAMANAKA – POSTER 20
Osaka University
hiroyama@fbs.osaka-u.ac.jp

HUIJUAN YOU – POSTER 21
Mechanobiology Institute, National University of Singapore
mbiyouh@nus.edu.sg

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Website: http://mbi.nus.edu.sg
Email: mbi@nus.edu.sg
Fax: (65) 6872 6123

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