RIKEN Center for Developmental Biology

2016 Annual Report

2016 Annual Report

RIKEN
Center for Developmental Biology

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The RIKEN Center for Developmental Biology was launched in April 2000 under the auspices of the Millennium Project Research Initiative, which was established to drive research in areas of vital importance to both Japan and the world in the 21st century. The challenges facing an aging society were among the issues addressed, and developmental biology was included within this remit as a scientific field with the potential to contribute to the development of regenerative medicine.

Located in Kobe, Japan, the Center's research program is dedicated to developing a better understanding of fundamental processes of animal development at the molecular

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and cell biological level, the more complex phenomena involved in organogenesis, as well as the biology of stem cells and regeneration. Scientists at the CDB conduct world-class studies in a truly international research environment, with ample access to core facilities and technical support and a critical mass of scientific talent and expertise. The CDB also benefits from belonging to RIKEN, Japan's largest basic scientific research organization, as well as its close ties to graduate and medical schools and research institutions in Japan and other countries, giving our scientists access to a broad network of talent and technology across the country and around the world.

Message from the Director

It has been a whirlwind, but rewarding second year serving as director of the RIKEN Center for Developmental Biology (CDB) as we prepare for impending changes laid out by our parent organization RIKEN, following its designation as one of the three Designated National Research and Developmental Institutes by the Japanese government. But rest assured that we have been working hard to ensure that the high quality world class research for which CDB is known for will not be interrupted or lost by the imminent changes.

The laboratories at the CDB continued to have a productive year in 2016, publishing new findings that extend our knowledge on the fundamental principles of organismal development, how to harness these principles to recapitulate developmental phenomena *in vitro*, as well as reveal insights into disease mechanisms. These include findings on mechanisms of cell specification and differentiation, those controlling morphology, to reports of *in vitro* generation of pituitary tissue, generation of complex skin tissue, and revealing underlying pathogenesis of the olfactory disorder dysosmia. There were major developments on technological fronts as well, with several cutting-edge publications on new tools and methods that not only assist scientists working in the basic sciences but also have potential applications in medicine, such as protocols for turning tissues transparent, and novel genome-editing technologies.

We were also delighted to welcome two new talented team leaders, Minoru Takasato and Li-Kun Phng, to our Center this year, who will no doubt in due course make great contributions to our scientific programs. As part of our commitment to support the careers of young capable researchers, we will continue to search for and recruit young talent to head new laboratories in a research-intensive setting, and strive to strengthen educational programs geared toward undergraduate and graduate students, including medical students, to foster the next generation of scientists.

In July, the CDB's research and management activities underwent an intensive evaluation and review process by members of the CDB Advisory Council, a panel of nine prominent scientists from around the world working in fields related to developmental and stem cell biology. I am pleased to say that, overall, the report submitted by the Council was positive and encouraging, and helped to renew my resolve to lead the Center to achieve higher levels of excellence.

One of the key initiatives implemented this year to this end was the launch of the RIKEN CDB–Otsuka Pharmaceutical Collaborative Center (COCC), a partnership with Otsuka Pharmaceutical Co. Ltd. (Otsuka) that will draw on CDB's strength in basic research in developmental and regenerative biology and Otsuka's expertise in developing medicines and health-related products, to develop seeds that can be translated into practical applications, focusing on areas related to neurodegenerative and kidney diseases. Other new joint research announced with major industry partners this year include Kyocera Corporation for hair follicle regeneration, and Santen Pharmaceutical Co. Ltd, for retinal diseases.

Keeping pace with changing tides of science, we recognize that, now more than ever, the government and society have high expectations for basic science to produce advances to ameliorate our daily lives, and in this regard the CDB is committed to developing a stronger pipeline from basic to translational science. Taking advantage of our location in the Kobe Biomedical Innovation Cluster, we have been working to forge stronger ties with clinicians at nearby medical institutions, with the aim of identifying findings with translational potential. We have also established a clinical translational program for physician scientists that would allow them to work alongside CDB scientists to exchange insights and expertise to promote translation of basic science. This will complement the pioneering research by the Laboratory for Retinal Regeneration that is working to establish an innovative therapy to treat retinal diseases using pluripotent stem cells.

Looking to the future, we pledge to continue laying down a strong foundation of basic science in understanding how bodies arise and are maintained, but at the same time, keep our eyes open to spot translational opportunities through closer collaboration with industry and clinical counterparts. I ask for your continued support on our quest to open new frontiers in development and regeneration.

Hiroshi Hamada
Director, RIKEN Center for Developmental Biology

Center for Developmental Biology

The Center for Developmental Biology (CDB) is a research center within the RIKEN Kobe Branch, which also includes the Center for Life Science Technologies (CLST), the Quantitative Biology Center (QBiC), the Advanced Institute for Computational Science (AICS) and the Kobe Administrative Division, which provides administrative services. The CDB underwent an organizational restructuring in 2014, resulting in major changes to laboratory designations and departmental affiliations. The majority of laboratories are now designated as teams, and assigned to one of five programs, described in detail to the right. In September 2016, the CDB partnered with Otsuka Pharmaceutical Co. Ltd to launch the RIKEN CDB–Otsuka Pharmaceutical Collaborative Center, intended to facilitate transfer of scientific findings to commercial applications. The CDB Director is assisted by a Deputy Director and advised by the Advisory Council, while governance issues are discussed by the Management Committee.

- Center for Life Science Technologies
- Quantitative Biology Center
- Advanced Institute for Computational Science
- Developmental Biology Planning Office
- Life Science Technologies Planning Office
- Quantitative Biology Planning Office
- Computational Science Planning Office
- Kobe Administrative Division

Deputy Director

Cellular Environment and Response Research Program

This program includes labs focused on developing platforms for gaining a better understanding of fundamental control principles of animal development, aimed at learning new ways of overcoming developmental disorders.

- **Morphogenetic Signaling**
  Shigeo HAYASHI Ph.D.
- **Developmental Epigenetics**
  Ichiro HIRATANI Ph.D.
- **Chromosome Segregation**
  Tomoya KITAJIMA Ph.D.
- **Growth Control Signaling**
  Takashi NISHIMURA Ph.D.
- **Vascular Morphogenesis**
  Li-Kun PHNG Ph.D.

Organogenesis Research Program

Labs in this program study mechanisms underlying the formation of complex model systems, in the hopes of gaining insight into the bases of the higher order structure of organs and functional development.

- **Neocortical Development**
  Carina HANASHIMA Ph.D.
- **Sensory Circuit Formation**
  Takeshi IMAI Ph.D.
- **Cell Asymmetry**
  Fumio MATSUZAKI Ph.D.
- **Cell Adhesion and Tissue Patterning**
  Masatoshi TAKEICHI Ph.D.
- **Epithelial Morphogenesis**
  Yu-Chiun WANG Ph.D.

Advisory Council

The CDB Advisory Council (DBAC) convenes regularly to review the Center's performance and make recommendations on the direction of its programs and governance. The DBAC reports its findings to assist in guiding future activities and decision-making. The full texts of DBAC reports can be viewed on the CDB website (www.cdb.riken.jp).

The nine-member Council comprises top international scientists working in developmental biology, stem cells, and related fields.

- Janet Rossant
  The Hospital for Sick Children, Canada
- Anne Ephrussi
  European Molecular Biology Laboratory, Germany
- Ryoichiro Kageyama
  Kyoto University, Japan
- Gordon Keller
  Princess Margaret Cancer Centre, University Health Network, Canada
- Hiromitsu Nakauchi
  Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, USA
- Daniel St Johnston
  Gurdon Institute, University of Cambridge, UK
- Clifford Tabin
  Harvard Medical School, USA
- Patrick Tam
  Children's Medical Research Institute, University of Sydney, Australia
- Shosei Yoshida
  National Institute for Basic Biology, Japan

Center Director Hiroshi Hamada

Office of the Director

Research Ethics Education Officer

Stem Cells and Organ Regeneration Research Program

Labs in this program work on identifying methods for controlling organ formation and regeneration through the study of stem cells and organogenesis.

- in vitro Histogenesis
  Mototsugu EIRAKU Ph.D.
- Tissue Microenvironment
  Hironobu FUJIWARA Ph.D.
- Lung Development
  Mitsuru MORIMOTO Ph.D.
- Pluripotent Stem Cell Studies
  Hitoshi NIWA M.D. Ph.D.
- Human Organogenesis
  Minoru TAKASATO Ph.D.
- Organ Regeneration
  Takashi TSUJI Ph.D.

Developmental Biology and Mathematical Science Program

This program aims to develop a systematic understanding of the complex cellular interactions underlying tissue morphogenesis through quantitative mathematical approaches and the development of computational models of gene expression, signal modulation, and dynamic processes.

- Organismal Patterning
  Hiroshi HAMADA M.D. Ph.D.
- Axial Pattern Dynamics
  Hidehiko INOMATA Ph.D.
- Histogenetic Dynamics
  Erina KURANAGA Ph.D.

Research and Development Project

This program conducts research using induced pluripotent (iPS) and other types of stem cells to develop approaches to regenerate retinal function, in active collaboration with the Kyoto University Center for iPS Cell Research and Application (CiRA) and various Kobe-based research initiatives.

- Retinal Regeneration
  Masayo TAKAHASHI M.D. Ph.D.

Collaboration Centers Program

This program aims to facilitate collaborations with industry by pursuing longer term projects in areas related to neurodegenerative and kidney diseases, with a view to develop seeds of innovation that can be translated into practical applications.

- RIKEN CDB–Otsuka Pharmaceutical Collaboration Center
  Hiroshi HAMADA M.D. Ph.D.

Management Committee

The Management Committee was established in November 2014 following the restructuring of the CDB. The Committee comprises the CDB Director, Deputy Director and several laboratory heads in addition to scientists outside the CDB, and convenes monthly to discuss and make decisions on governance issues such as recruitment of new laboratory heads and personnel, and budgetary allocations.

- Hiroshi Hamada
  Director, CDB
- Toshihiko Oguru
  Director, RIKEN Kobe Branch; Deputy Director, CDB
- Akihiko Koseki
  RIKEN Center for Integrative Medical Sciences
- Yoichi Shinkai
  RIKEN Cellular Memory Laboratory
- Hisato Kondoh
  Kyoto Sangyo University
- Tomoya Kitajima
  Lab. Chromosome Segregation, CDB
- Tatsuo Shibata
  Coordinator, CDB
- Masayo Takahashi
  Lab. Retinal Regeneration, CDB
- Takashi Tsuji
  Lab. Organ Regeneration, CDB
- Takashi Nishimura
  Lab. Growth Control Signaling, CDB
- Carina Hanashima
  Lab. Neocortical Development, CDB
- Yasuhide Furuta
  Coordinator, CDB
- Katsutoshi Nukui
  Developmental Biology Planning Office

2016 Highlights

Team Leader Tomoya Kitajima receives MEXT Prize

Tomoya Kitajima, team leader of the Laboratory for Chromosome Segregation was honored with a commendation for Science and Technology from the Minister of MEXT at a ceremony held in Tokyo on April 20. He received the Young Scientist's Prize for his study of the mechanisms of chromosome segregation in mammalian oocytes using high-resolution live-imaging in combination with micromanipulation and genetic engineering techniques.

CDB Director elected associate member of EMBO

Director Hiroshi Hamada was elected as an Associate Member of the European Molecular Biology Organization (EMBO) following this year's membership elections, an honor reserved for a small number of leading scientists residing outside Europe in recognition of their contributions to research in the life sciences.

CDB signs MOU with Chulalongkorn University

The CDB established an MOU with the Faculty of Pharmaceutical Sciences of Chulalongkorn University, in Thailand. The signing ceremony was held at Chulalongkorn University in May, and a joint symposium was also held after the ceremony.

New organizational framework for iPSC clinical research

RIKEN CDB concluded an agreement with the Kobe City Medical Center General Hospital, Osaka University Hospital and Kyoto University on May 30, to launch a new clinical research project aimed at establishing an iPSC-based treatment for wet-type age-related macular degeneration. Under the new partnership, Kyoto University will establish autologous and allogeneic iPSC lines, CDB will generate RPE cells that will be used for transplantation from iPSCs, and Kobe General Hospital and Osaka University Hospital will perform the transplantation.

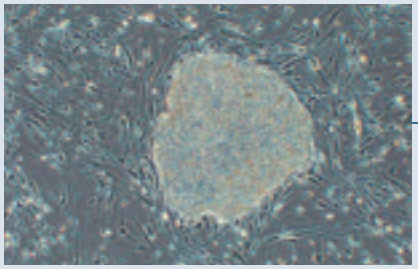
CDB Advisory Council convenes

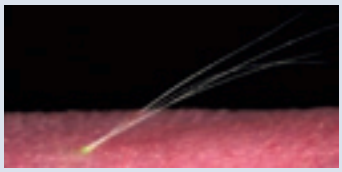
The biennially organized CDB Advisory Council (AC) was held on July 14–16 at the CDB. AC members convened to hear about scientific progress of each of the laboratories, as well as discuss and make suggestions on the research activities at CDB, and its directions for the future.

New faces at the CDB

The RIKEN CDB welcomed two new team leaders to its research programs. Minoru Takasato was appointed Team Leader of the Laboratory for Human Organogenesis in August, and Li-Kun Phng joined the CDB in October as Team Leader of the Laboratory for Vascular Morphogenesis.

2016 Highlights





Strengthening research collaborations with industry

CDB announced the launch of several new collaborative research projects with industry. The Laboratory for Organ Regeneration has teamed with Kyocera Corporation and Organ Technologies Inc. with the aim of developing technologies and products for treating alopecia (hair loss) through the regeneration of hair follicles, and the Laboratory for Retinal Regeneration has joined hands with Santen Pharmaceutical Co. Ltd. and FBRI to identify new drug candidates for treating retinal degenerative diseases using retinal cells derived from iPSCs.

Team leader wins Australian Museum Eureka Prize

Minoru Takasato, team leader of the Laboratory for Human Organogenesis, was announced as one of the winners of the 2016 Australian Museum Eureka Prizes. At a gala event held in Sydney, Australia on August 31, he along with his colleague, Professor Melissa Little from the Murdoch Childrens Institute, received the 2016 UNSW Eureka Prize for Scientific Research for their work generating human kidney organoids from stem cells. This work was carried out by Takasato during his previous post at the Murdoch Childrens Institute.

RIKEN CDB and Otsuka Pharmaceutical launch new collaborative research center

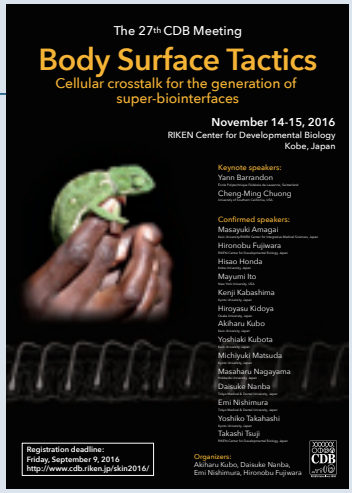
CDB signed an agreement with Otsuka Pharmaceutical Co. Ltd to establish the RIKEN CDB-Otsuka Pharmaceutical Collaborative Center (COCC) starting September 1. The new collaborative research center is headed by Hiroshi Hamada, and will initially focus on the areas of kidney and neurodegenerative diseases.

G7 Ministers visit RIKEN CDB

Health ministers of countries in the Group of Seven (G7) visited the CDB on September 12 to learn about RIKEN, and hear a talk by Masayo Takahashi on her research to establish iPSC-based treatment for age-related macular degeneration. The ministers also had the opportunity to look at samples of iPSCs and retinal pigment epithelial cell sheets generated from iPSCs.

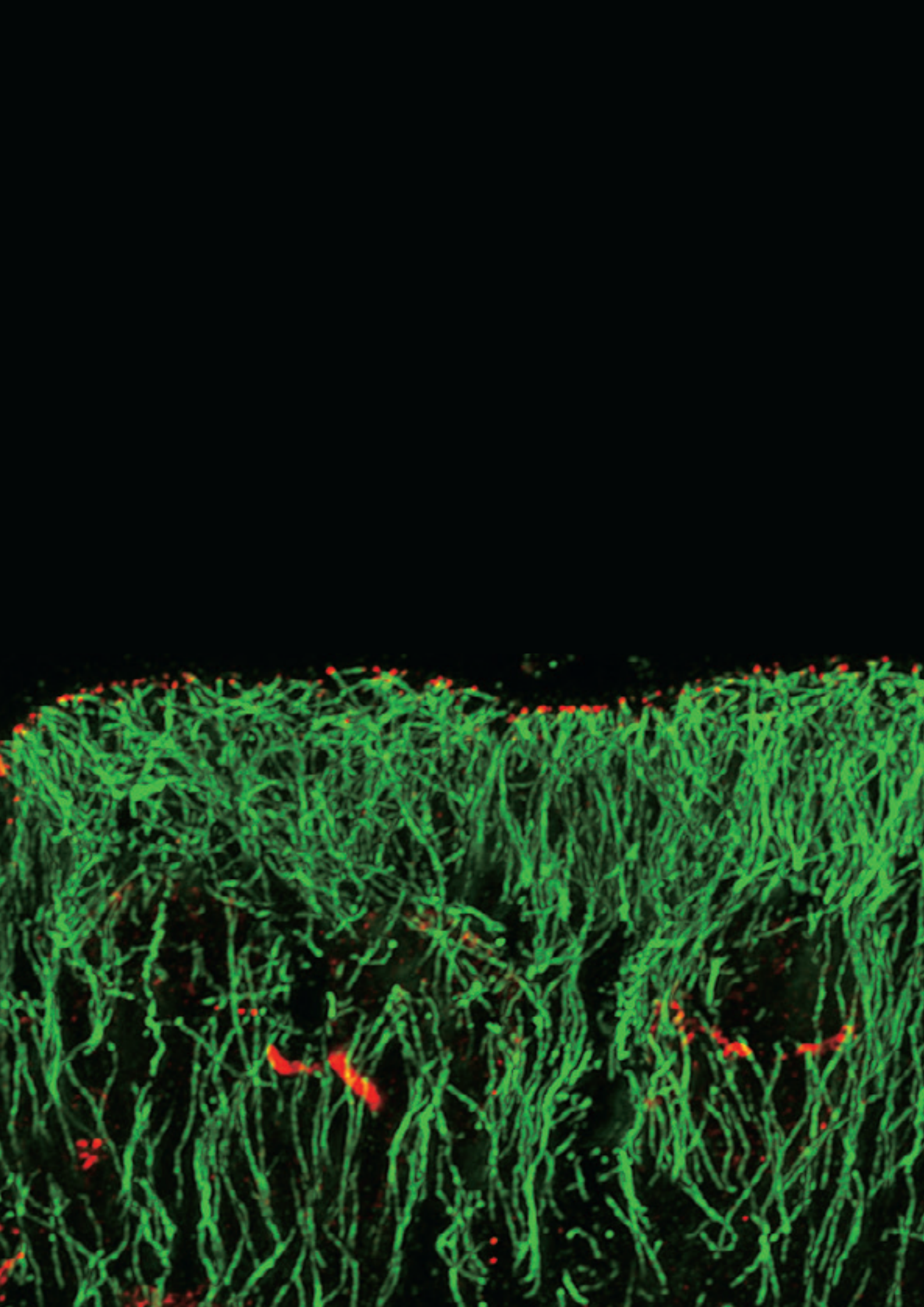
Going beyond skin-deep

The 27th CDB Meeting, “Body Surface Tactics: Cellular crosstalk for the generation of super-biointerfaces,” was held November 14–15. Scientists and students with an interest in some aspect of the skin assembled at the CDB to take part in the two-day meeting that focused on themes such as epithelial formation, maintenance and regeneration, new technologies, and skin dysfunction and therapeutics.

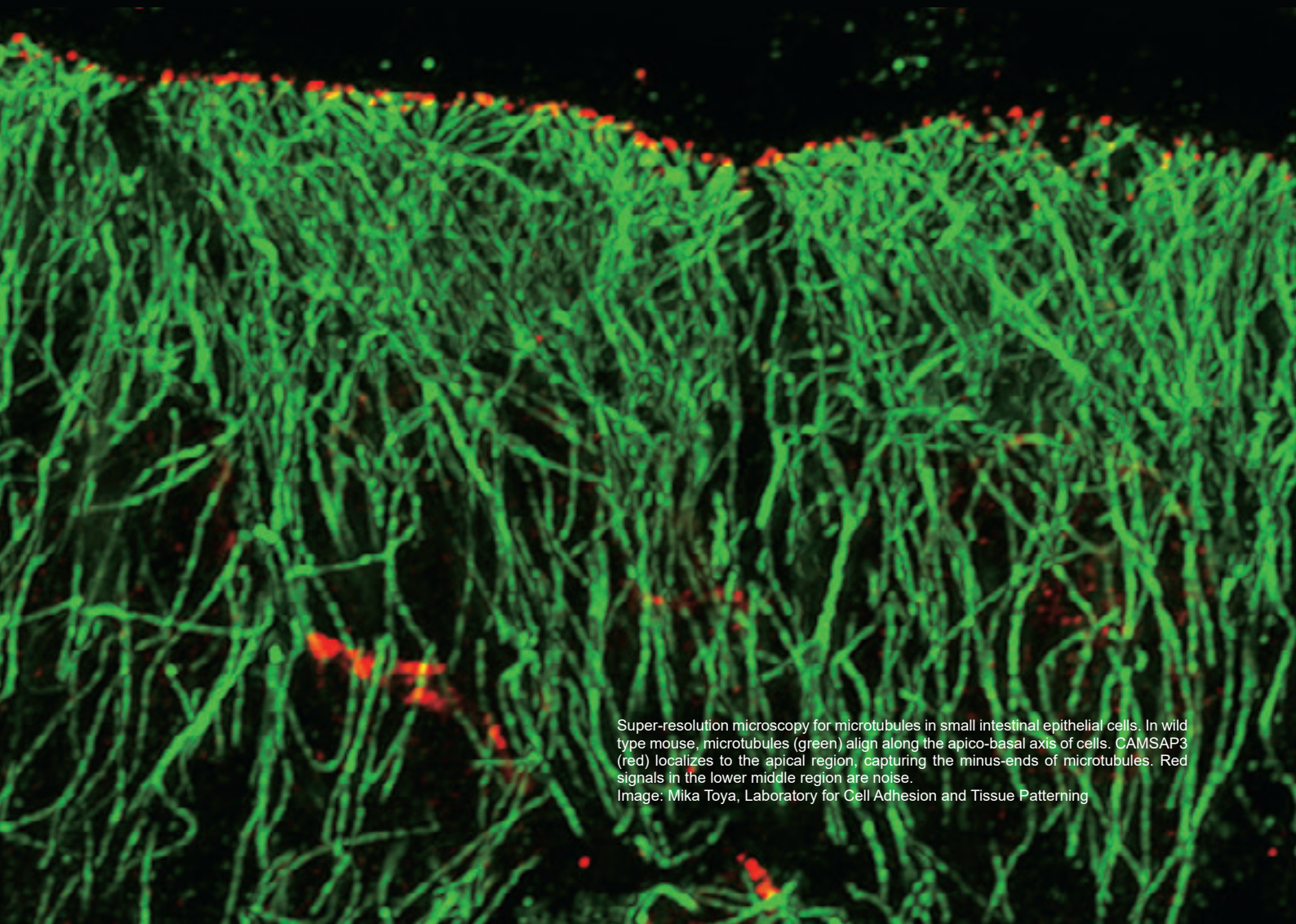


Seeking new frontiers in cilia and centrosome research

The 28th CDB Meeting, “Cilia and Centrosomes: Current Advances and Future Directions,” was organized November 27–29. Over 100 participants were in attendance to discuss roles of cilia and centrosomes in cell cycle, embryogenesis, and disease pathogenesis.



Research Highlights



Super-resolution microscopy for microtubules in small intestinal epithelial cells. In wild type mouse, microtubules (green) align along the apico-basal axis of cells. CAMSAP3 (red) localizes to the apical region, capturing the minus-ends of microtubules. Red signals in the lower middle region are noise.
Image: Mika Toya, Laboratory for Cell Adhesion and Tissue Patterning

Mechanism orienting microtubules in epithelial cells

Mika TOYA

Epithelial cells are found covering flat surfaces and lining tubular organs of the body. They exhibit an apicobasal polarity, a characteristic that is essential for carrying out their numerous functions such as secretion, absorption, and protection. One feature contributing to epithelial cell polarization is the distinct intracellular organization of microtubules, a key component of the cytoskeleton. In polarized epithelial cells, microtubules are arranged in longitudinal rows parallel to the apicobasal axis, appearing like a string curtain, but the mechanisms at work to properly orient the microtubules remained unclear.

In a study led by research scientist Mika Toya in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader), the team demonstrated, using mouse intestine epithelial cells and human colorectal adenocarcinoma-derived cell line (Caco-2) as models, that microtubule-binding protein CAMSAP3 (calmodulin-regulated–spectrin-associated protein 3) is important for microtubule orientation in polarized epithelial cells. They also showed that microtubule orientation is critical for correctly positioning organelles within the cell. Their work was published in the *Proceedings of the National Academy of Sciences USA*.

Microtubules are involved in many cellular functions such as chromosome segregation, intracellular transport, cell motility and cell structure maintenance. The tubular structure of the microtubule has polarity, a minus- and plus-end, with the plus-end being associated with rapid elongation. In epithelial cells, microtubules are aligned along the apicobasal axis, with minus-ends oriented near the apical side and plus-ends toward the basal side. Past studies from the same laboratory using cultured cells identified CAMSAP3 (also called Nezha) as a protein binding specifically to microtubule minus-ends. In the current study, the team focused on revealing the mechanism underlying microtubule orientation in polarized epithelial cells.

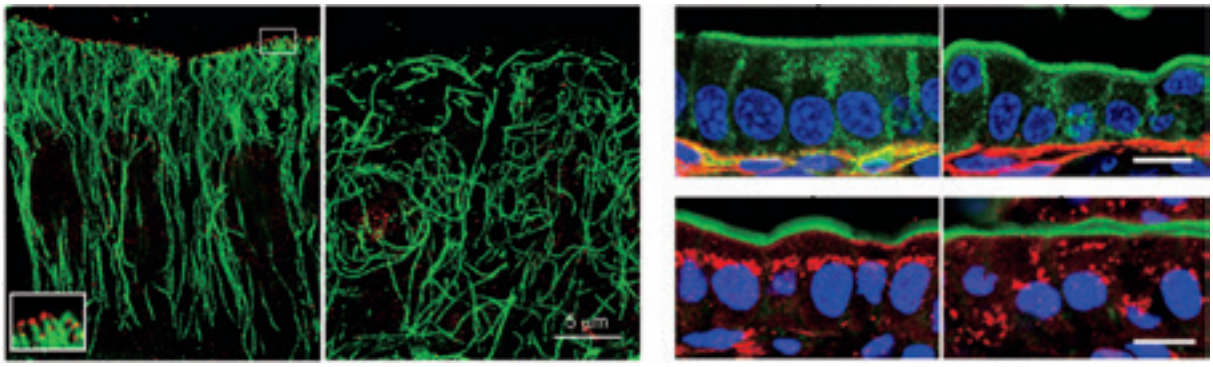
CAMSAP3 is known to bind to microtubule minus-ends through its CKK-domain at the C-terminus, so the team first generated a *Camsap3* mutant mouse line, which expresses a CAMPSAP3 lacking the CKK-domain. Homozygous mutants displayed growth impairments, and about 15% of these mice died before postnatal day 30. When epithelial cells of the small intestine were examined, they noticed those from *Camsap3* mutants lacked the normally punctate distribution of CAMSAP3 on the apical side. Comparisons of microtubule distribution in cells using stimulated-emission depletion (STED) super-

resolution microscopy revealed that microtubules in cells from wildtype were bound to the apically distributed CAMSAP3 and aligned longitudinally along the apicobasal axis, whereas in mutant cells, microtubules were disorganized, displaying no specific orientation. Thus, these experiments suggested that apically distributed CAMSAP3 steers proper microtubule orientation by binding to the microtubule minus-ends, anchoring them in place.

They next examined arrangements of organelles in cells from *Camsap3* mutant mice. In wildtype mice, cell nuclei were found in near the basal side at relatively similar distances from the basal membrane, whereas in *Camsap3* mutants, nuclei positions were disorganized, with some even positioned close to the apical side. Position of the Golgi apparatus, generally found just above (apical to) the nucleus, was also abnormal in *Camsap3* mutants, scattered to different areas of the cytoplasm. Cells from mutants also appeared to have problems maintaining consistent cell height along apicobasal axis. Electron microscopy revealed mitochondria elongation, and in some cells, the normally apically positioned adapter protein ezrin was mislocalized. Thus, it appears that organelles cannot be correctly localized when microtubules are misoriented as seen in *Camsap3* mutant cells.

Toya also analyzed CAMSAP3 function in Caco-2 cells, a human colorectal adenocarcinoma-derived cell line, culturing them in a media that allowed the cells to maintain epithelial-like polarity. When CAMSAP3 was depleted in Caco-2 cells, they found microtubule misalignment and mislocalization of organelles. Analyses of the CAMSAP3 domains revealed that the CC1 domain contained an amino acid sequence that was critical for CAMSAP3's apical localization. CAMSAP3 also requires binding to microtubules through its microtubule-binding CKK domain for apical localization.

"Our combined experiments using STED super-resolution microscopy and generating *Camsap3* gene knockouts has allowed us to analyze microtubule alignment in epithelial cells in greater detail than ever before," explains Takeichi. "We hope to look at CAMSAP3 function in other cell types, and understand how microtubules bound to CAMSAP3 control intracellular structure. It will also be interesting to determine the underlying causes of the growth defects observed in *Camsap3* mutant mice."



Left: Distribution of CAMSAP3 (red) and microtubules (green) in mouse intestinal epithelial cells. Upper right row: Position of nuclei (blue, nucleus; green, apical protein ezrin; red, basal membrane) Lower right row: Position of Golgi apparatus (blue, nucleus; green, apical cortex; red, Golgi). Scale bar for both rows, 10 µm. All panel sets, wildtype is on left and *Camsap3* mutant is on right.

Modified from article © 2016 Toya M, et al.

Functional pituitary tissue generated from human ESCs

Chikafumi OZONE

The pituitary, located at the base of the brain, is the central regulator of the endocrine system, controlling hormone secretions within the body. It secretes different hormones in response to signals from the hypothalamus, and also controls hormone secretion levels in response to feedback signals from the body. Disruption of hormone production in the pituitary can lead to problems with blood pressure, electrolyte balance, growth, and fertility, highlighting the importance of this tissue in maintaining homeostasis. While hormone replacement therapies are currently used to treat some of these conditions, they need to be administered throughout the patient's life and cannot emulate the precise regulatory control of the natural endocrine system, which responds to the ever changing needs of the body. Thus, a method to produce pituitary tissue that is capable of responding to regulatory signals would be a step forward developing an effective therapy for pituitary diseases.

A research collaboration between research associate Chikafumi Ozone of the Laboratory for Organ Regeneration (Takashi Tsuji, Team Leader), associate professor Hidetaka Suga of Nagoya University and others has culminated in the successful generation of anterior pituitary tissue from human embryonic stem cells (ESCs). Furthermore, they were able to steer the induction of various functional hormone-producing cells of the anterior pituitary. Their achievements were published in the online journal, *Nature Communications*.

The current work builds on previous work by Suga and former group director Yoshiki Sasai (dec.) of the Laboratory for Organogenesis and Neurogenesis, in which they generated self-organizing pituitary tissue *in vitro* from mouse ESCs, using a 3D floating cell culture method called SFEBq which was developed by the same lab.

The pituitary primordium, also known as Rathke's pouch, emerges from the oral ectoderm upon receiving inductive signals from the neighboring hypothalamic neural epithelia (NE). The team began by determining the optimal conditions for inducing both hypothalamic NE and oral ectoderm in the same culture. After many attempts, they discovered that the addition of a hedgehog pathway agonist and BMP4 to cultured human ESC-aggregates led to the efficient induction of oral ectoderm-like tissue around hypothalamic NE. After three weeks, they noticed a thickening of some sections of the oral ectoderm that expressed an early pituitary progenitor marker, LHX3, which eventually invaginated to form a Rathke's pouch-like structure, and the addition of FGF, important in early pituitary forma-

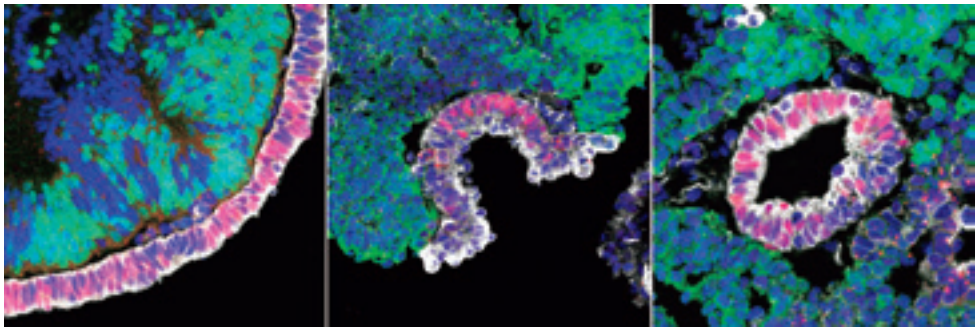
tion, produced Rathke's pouch-like structure at higher frequencies.

The group then examined whether their derived pituitary primordium could differentiate into mature pituitary hormone-producing cells. By culture day 67 to 70, they identified differentiation of adrenocorticotropic hormone (ACTH)-producing cells (corticotrophs) through immunostaining analyses and the observation of secretory granules in the cell cytoplasm. They also found that, consistent with reports from past studies, treating their derived pituitary tissue with glucocorticoids induced differentiation of somatotrophs, which produce growth hormone, and at much lower frequencies, of cells producing prolactin and thyroid stimulating hormone. When a Notch signaling inhibitor was added, they saw induction of gonadotrophs. Thus, their results show human ESC-derived pituitary primordium could differentiate into different hormone-producing cells of the anterior pituitary.

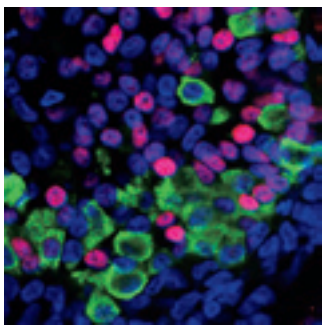
They also examined the regulatory responses of their derived corticotrophs and found that, similar to *in vivo* responses, ACTH release was stimulated with corticotropin-releasing hormones whereas glucocorticoids inhibited ACTH release. Likewise, they found their human ESC-derived somatotrophs responded similarly to natural ones *in vivo*.

Finally, they analyzed whether their hESC-derived pituitary could function *in vivo* by transplanting their pituitary tissue into the subrenal capsule of a hypopituitary mouse model. Extraction of the pituitary in mouse is lethal, causing death within a matter of weeks due to glucocorticoid deficiency arising from lack of ACTH. Ten days after transplantation, they observed engraftment of corticotrophs as well as a rise in ACTH levels stimulating glucocorticoid release. Comparisons between mice that received transplants and those that did not, revealed improvements to some hypopituitary symptoms, such as activity levels, which had dropped due to glucocorticoid deficiency, weight stability and length of survival. Three to four months after transplantation, the tissue graft showed signs of a vascular system and retained its hormone-secreting function.

"While there have been previous reports of pituitary induction from human ESCs, our study differs in that we have recapitulated the *in vivo* pituitary development *in vitro*, and are the first to demonstrate that our derived pituitary tissue can respond to regulatory signals, and show some therapeutic effects when transplanted into hypopituitary mouse model," says Suga. "Our work provides a platform for understanding pathogenesis of pituitary diseases and for developing future therapeutic applications."



Co-induction of ventral neural epithelium (green) and non-neural ectoderm (white) from hESCs. A Rathke's pouch-like structure (LHX3+, red) began to form between culture days 26 (left) to 27 (middle and right).



Corticotrophs differentiated from human ESC (green, ACTH-positive)

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Origins of complex vertebrate brain older than previously thought

(From left) Wataru TAKAGI, Tamami HIRAI, Noritaka ADACHI, Shigeru KURATANI, Juan PASCUAL ANAYA, Fumiaki SUGAHARA

Cyclostomes are jawless vertebrate fish that lack paired fins and have only one nostril. They are regarded as being more primitive forms of vertebrates, and there are presently only two surviving groups of cyclostomes—hagfish and lampreys. The latest common ancestor they shared with gnathostomes, the jawed vertebrate species including humans, was thought to be 500 million years ago. The acquisition of a highly regionalized complex brain is considered the hallmark of vertebrates, and the brain of cyclostomes have been characterized as being ancestral forms of the vertebrate brain due its more simple regionalization. The actual evolutionary origins of the vertebrate, however, remain unclear.

RIKEN visiting scientist Fumiaki Sugahara and research scientist Juan Pascual Anaya of the Evolutionary Morphology Laboratory (Shigeru Kuratani, Chief Scientist) and others performed detailed analyses of the embryonic brain of cyclostomes and discovered the existence of two key brain domains—the medial ganglionic eminence (MGE) and the rhombic lip—common across all gnathostomes but previously thought to be absent in cyclostomes. Their discovery provides strong evidence that the fundamental plan for vertebrate brain development was already established before the divergence of cyclostomes and gnathostomes over 500 million years ago. This work, published in *Nature*, was carried out when the laboratory was part of the CDB. Sugahara has since moved to and continues his research at the Hyogo College of Medicine, Japan.

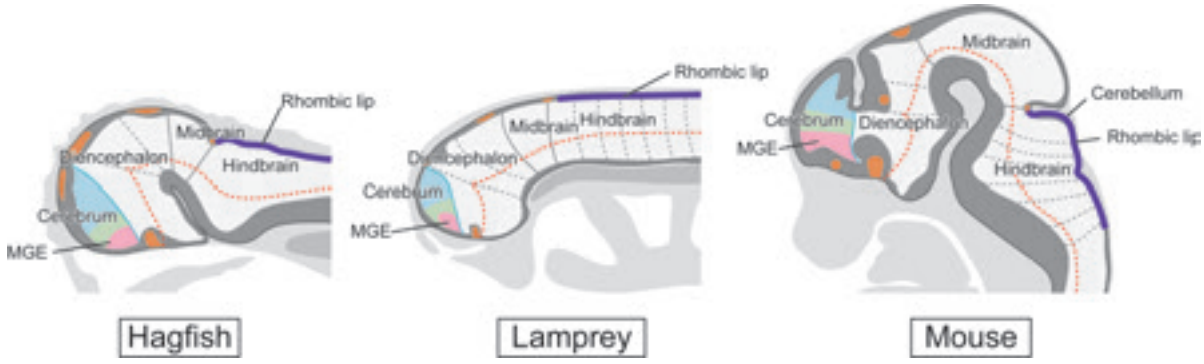
The blueprint for brain development is remarkably well conserved across gnathostome species. In the embryonic brain, the MGE emerges from the ventral region of the subpallium (future cerebrum), from which GABAergic interneurons migrate to the cerebral cortex. The rhombic lip, which gives rise to the cerebellum important for motor control, appears along the dorsal hindbrain. The apparent absence of the MGE and rhombic lip in developing lamprey brains suggested that these domains emerged in gnathostomes after divergence of cyclostomes and gnathostomes. However, GABAergic interneurons, presumed to be of MGE origin, have been found in adult lampreys, which alludes to the existence of an MGE region in embryonic lamprey brains, conflicting with conventional evolutionary models.

Sugahara and his collaborators began by making a detailed examination of brain development in hagfish, the only other extant cyclostome groups. They made serial sections of the embryonic head region at several different developmental stages, and used immunostaining and

in situ hybridization methods to stain for specific gene-expressing regions and tissues, and then reconstructed the brain in 3D using computer software. These results were then compared to those obtained from brains of lamprey and cloudy cat sharks, an example of a gnathostome offshoot to cyclostomes, at similar developmental stages. Hagfish had many common developmental brain features such as the positioning of key structures and nerve fibers as lamprey and shark. However, unlike in lamprey, the team identified expression patterns of MGE marker genes, *Nkx2.1* and *Hedgehog*, in the subpallium region, and of rhombic lip marker genes, *Pax6* and *Atoh1*, along the dorsal hindbrain region, confirming that hagfish form an MGE and rhombic lip domain during brain development.

Then how can the absence of the MGE and rhombic lip in lamprey, which exist in the hagfish and gnathostomes, be explained? One possibility is that common brain features between cyclostomes and gnathostomes were lost secondarily in lamprey, and another is that the gnathostomes and hagfish evolved similar features independently through convergent evolution. The team revisited the embryonic lamprey brain armed with newly published genomic data for lamprey, and uncovered additional orthologues of MGE and rhombic lip marker genes that were previously undetected. They confirmed that orthologous genes for *Nkx2.1*, *Pax6*, and *Atoh1* in the lamprey were expressed in patterns comparable to those seen in hagfish and gnathostomes, indicating that an MGE and rhombic lip-like domain exist in the embryonic lamprey brain.

"The brain architecture of cyclostomes were presumed to be less complex than those of gnathostomes because two key brain domains common across all vertebrate brains could not be found. But our current study shows that these brain domains are indeed present in cyclostomes, indicating that the fundamental vertebrate brain plan was established well over 500 million years ago," says Kuratani. "Most gnathostomes subsequently developed more elaborate brain regions leading to the forms seen today, so our next step is to understand how each brain region was acquired and evolved. Also of interest is the origins and evolution of cerebral neocortex and cerebellum."



Embryonic brain regionalization in cyclostomes (hagfish, lamprey) and gnathostomes (mouse)

Hagfish (behind) and lamprey (front)

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Improved tissue clearing solution for super-resolution imaging

Meng-Tsen KE (with Ying-Chu)

As biologists seek to gain further insights on subcellular structures deeply embedded in various tissues, high-resolution fluorescence microscopy using various fluorescent markers has emerged as a powerful tool to 'see' inside tissues in three-dimension (3D). High-resolution imaging remains challenging, however, as it is sensitive to light scattering and spherical aberrations, which in turn creates blurred or inaccurate images. To facilitate 3D imaging, especially of thicker tissue samples, several protocols for turning tissues transparent in a process called optical clearing have been developed to reduce light scattering and spherical aberrations. While some optical clearing agents achieve very high tissue transparency, many include use of harsh chemicals which can cause tissue damage or swelling resulting in morphological changes to more delicate structures. Thus, these clearing agents are unsuited for visualizing subcellular features at high resolution.

In a study by Foreign Postdoctoral Researcher Meng-Tsen Ke in the Laboratory for Sensory Circuit Formation (Takeshi Imai, Team Leader) and other collaborators, they report the development of a tissue-clearing solution SeeDB2 that can be used in combination with different types of high-resolution fluorescence microscopes, allowing them to analyze tiny fine structures lodged deep within thick tissue samples at super-resolutions. Published in *Cell Reports*, their study also demonstrated the applications of their tissue clearing solution for large-scale detailed 3D imaging of neuronal circuits in the mouse and fruit fly brain at synaptic-scale resolutions.

The Laboratory for Sensory Circuit Formation previously developed SeeDB (see deep brain), a non-hazardous, fructose-based clearing agent that can rapidly clear tissues within three days with minimal damage to tissue structures simply by soaking the tissue. Despite the well-preserved state of structures in samples treated with SeeDB, obtaining high resolution images of these embedded structures have been hampered by the resolution limit of the objective lenses.

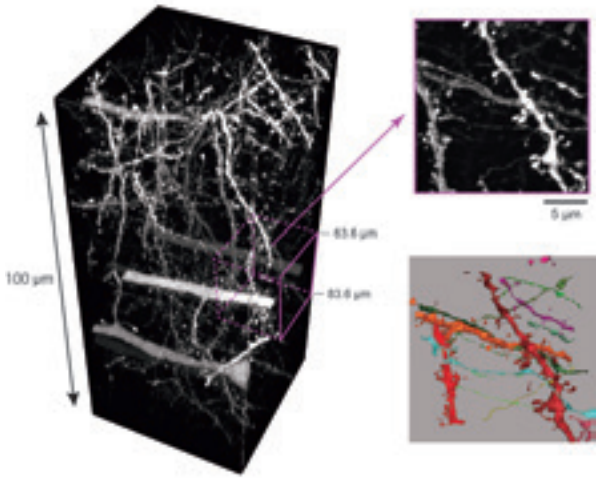
To increase resolution power of fluorescence microscopy, immersion oil, which has a high refractive index (RI), is normally placed between the glass coverslip and objective lens. RIs of clearing agents, including SeeDB, are lower than that of the coverslip and immersion oil, thus when the lens focus shifts deeper in a tissue, the light passing through tissue can no longer converge resulting in spherical aberrations. Spherical aberrations can be minimized when RI of cleared tissues matches that of immersion oil.

The team searched for aqueous solutions with high RIs and came across iohexol, an iodine compound used as a contrasting medium for X-ray imaging. After testing various formulas, they found that the combination of iohexol, small amounts of saponin (weak detergent) and Tris-EDTA buffer improved its permeation into tissues as well as the transparency of tissues to match the RI of immersion oil and the glass coverslip. This improved formula was named SeeDB2, and tissues could easily be cleared within two days, in some cases as short as a few hours, depending on the thickness of the sample by simple soaking. Like its predecessor, SeeDB2 also minimized tissue swelling during the clearing process, and could stably maintain bright fluorescence signals emitted by several different fluorescent proteins and fluorophores used for high-resolution fluorescence microscopy.

The performance of SeeDB2 was tested using different biological samples in combination with several commercial super-resolution microscopes. When imaging SeeDB2-treated mouse oocytes, which are relatively larger than the average cell, they were able to see individual microtubule strands in fine detail, where previously it had been difficult to do so. In mouse brain tissues cleared with SeeDB2, they were able to visualize neurons located at depths approximately 10 times deeper than previously possible as well as resolve fine neuronal features including spines on the dendritic processes which have diameters less than 1µm.

The team also demonstrated applications of SeeDB2 and super-resolution microscopy for imaging neuronal circuitry in brain tissues of mouse. They focused on the neuronal synapses, structures where electrochemical signals between neurons are exchanged, and which due to their small size (< 1µm) have been difficult to observe directly. Many cognitive disorders have been linked to abnormalities of neuronal synapses, thus, understanding synaptic structure will also add to our comprehension of how these disorders arise. They compared the dendrites of normal neurons with those in which an essential subunit of a glutamate receptor NMDAR was knocked out. In normal neurons, dendritic spines displayed different morphologies, some with pointed spines and others that were mushroom-shaped. Whereas excitatory synapses are known to localize at tips of dendritic spines, examination of inhibitory synapses clearly revealed they localize to the shaft of dendritic spines as well as the tips. In knockout neurons, while the spine density was comparable to normal neurons, the team observed that sizes of some spines were larger than those of normal neurons and that inhibitory synapses were densely localized at tips of spines. They also successfully imaged the neural circuitry in brain of *Drosophila*, revealing unique synaptic structures in 3D that could not be distinguished before.

“SeeDB2 is a simple and highly versatile clearing agent, particularly useful for imaging fine structures in tissues at high resolutions. The other clearing agents each have their own advantages, therefore scientists should choose the one that best suits their purpose," says Imai. “Electron microscopy has been the only means available until now to study neural synaptic structures, a time-consuming and laborious method. Combining the use of SeeDB2 with super-resolution microscopy will facilitate the study of connectomics of neural networks by revealing a manifold of detailed information on these delicate subcellular structures at synaptic level.”



Reconstructed image of mouse cerebral cortex cleared with SeeDB2 using super-resolution microscopy (Airyscan). Fine structures of the dendritic spines and synapses can be clearly seen.

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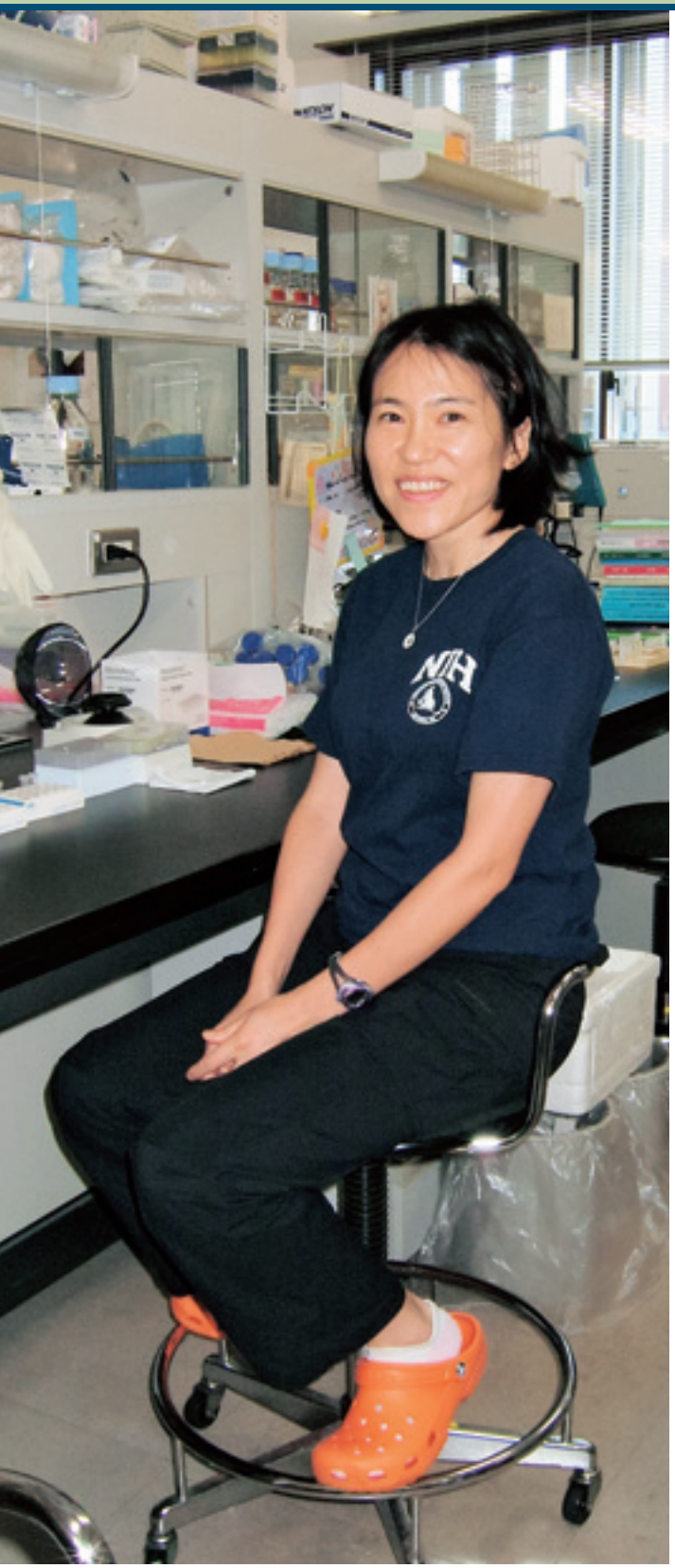
New insights into telomere maintenance mechanism in embryonic stem cells



Yoko FUTATSUGI

Embryonic stem cells (ESCs) are derived from cells extracted from the inner cell mass of early stage embryos. When cultured under the right conditions, they are capable of dividing and proliferating indefinitely, all the while retaining the potential to become any somatic cell type in the body. In most somatic cells, the terminal ends of DNA called telomeres become shorter after each successive round of cell division. Once telomere shortening reaches a critical limit, the cell stops dividing, eventually progresses into senescence (aging) and finally cell death. The exceptions to this are germ cells, stem cells and cancer cells, which can repair their telomeres through telomere elongation and continue to proliferate. In ESCs, a DNA-binding protein called ZSCAN4 is known to play an important role in regulating telomere elongation and genomic stability; however, its gene is not expressed in all ESCs, and it was unclear what triggered its expression.

In a study by research scientist Yoko Futatsugi and Team Leader Hitoshi Niwa in the Laboratory for Pluripotent Stem Cell Studies, they investigated the conditions leading to the activation of *Zscan4* expression in mouse ESCs by monitoring its activity at the single-cell level and found that it is activated in response to telomere shortening. They also discovered that the cell-cycle lengths were longer when *Zscan4* was expressed, presumably reflecting the time necessary for telomere maintenance. Their findings were published in the journal *Stem Cell Reports*.

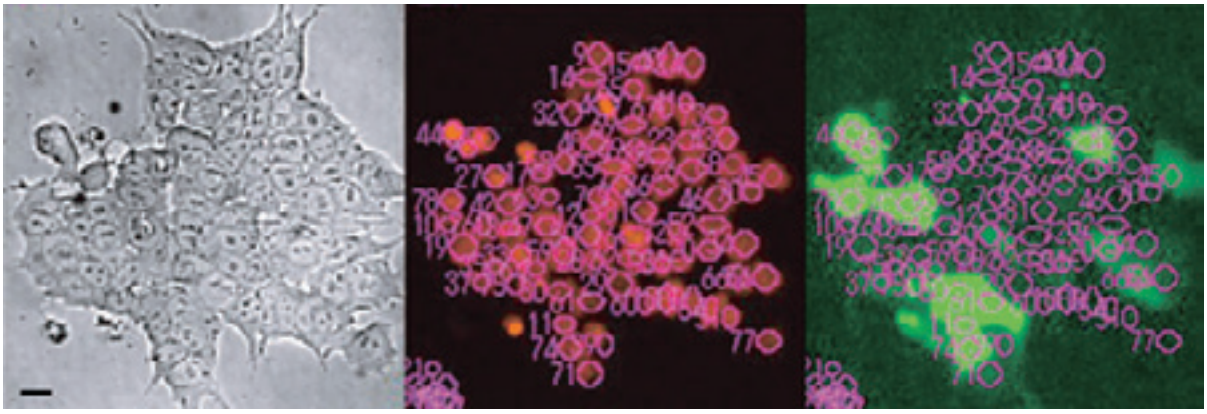
Futatsugi and Niwa first transfected mouse ESCs with two transgenes, each expressing different colored fluorescence when activated. They then established an experimental system where a nuclear marker was used to identify and track individual cells as well as analyze the *Zscan4* activity quantitatively over time. Individual mouse ESCs were tracked over a period of 120 hours, and a cell lineage tree was constructed based on the tracking data. The team also analyzed the cell-cycle length, and telomere length in these cells, and looked for correlations between these variables and *Zscan4* expression levels.

They were surprised to find that the cell-cycle length of mouse ESCs, which were thought to be approximately 12 hours, in fact displayed considerable variation, ranging between 10–30 hours. When they examined whether a correlation existed between cell-cycle length and *Zscan4* expression levels, cells with longer cell cycles were discovered to show higher expression levels of *Zscan4*. Those cells with high *Zscan4* expression levels also had shorter cell-cycle lengths in the following round of cell division. Searching for a link between cell-cycle length and telomere length, they found that cells with longer cell cycles had shorter telomeres. Thus, these results

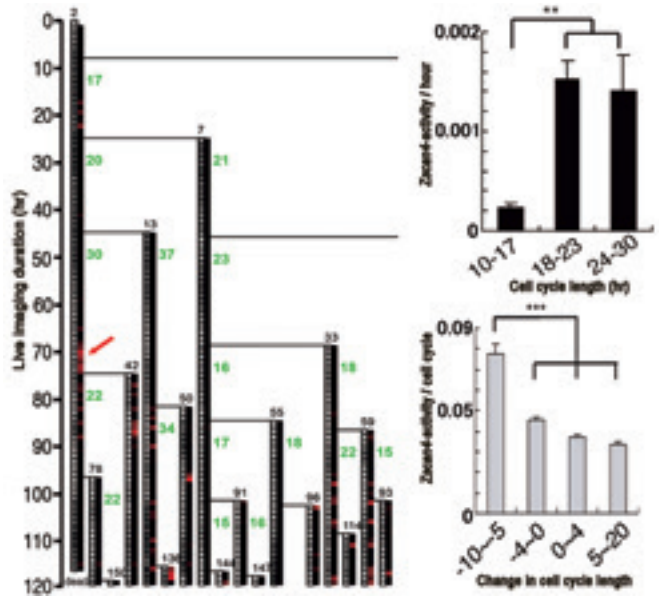
strongly suggested that as the ESCs undergo successive rounds of cell division, their telomeres become shorter, which triggers activation of *Zscan4* expression. *Zscan4* activation, in turn, functions to elongate the shortened telomeres, resulting in extended cell-cycle lengths.

Futatsugi and Niwa also examined the relationship between pluripotency marker gene *Rex1* and expression of *Zscan4*, but they did not find any correlations between these two factors. This suggested that telomere elongation by *Zscan4* functions independent to the mechanism maintaining ESC pluripotency.

"ESCs are known to be a heterogeneous population of cells. In this study, our single-cell tracking method uncovered surprising revelations about the telomere elongation mechanism that could not be identified simply through examinations of ESC colonies," explains Futatsugi. "As *Zscan4* appears to be activated through some mechanism sensing telomere length, our next step is to try understanding the details of this regulatory mechanism. We hope that further unveiling of innate characteristics of ESCs will lead to the development of more stable culturing methods of pluripotent stem cells, including iPS cells."



Expression of *Zscan4* in cultured mouse ESCs. Using nuclear marker H2B (middle, red) as a reference marking individual cells, expression levels of *Zscan4* (right panel, green) were analyzed temporally and quantitatively in each individual cell. Notice only one portion of cells expresses *Zscan4*.



Left: Example of cell lineage tree. Cells were sequentially numbered in the order they emerged (small black numbers). Numbers in green indicate cell-cycle length (hours). *Zscan4* activity is indicated on a scale of red intensity. Cells expressed *Zscan4* transiently. Top, right: *Zscan4* activity is higher when cell-cycle length is longer. Bottom, right: When *Zscan4* expression levels are high, the next round of cell division tends to be shorter.

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Making skin from iPS cells

(From left) Ryohei MINAMIDE, Koh-ei TOYOSHIMA, Takashi TSUJI, Miho OGAWA, Kyousuke ASAKAWA

The skin is a complex organ system comprising multiple organs. In addition to the epithelium, dermis and subcutaneous fat—the three tissue layers making up the bulk of the skin—it includes appendage organs such as sweat glands, sebaceous glands and hair follicles. These organs are all connected to structures such as sensory nerves or muscles, and work together to produce physiological responses. Many scientists have been working to develop artificial skin that closely resembles the function and structure of natural skin for therapeutic use to restore skin function in patients suffering from different skin-related diseases or injuries and for use in testing the safety or effects of cosmetics or drugs. Thus far, it has been possible to induce pluripotent stem cells to differentiate into specific cell types of the skin, but generating a three-dimensional (3D) skin structure complete with appendage organs has proven to be difficult.

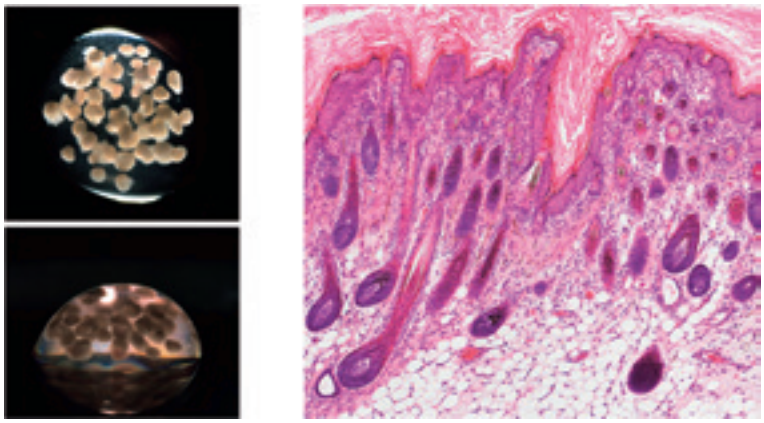
A research team headed by Takashi Tsuji, Team Leader of the Laboratory for Organ Regeneration, reported the successful generation of skin tissue complete with appendage organs such as hair follicles and sebaceous glands from mouse induced pluripotent stem cells (iPSCs). They developed a new approach to efficiently induce generation of 3D skin tissues from iPSCs by transplanting them into an *in vivo* system. Furthermore, they demonstrated that their derived skin tissue was fully functional when implanted into the skin of a nude mouse, with the transplanted skin engrafting with the host tissue and its hair follicles exhibiting regular hair eruption cycles similar to that of natural skin. This work was published in the online journal *Science Advances*.

Generally, the transplantation of iPSCs or embryonic stem cells into an *in vivo* system leads to the formation of teratomas, a tumor consisting of a mix of tissue types derived from the three embryonic germ layers. Tsuji's group began by searching for conditions favorable for inducing the formation of epithelial tissues, which is essential for organogenesis, from iPSCs at high efficiencies. They developed a novel method to transplant numerous embryoid bodies (EBs) formed from culturing iPSCs clustered in drop of collagen gel into a live mouse, calling this the clustering-dependent embryoid body (CDB) transplantation method. The team found that transplantation of clustered EBs in collagen drop produced four times as many cysts containing epithelial tissues than observed from transplantation of single iPSCs or EBs. These cystic epithelia possessed the three epithelial tissue layers as well as hair follicles and sebaceous glands that make up whole skin tissue all arranged akin to that of natural skin. They also discovered that stimulating EBs

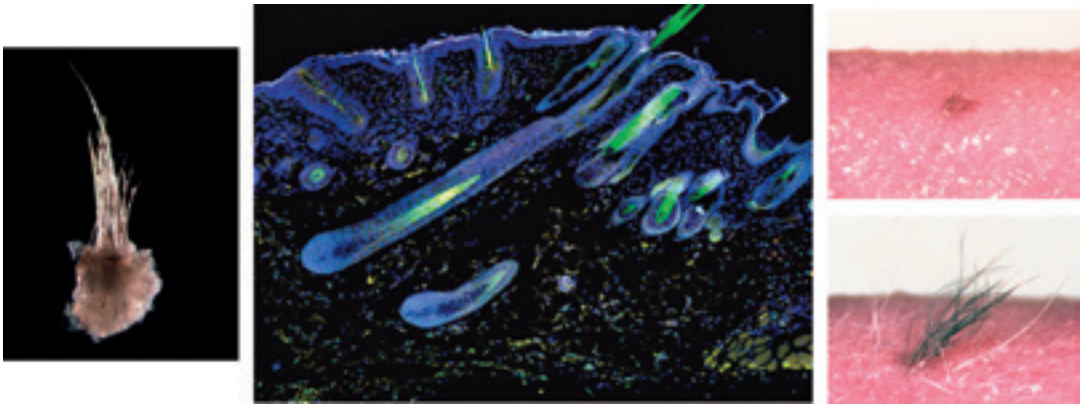
with Wnt10b, a signaling factor important for hair follicle formation, markedly increased the number of hair follicles formed within iPSC-derived skin.

Next, the team cut out small units of skin tissue with hair follicles from the cystic epithelia, implanted them subcutaneously into the back skin of a mouse. To trace engraftment of the transplanted skin unit, skin tissue made from male mouse-derived iPSCs was transplanted into a female mouse, and using Y-FISH analysis, which labels Y-chromosome positive cells, they verified that their iPSC-derived skin engrafted with the surrounding host tissue and showed no signs of tumorigenesis. Close analysis of the transplanted skin tissue revealed that it formed proper connections to nerves, muscles, and other structures of the host, and the hair follicles themselves underwent repeated hair cycles consistent with natural skin. The color, shape, type, pattern and density of the erupted hair was also confirmed to be similar to that of normal mice skin.

"Our CDB method appears to induce complex skin tissue through the interactions between outer epithelial surfaces of neighboring EBs, which may be creating epithelial niches with increased surface areas that undergo epithelial-mesenchymal interactions needed for skin organogenesis," says Tsuji. "The finding that Wnt10 signaling increases induction frequency of hair follicles provides an important hint for future studies attempting to replicate skin organogenesis in a culture system. We aim to understand the basic principles at work during organ induction as well as strive to develop artificial skin that can be used for human clinical applications and in industry."



EBs transplanted into adult mouse using CDB method (upper left, view from top; lower left, side view) resulted in formation of various structures making up the skin tissue inside the explants (left).



Skin tissue units including hair follicles (left) from iPSC-derived cystic epithelia were excised and transplanted subcutaneously in a nude mouse. Transplanted tissue unit became engrafted in host tissue (middle: explant-derived Y-chromosome-positive cells (green) and nucleus (blue)) and displayed normal repeating hair cycles (right).

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How cells can turn into donuts

Kagayaki KATO

From a topological perspective, a ball and a fork are considered equivalent forms as one can be deformed into other by stretching or shrinking the form of the outer surface. They are inequivalent to a donut (torus), as neither forms can be continuously deformed into a torus. In the natural world, however, where cells making up the animal body are found in diverse shapes, there appears to be way to turn a sphere-like cell into a torus. This extreme example is seen in tip cells of the developing *Drosophila* tracheal system. Tracheal primordia arise in each segment of the *Drosophila* embryo to form an epithelial tube unit, and tubular branches of each unit must connect to those in neighboring segments to form a continuous network circulating the body. Tip cells are located at the tips of migrating tubules and lead the branches to pair and connect with a neighboring branch. As tip cell pairs begin adhering to one another, they also undergo dramatic shape changes to facilitate luminal fusion, but how this shape change occurs was not well understood.

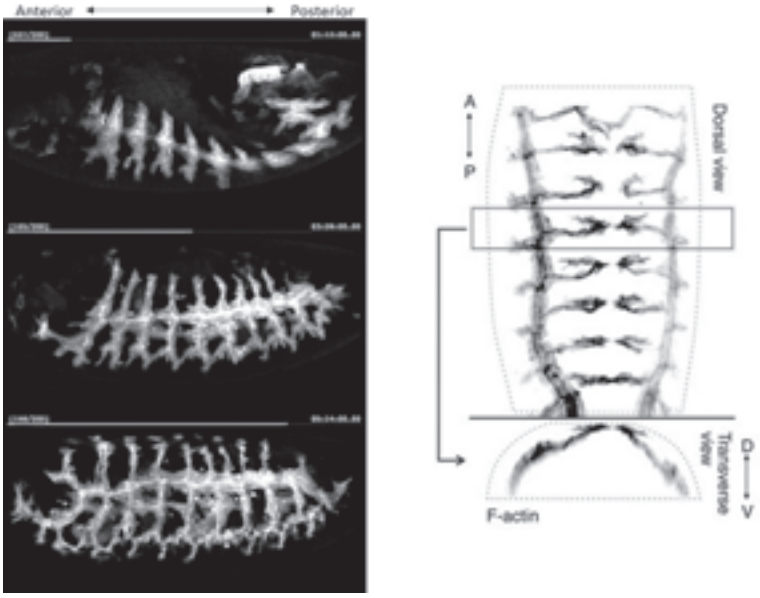
In a paper published in *Nature Communications*, research scientists Kagayaki Kato (now at National Institutes of Natural Sciences, Okazaki, Japan) and Bo Dong (now at Ocean University, China) of the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader) reveal a mechanism underlying topological changes of the tip cells observed in the developing branches of the *Drosophila* tracheal system. Their study demonstrates that microtubules within the tip cells play an important role in bringing about the dramatic shape changes.

To change the cell topology from sphere-like into a torus requires opposing sides of the cell to compress inwards, followed by fusion of the cell membrane. The team employed live imaging technology to track the behavior of tip cells destined to pair and fuse, from migration, to cell-cell contact, and finally fusion to connect the tracheal branches. During migration, the leading edge of tip cells extended a radial array of filopodia, with microtubules actively elongating in the direction of the target cell and punctate distribution of the cell adhesion molecule E-cadherin. When tip cells came into contact with their partner, they saw E-cadherin accumulating near the tip cell interface and forming new cell adhesion sites. Furthermore, dense central bundles of actomyosin and microtubules were found to span across the pairing tip cells. Morphological changes in tip cells were compromised when myosin activity was blocked, indicating that intrinsic actomyosin forces were driving the contraction of tip cells.

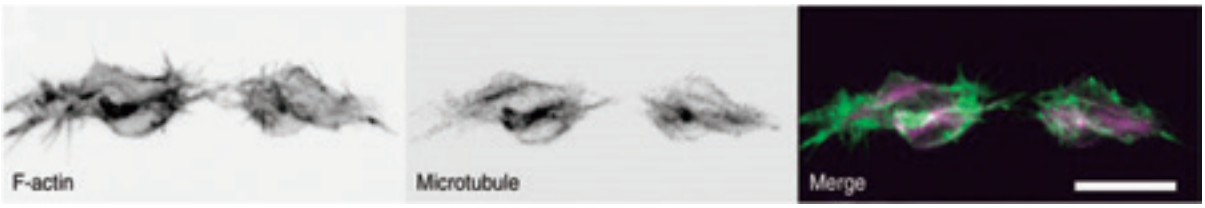
The team also investigated the effects of disrupting microtubule organization by treating the tip cells with different microtubule severing factors. They found that contraction of interfacing tip cells showed a range of problems, from an imbalance of contractile forces between tip cell leading to asynchronous contraction, to delays in or prolonged contraction phase, or even failed tip cell fusion. Thus, these observations suggested that a microtubule mechanism underlies the synchronization of cell fusion of the cells.

Microtubules also serve as transport routes to shuttle molecular cargo within the cell, and the lab previously reported that in fly tracheal formation, the chitin deacetylase Serpentine (Serp) is shuttled by vesicular transport to be secreted in the tracheal lumen to promote luminal formation. When microtubules were disrupted in the tip cells, the Serp transport machinery was also disrupted and tracheal branch fusion was arrested before luminal fusion was complete.

"A cell changing its topology to assume a torus-shape is an extremely rare occurrence, but our current study shows that even these radical morphological changes can be realized by skillful manipulation of cellular components found in any cell. Studying these extreme examples may shed light on the universal principles guiding morphological changes in cells," explains Hayashi. "It is still unclear how microtubules can sense and balance the contractile and tensile forces. We would like to understand the mechanism linking the sensing of these forces by microtubules to cell morphogenesis."



Tubular arrangement of epithelial tissues in the fly tracheal system. The tracheal network extending to all regions of the body is generated by connections formed between tracheal branches (left, lateral view; right, dorsal view). Bottom left and right panels were taken at stage 14 of development.



Microtubules (middle panel and magenta in right panel) formed bundles in the contact area of the paired tip cells. In this area, the actin filaments (left panel and green in right panel) overlapped with microtubules and formed dense bundles used for cell contraction.

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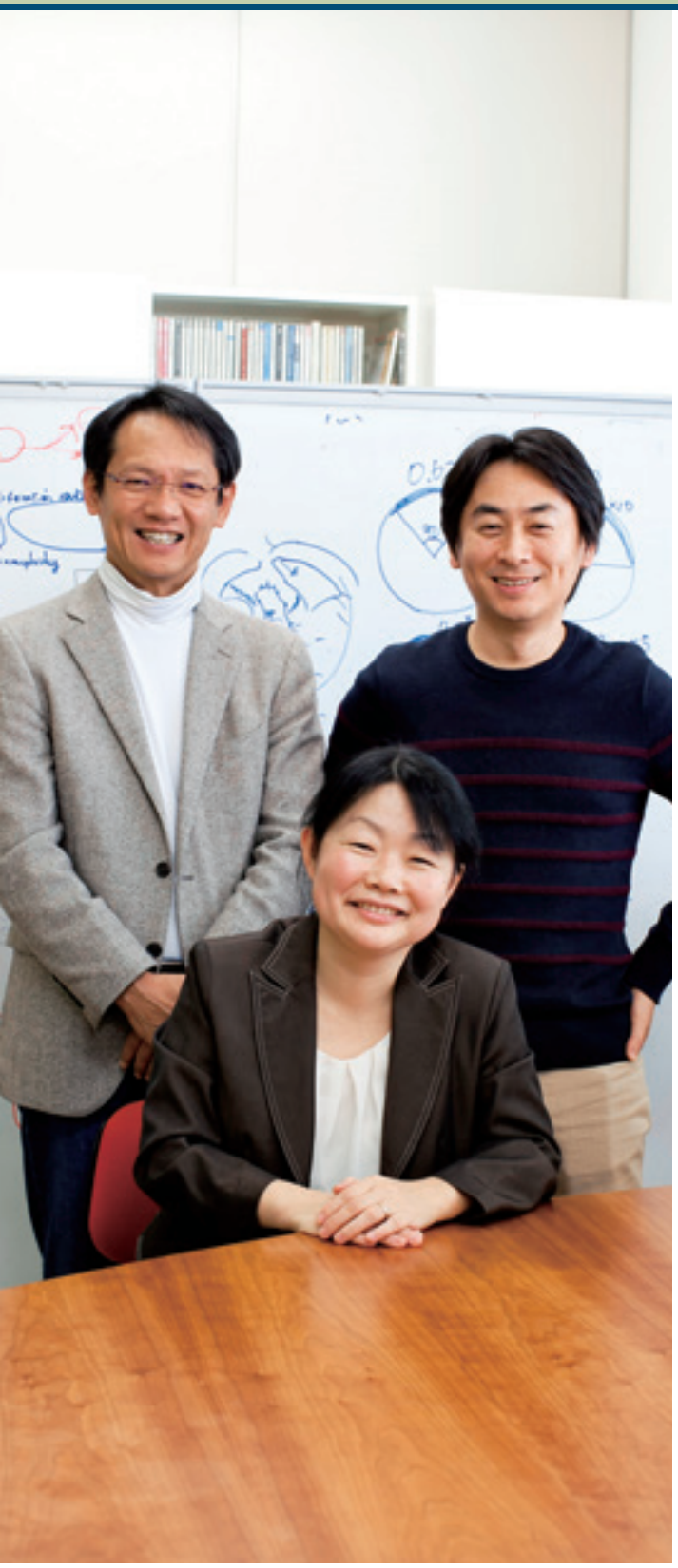
There’s no stopping time in apical progenitors



Fumio MATSUZAKI, Ayano KAWAGUCHI, Daijiro KONNO

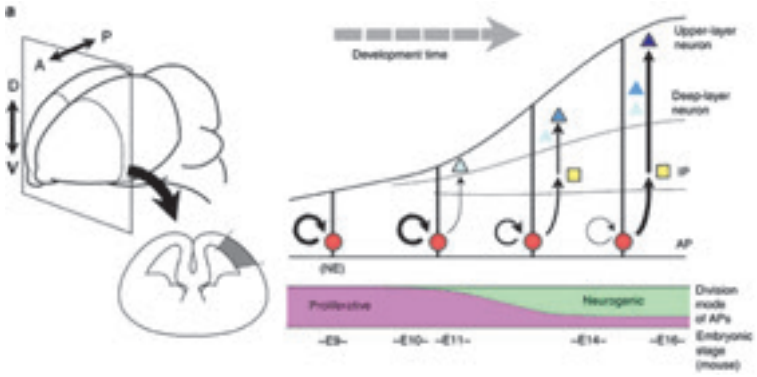
The most striking feature of the mammalian brain is the presence of a large, complex cerebral cortex, a six-layered structure comprising different neuronal types and each layer being derived from self-renewing neuroepithelial cells, called apical progenitors (APs), found in the ventricular zone. APs initially proliferate through symmetric cell division, but later switch to differentiation mode, undergoing asymmetric cell division to produce one AP that will continue to self-renew and another daughter cell that becomes an intermediate progenitor (IP) which will differentiate to give rise to neurons. As the type of neurons born from IPs changes according to progression of time, from deep-layer neurons to more upper-layer neurons, the precursor APs are thought to “sense” temporal progression of development to change fate of their differentiating progeny, but the underlying mechanism regulating time in the cell remains unknown.

A collaborative study between research scientist Daijiro Konno of the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Team Leader), Ayano Kawaguchi, an alumnus of the laboratory and currently associate professor at Nagoya University, and others has identified a group of so-called “temporal-axis genes” in neural progenitors—genes that show fluctuations in expression levels only with respect to progression of developmental time—using the mouse model. They used single cell transcriptomics to analyze changes in gene expression at several different developmental stages, and also revealed that temporal expression patterns of the temporal-axis genes were unaffected by cell-cycle arrest, thus functioning independent to cell cycle. Their findings were published in the online journal *Nature Communications*.

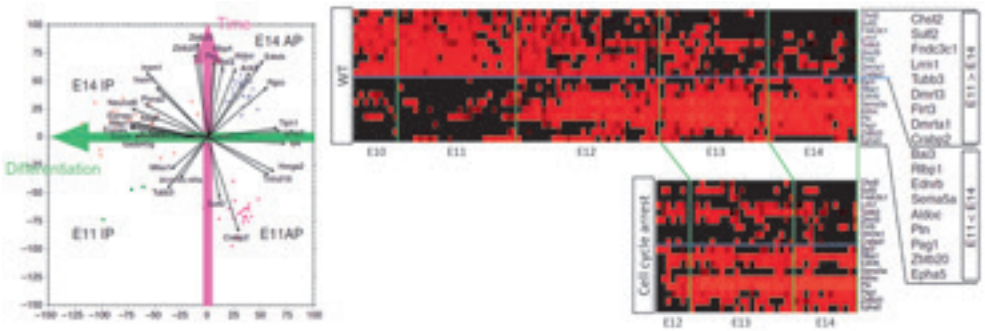
Identifying the molecular factors that regulate the timing when APs temporally change their properties, including switch from proliferation to neuronal production, has been a challenge as this process is not synchronized. Thus it is difficult to make a distinction between genes involved in temporal change of degree of differentiation and those involved only in shift of temporal pattern of AP identity. In this study, the research group decided to apply single cell transcriptomics to comprehensively analyze and compare all genes transcribed within a cell at several different developmental stages (E11, E14 and E16). From their transcriptome data, they used a statistical method called principal component analysis (PCA) to identify a set of genes that showed changes in gene expression only in correlation to temporal progression. These changes in gene expression patterns were independent to differential fate of progenitors (whether the cell is an AP or an IP), and most of the temporal-axis genes displayed the largest changes in expression around E12.

The cell cycle would appear as the most likely mechanism involved in regulating time within a cell since it is a fundamental mechanism found in all cells, with genes being activated in turn to prompt cells to steadily advance to the next stage of the cell cycle. The group devised an experimental system to transiently arrest the cell cycle of APs in the embryonic mouse brain by activating and terminating expression of genes involved in cell-cycle progression via electroporation, and then tracked the effects of transient cell-cycle arrest on expression patterns of temporal-axis genes. They found that, despite the temporary cell-cycle arrest, expression patterns of the temporal-axis genes, which displayed considerable changes in expression around E12, remained unchanged. Furthermore, when neuronal types that are born after transient cell-cycle arrest were examined, the team noticed that these APs failed to produce deep-layer neurons, and instead differentiated into the same type of upper-layer neurons that would be produced under normal conditions if cell cycle had not been arrested. Together, these findings suggest that the ability of APs to produce sequentially diverse neurons is independent to the cell cycle.

"Our experiments also suggested the existence of two types of temporal-axis genes, those that can be controlled cell intrinsically and those that require an extrinsic signal to trigger gene expression changes," explains Matsuzaki. "Whereas the question of how living organisms keep time has remained largely a mystery, our present study presents evidence refuting the widely-held hypothesis of the involvement of the cell cycle, at least in the context of brain development. Our next step is to identify the key temporal-axis genes, and eventually reveal the complete mechanism controlling temporal progression in cells."



Schematic of mammalian cerebral development. Self-renewing APs gradually switch to differentiation mode, producing IPs which will differentiate into different types of neurons. The fates of differentiating neurons transitions over time, from deep-layer neurons to upper-layer neurons.



Left: PCA was used to identify temporal-axis genes (those closely aligned to pink arrow), which exhibit changes in expression in correlation with temporal progression and independent from differentiation state. Right: Temporal-axis gene profile. Temporal change of gene expression occurs mainly around E12 (top panels). Transient cell-cycle arrest does not affect the temporal change of gene expression.

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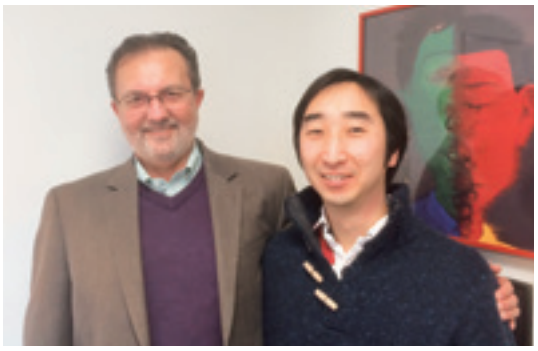
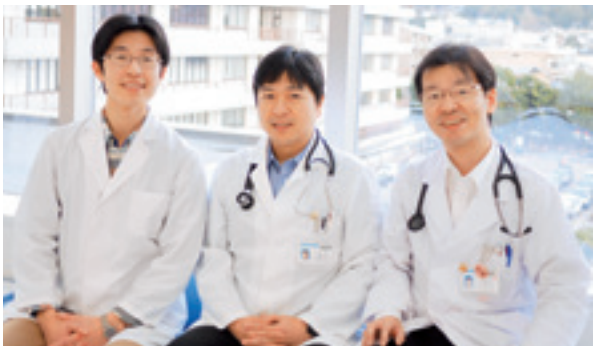
Notch signaling critical for postnatal alveolar morphogenesis

(From left) Keishi KISHIMOTO, Akira YAMAOKA, Yuki KIKUCHI, Mitsuru MORIMOTO, Chisa MATSUOKA, Hirofumi KIYOKAWA, Yuka NODA

Po-Nien TSAO (Taiwan University)



Wellington CARDOSO, Munemasa MORI (Columbia University Medical Center)



Koichi HASEGAWA, Atsuyasu SATO, Susumu SATO (Kyoto University)

Pulmonary alveoli are small, spongy, sac-like structures found at the terminal ends of the branching respiratory system and is the region of the lung where gas exchange takes place. They comprise the planar type I alveolar epithelial cells involved in gas exchange, the round type II alveolar epithelial cells that secrete surfactants to regulate humidity inside alveolar sacs, and the myofibroblasts that form the septa between alveolar ducts and help maintain the lung structure, which undergoes significant expansion and contraction during respiration. Alveolar development (alveologenesis) is unique in that it spans both embryonic and postnatal stages of development, and the distinctive morphology of the alveoli arises postnatally following the initial contact with air inhaled through respiration. The molecular mechanisms underlying alveologenesis, especially those involved postnatally, however, remain largely unknown.

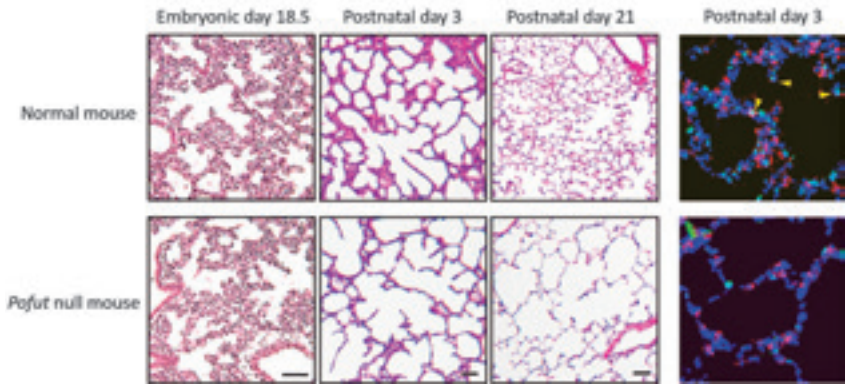
A study by technical staff Chisa Matsuoka and Team Leader Mitsuru Morimoto of CDB's Laboratory for Lung Development, published in the *Proceedings of the National Academy of Sciences USA*, demonstrated the critical role of Notch signaling in postnatal alveolar development using the mouse as a model. They showed that disruption of Notch signaling, Notch2 in particular, in epithelial cells lining the airways leads to abnormal alveolar morphology reminiscent of the phenotypes of bronchopulmonary dysplasia, with enlarged alveolar airspaces and decreased cellularity, reducing efficiency of gas exchange. This study was carried out in collaboration with National Taiwan University and Columbia University.

The Notch signaling pathway is important in lung morphogenesis, particularly in the differentiation of bronchial epithelial cells, and mice lacking epithelial Notch die shortly after birth, making it difficult to study the role

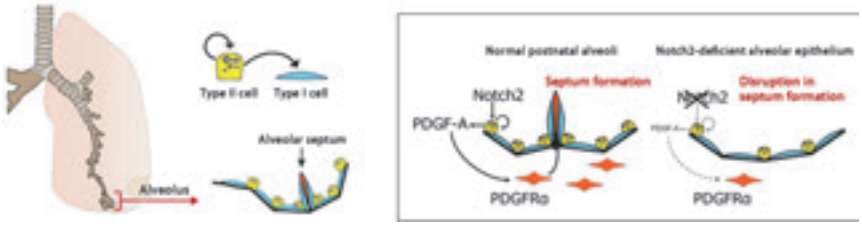
of Notch in postnatal alveolar development. Thus, to carry out a more detailed analysis of alveolar development, the research team examined a Notch-deficient mouse line which shows moderate lethality, as well as a few knockout mouse lines for Notch signaling-related genes that partially impair Notch function. Analysis of a *Pofut1* knockout mouse line which leads to inactivation of epithelial Notch signals revealed that progression of prenatal pulmonary development is relatively normal, but abnormalities arise postnatally during alveologenesis. The lungs of *Pofut1* mutants were reminiscent of the emphysema-like phenotype with enlarged airspaces in the alveolar structure. Notch2 was discovered to be the critical signal required for postnatal alveologenesis, with the lungs of *Notch2* mutants displaying significantly reduced numbers of type II cells in the alveoli. The team determined that a drop in proliferation of type II cells in *Notch2* mutants that may lead to overall reduction numbers of type II cells seen in alveoli, as type II cells serve as progenitors to generate both type I and type II cells.

Notch-deficient mice also exhibited abnormalities in the mesenchymal cells of alveoli in addition to the epithelial cells. During postnatal alveolar development, the alveolar septa begin to form from the mesenchymal myofibroblast cells lining the pulmonary epithelium, increasing pulmonary surface area for gas exchange. In the *Notch2*-deficient mouse, the number of myofibroblast cells found in the distal respiratory branches were reduced which in turn resulted in fewer septa formation. These myofibroblast cells are known to be activated to proliferate through PDGF-A, a growth factor secreted by the epithelial cells and known as a downstream factor of Notch signaling. PDGF-A expression was indeed reduced in Notch2-defective epithelium. Thus, loss of Notch signals disrupted epithelial-mesenchymal interactions, causing severe abnormalities in alveolar morphogenesis.

“Our present study focused on developmental events occurring postnatally, rather than those seen prenatally, and has opened up new avenues of research. Emphysema is caused not only by impaired alveolar development, but can arise during adulthood triggered by external factors or from aging. Thus, understanding postnatal lung morphogenesis may help us to discover ways to regenerate damaged lung tissues,” explained Morimoto. “There are many factors at work in an *in vivo* system that make it difficult to gain clear insights into normal developmental processes. We are now working to develop an *in vitro* system to recapitulate pulmonary development, which would allow us to clue in on the key mechanisms required for this process.”



Histological comparison of the lungs in wildtype and in Notch-deficient (*Pofut1* mutant) mice. Whereas embryonic lungs were indistinguishable, after birth, the mutant lungs displayed abnormal alveologenesis, with airspaces within tissue becoming enlarged and tissue becoming less dense (left). Mutant lungs had fewer numbers of proliferating type II cells (light blue and arrowheads) (right).



Role of Notch2 in postnatal alveologenesis

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Trehalose important for maintaining body water homeostasis

Miki YOSHIDA

Trehalose is a disaccharide sugar synthesized and used as a source of energy in many plants and insects. In insects, trehalose is the primary sugar compound circulating in the hemolymph (insect circulatory system), and in addition to being an energy source, is thought to play an important role in preventing desiccation due to its stability and chemical inertness. There have been some reports of insects in dry environments having higher levels of trehalose in the hemolymph than their counterparts in other environments. However, many aspects of the physiological role of trehalose and its metabolism remain largely unknown.

A study carried out by CDB student trainee Miki Yoshida of the Laboratory for Growth Control Signaling (Takashi Nishimura, Team Leader) and others revealed the physiological functions of two enzymes involved in trehalose metabolism in *Drosophila*—the trehalose-synthesizing enzyme, trehalose-6-phosphate synthase (Tps1), and the trehalose-hydrolyzing enzyme, trehalase (Treh). They also show the importance of trehalose metabolism for normal growth and development, maintaining systemic water homeostasis, and tolerating desiccation. Their work was published in the online journal, *Scientific Reports*.

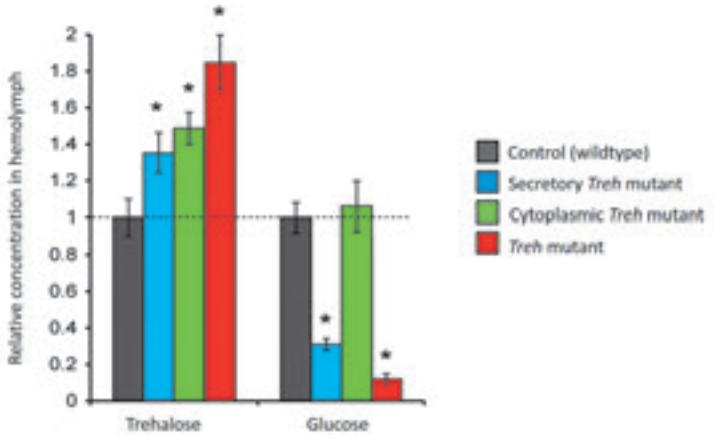
In insects, trehalose is synthesized in the fat body, the insect-equivalent of the mammalian liver, by Tps1, and then released into the hemolymph. Using a *Drosophila Tps1* mutant strain, the lab previously demonstrated that lack of trehalose under starvation or nutrient-deficient conditions has significant effects on growth and viability of the fly. The Treh enzyme, in contrast to Tps1, functions to breakdown trehalose into two glucose molecules through hydrolysis. Treh is found in two different forms in the body, a secreted form and a cytoplasmic form, due to alternative splicing, but their respective physiological roles remained unclear.

The group began by taking a close look at *Drosophila* carrying mutations in the gene encoding Treh. Using the CRISPER/Cas9 system, they generated different *Treh* mutant strains—for the secreted form, for the cytoplasmic form, and for both forms of Treh. *Treh* mutants for both forms did not survive past pupal stages. Cytoplasmic *Treh* mutants also displayed lethality during pupal stages, whereas secreted *Treh* mutants produced viable adults, indicating that cytoplasmic Treh plays a more critical role in development, especially beyond the pupal stages.

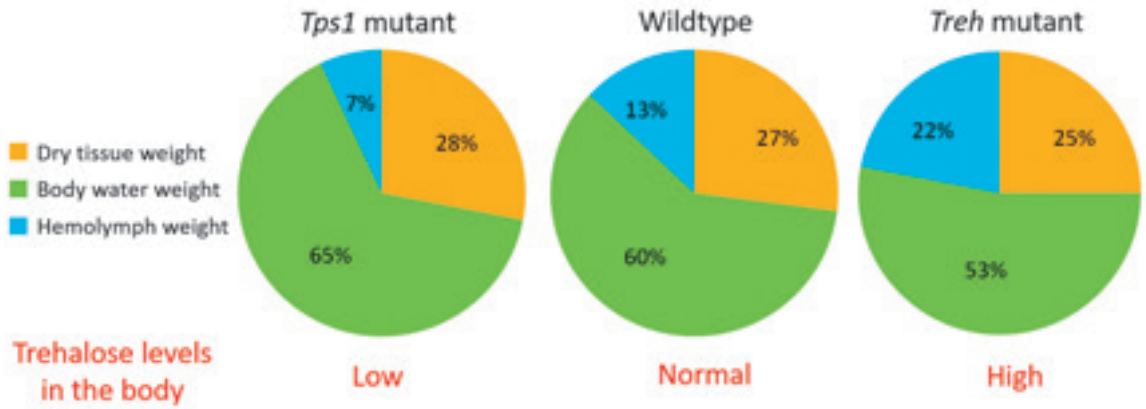
The team next examined the *Tps1* mutant strain and *Treh* mutant strain to determine the function of trehalose in

Drosophila. *Tps1* mutants are unable to synthesize trehalose, and consequently have lower levels of trehalose in the body than found in wildtype. *Treh* mutants on the other hand are unable to breakdown trehalose, and thus have higher levels of trehalose than wildtype. They found that both mutants showed markedly reduced levels of glucose, and had lower tolerance for starvation and nutrient-deficient conditions. The group also analyzed the relative water content distribution within the body, and found that when trehalose levels were high, water content increased in the hemolymph but decreased in tissues. Thus, the hemolymph of *Tps1* mutants appeared more viscous than seen in *Treh* mutants. Interestingly, when fly mutants were analyzed for tolerance to desiccation conditions, *Tps1* and *Treh* mutants both showed higher lethality than wildtype, indicating that normal trehalose metabolism as well as high levels of trehalose is essential for protecting the fly from desiccation.

"The hemolymph does not function solely as the circulatory system to transport factors within the body. It also serves as the medium where catabolic reactions take place to produce physiologically important molecules," says Nishimura. "Here, we revealed conditions when impairment of Tps1 and Treh, two enzymes that have opposing functions, produce similar phenotypes. Our next step will be carry out a closer analysis of *Treh* mutants, focusing on features specific to this mutant. Mutants with high trehalose levels may be useful as a disease model for diabetes, which is characterized by high sugar levels in the body."



Different *Treh* mutants (secretory, cytoplasmic, null) all exhibit higher trehalose levels than wildtype. However, levels of glucose, which is the product of trehalose metabolism, is markedly reduced.



Water content distribution in wildtype, *Tps1* mutant and *Treh* mutant. Relative water content in hemolymph and tissues changes in correlation with fluctuations in trehalose levels.

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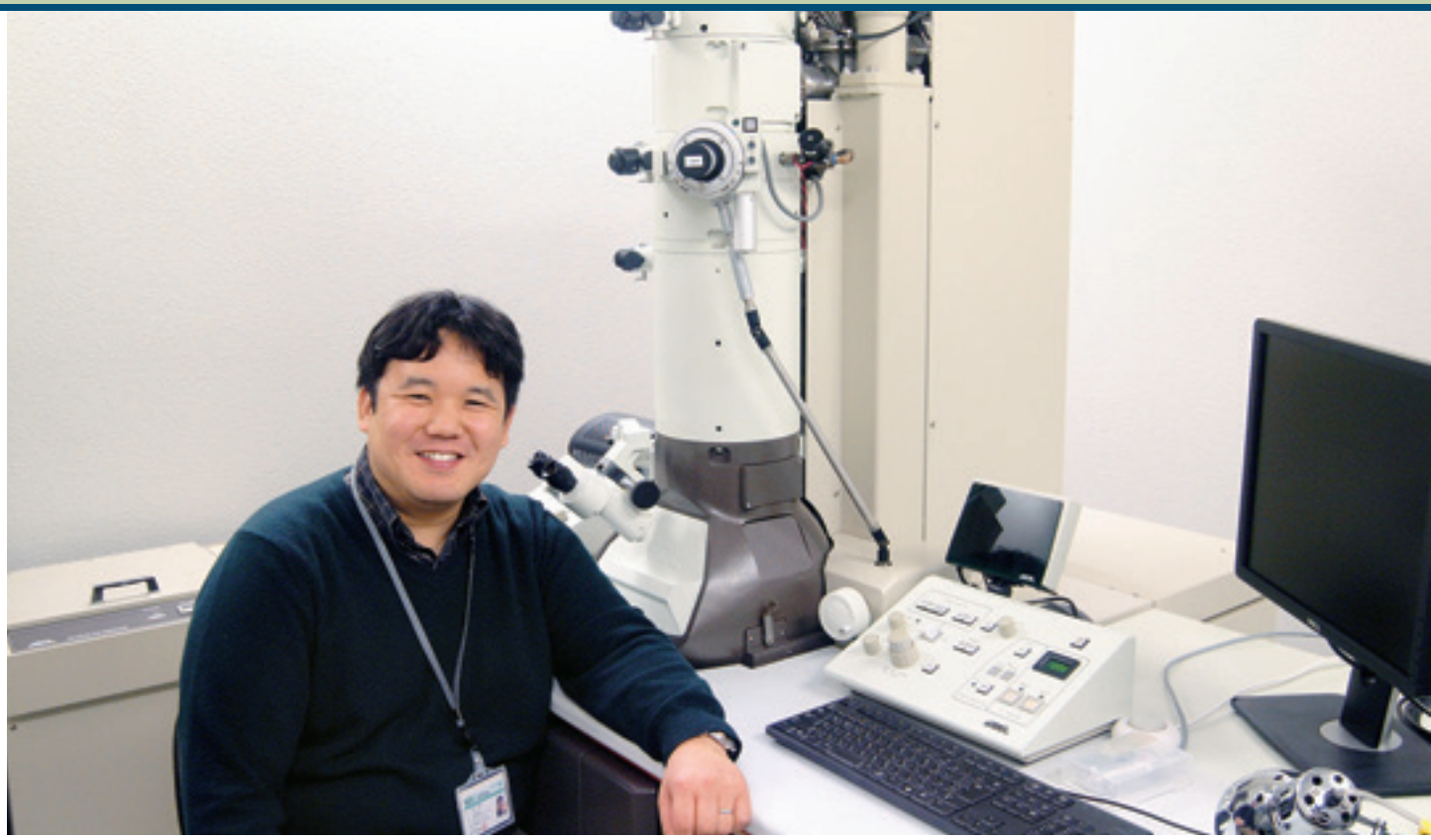
Keeping actin filaments in order in fly bristles



Tetsuhisa OTANI

The bristles on the external surface of the *Drosophila* fruit fly are mechanosensory organs that form from the robust elongation of single cells during pupal stages of development. On the inside of the bristle, parallel bundles of actin filaments are aligned in an orderly fashion along the long axis of the bristle shaft, nestled close to the cell cortex. A further magnified view of the bristle reveals that the actin filaments making up the actin bundles are aligned in hexagonal paracrystalline lattice, but how this distinct and orderly arrangement, which is essential for maintaining the robust yet supple structure of the bristle, is formed remained relatively unknown.

In a study published in *Development* by former CDB scientist Tetsuhisa Otani and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader), working in collaboration with the RIKEN Center for Life Science Technology (CLST)'s Ultrastructural Research Team (Shigenobu Yonemura, Team Leader), they reveal that protein kinase IKKε, important for distal tip elongation in *Drosophila* bristles, is also involved in promoting actin bundling by protecting the actin crosslinking protein Fascin from becoming phosphorylated by another protein kinase called, Protein Kinase C (PKC). Fascin, when phosphorylated by PKC is unable to crosslink actin filaments into hexagonal arrangement. IKKε uses a double inhibition mechanism to promote actin bundling by inhibiting PKC activity, which in turn prevents PKC from phosphorylating Fascin and facilitates bundling activity.

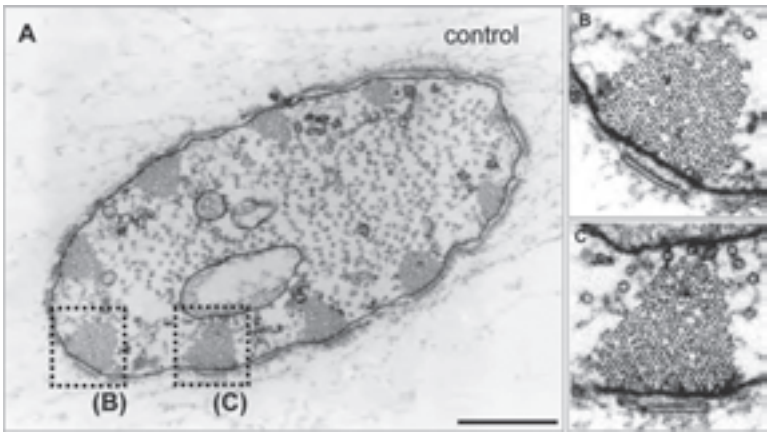
Otani previously reported that protein kinase IKKε is localized and activated at the distal tip of the elongating bristle, serving as a signaling center regulating the shuttling of molecular cargo required for elongation. They reported that fly mutants for *ikkε* showed kinked or branching bristles as opposed to straight smooth bristles because the signaling center could not be maintained at the elongating tip. These mutants were also seen to display morphological abnormalities along the bristle shaft, and the group turned their attention to uncovering other roles of IKKε in bristle morphogenesis.

The alignment of actin filaments is known to be mediated by two types of actin-binding proteins, Forked which bundles newly formed actin filaments at the bristle tip and Fascin which packages actin filaments into hexagonal arrangement. They found that of the two proteins, which normally distribute along actin filaments, in the *ikkε* mutants, only the localization of Fascin was disrupted, in addition to displaying disorganized actin filament bundles. These results led them to speculate that Fascin

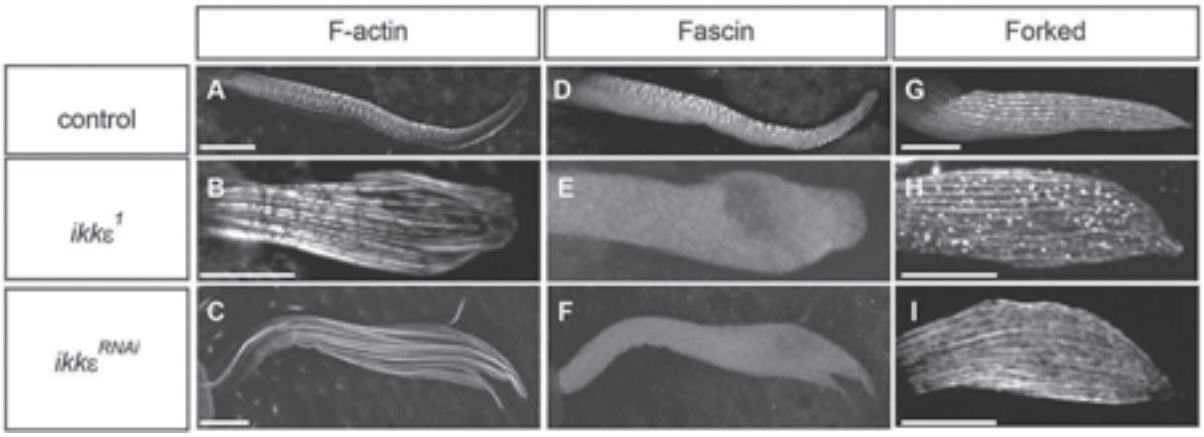
functions to bind actin filaments together, and that IKKε regulates Fascin-mediated actin bundling.

How exactly does IKKε regulate Fascin? Phosphorylation of Fascin by PKC was known to disrupt actin bundling activity in bristles. Thus, the team examined the different mutant fly strains to determine the relationship between IKKε, PKC, and Fascin. They determined that Fascin localizes along actin bundles only in the unphosphorylated state, and that phosphorylation by PKC disrupts Fascin localization along actin bundles. IKKε was also found to play a role in inhibiting phosphorylation of Fascin by PKC. As PKC is known to be activated when it translocates and binds to the cell cortex, the team showed, using cultured cells, that IKKε could prevent PKC activation. These results indicate that IKKε could indirectly maintain Fascin-mediated acting bundling through the inhibition of PKC activity.

"PKC activation is not required for normal bristle formation as *pkc* mutants show no visible abnormalities in bristle morphology. What then is the merit of adopting a double inhibitory mechanism to regulate bristle formation?" says Hayashi. "Having a factor like IKKε keeping PKC activity in check may be the key to maintaining safe and stable cell and body functions, as PKC plays a central role in many intracellular signaling pathways and errors in regulation of PKC activity can lead to cancer or lifestyle diseases, such as diabetes."



Electron-microscopic image of bristle cross section. Actin bundles spaced at regular intervals along the cell cortex (A). Magnified view of actin bundles reveal individual actin filaments are arranged in hexagonal paracrystalline arrangement (B,C).



In wildtype flies (top row), Fascin and Forked localize along actin filaments in wildtype flies. In *ikkε* mutant strains (middle and bottom rows), morphology of bristle is abnormal and Fascin localization was disrupted (E, F).

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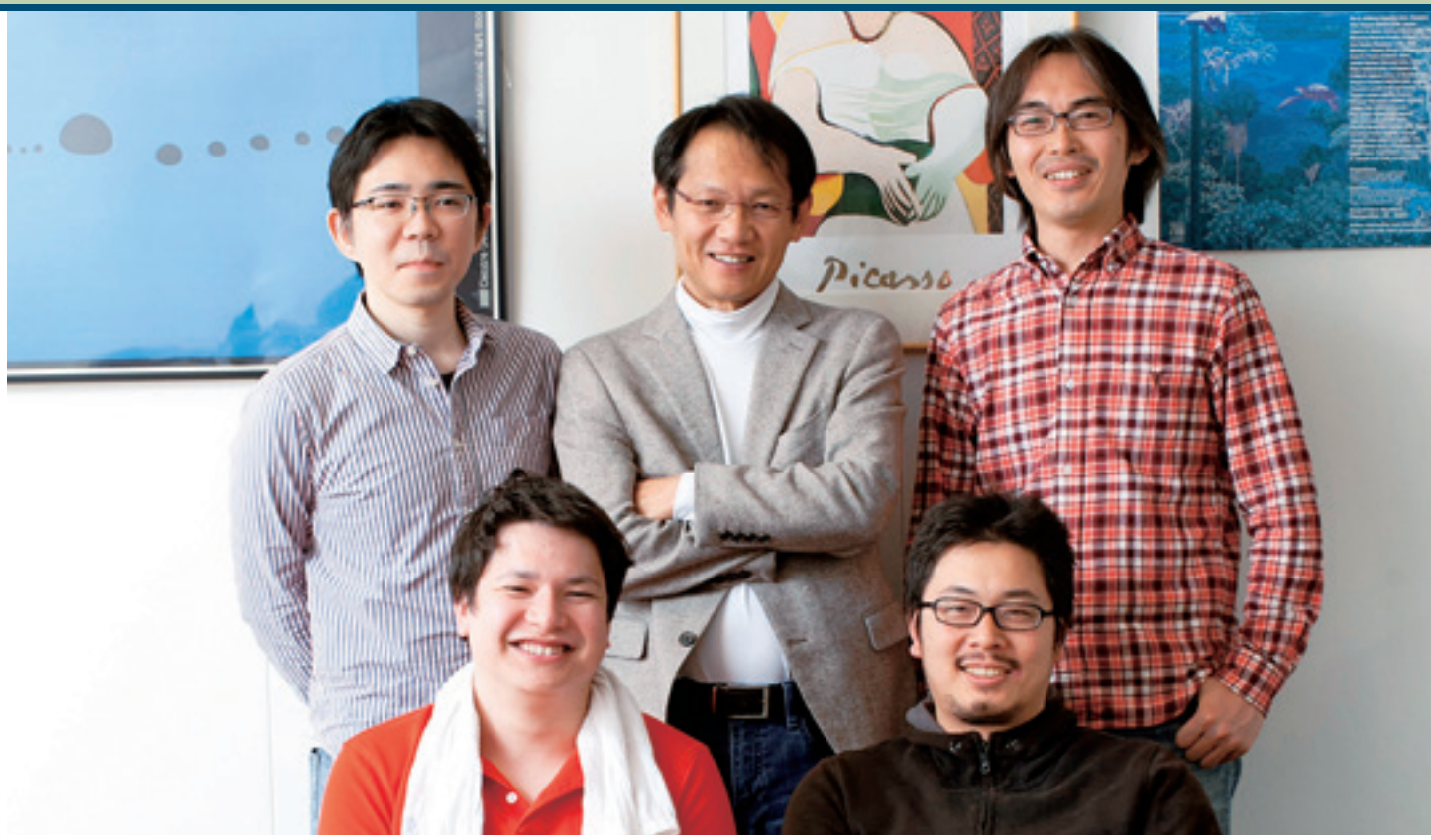
New tool for introducing genes into developing mammalian brain



(From left) Ikumi FUJITA, Raymond TERHUNE-KUNIKANE, Fumio MATSUZAKI, Yuji TSUNEKAWA, Atsunori SHITAMUKAI

In utero electroporation is a technique that is widely used in the study of brain development to introduce DNA or RNA into the brain of the developing embryo inside the uterus by applying electrical pulses. This method can be used to label cells by inducing fluorescent protein expression vectors, or to modify gene function by overexpression, mis-expression or knocking down expression of a specific gene and examining phenotypes at tissue level. It remained difficult, however, to modify the gene itself using *in utero* electroporation. If gene knock-in was made possible via *in utero* electroporation, it would be possible to alter the genome of a specific cell population in the brain during development, facilitating labeling of cells, lineage tracing, or analyses of the localization and dynamics of a protein at the cellular level *in vivo*.

Research scientist Yuji Tsunekawa, student trainee Raymond Terhune and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Team Leader) have now developed a new tool for introducing genes into the developing mammalian brain by combining the use of the CRISPR/Cas9 system and *in utero* electroporation. In a paper published in *Development*, they demonstrate the high efficiency of their gene knock-in method to insert transgenes into a target site in neural progenitors in the embryonic mouse brain. Furthermore, the team refined their protocol to insert two different colored fluorescent markers into a target gene in each homologous chromosome, which enabled them to visualize by color the cells with homozygous knocked-in alleles.

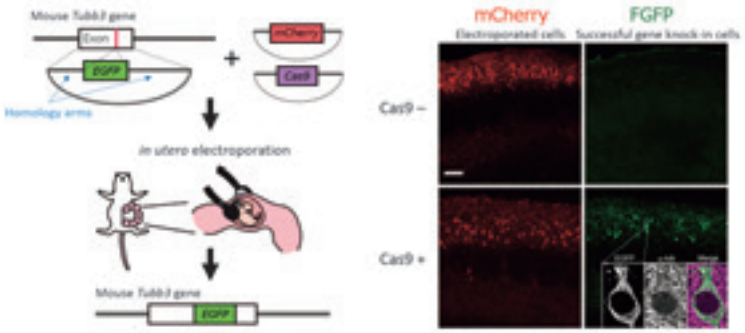
The success of a gene knock-in protocol is dependent on the efficacy of gene targeting events, which is usually very low. Invention of the CRISPR/Cas9 system has enabled the genome to be edited with ease and high precision. Tsunekawa and his collaborators have been working to develop CRISPR/Cas9-based gene-editing techniques that can efficiently modify the genome, and have more universal applications, one of which culminated in the development of HITI, which can efficiently knock-in genes in differentiated cells *in vivo* (Suzuki K, et al., *Nature* 2016). Concurrently with the development of HITI, the team tried developing a simple gene knock-in method targeting neural progenitor cells in embryonic brain *in utero* by combining the CRISPR/Cas9 method with *in utero* electroporation.

CRISPR/Cas9 system-based genome-editing technology exploits the two intrinsic DNA repair pathways of cells: homology-directed repair (HDR) and non-homologous end joining (NHEJ). Whereas the HITI approach used the NHEJ pathway to insert genes into both proliferating and

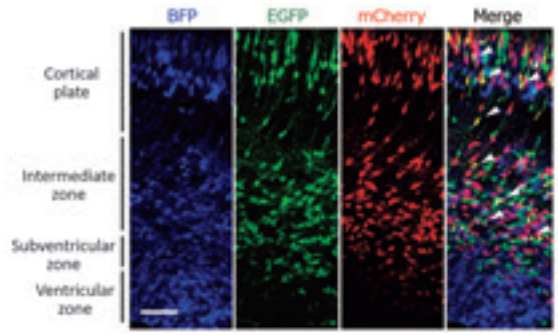
non-proliferating cells, to specifically target proliferating neural progenitors in the brain, the team elected to use the HDR pathway, which functions only in actively dividing cells but is more accurate and efficient. They first designed the vector such that the EGFP sequence to be inserted was flanked by homology arms and tested their method to knock-in EGFP (green) fluorescent marker targeted to the *Tubb3* gene, a neural marker. They found EGFP was knocked-in in approximately 20% of the electroporated cells. Proving that their concept did work, the team then determined the best homology arm lengths for efficient gene insertion and optimizing the vector template to minimize leaky expression.

Genes knocked-in to the cell are inserted at random into one target loci of homologous chromosomes, thus resulting in cells carrying gene insertions in either one of the two loci (heterozygous) or in both loci (homozygous) of homologous chromosomes. The team wondered whether it was possible to distinguish between heterozygous and homozygous targeted cells, and designed a protocol in which two targeting vectors, each with a different colored fluorescent marker, were electroporated simultaneously *in utero* into the mouse brain. The theory was that cells in which both loci had been knocked-in would fluoresce both colored signals to produce a different colored signal, thus indicating a cell carried homozygous knocked-in alleles. When the team tested their protocol targeting *Tubb3* gene with EGFP (green) and mCherry (red), they found cells expressing both EGFP and mCherry produced a yellow signal, indicating that donor sequence had been knocked-in to both *Tubb3* gene loci of those cells (approximately 5% of the electroporated cells).

"Efficient observation of simultaneous fluorescence of two different colored signals is made possible by the CRISPR/Cas9 system's efficiency for introducing transgenes. Using this method, we can now identify cells with homozygous knocked-in alleles, and also trace cell lineages with live-imaging," explains Matsuzaki. "We also showed that our new protocol can be used in ferret models, demonstrating that this can be used for non-rodent animals for which generating genetically modified animals is difficult, and spur research advances in neurosciences."



Left: Schematic of gene knock-in using *in utero* electroporation. Right: Efficient knock-in demonstrated using CRISPR/Cas9 system. Scale bar, 100 µm.



When two different colored fluorescent markers (EGFP, green; mCherry, red) were electroporated simultaneously, cells with homozygous knocked-in alleles can be identified by yellow fluorescence in the Merge panel. BFP (blue) indicates all electroporated cells. Scale bar, 50 µm.

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Transplanted iPSC-derived retinal cells not rejected if MHC-matched with recipient

Sunao SUGITA

Cells of an animal's immune system are capable of differentiating between self and non-self cells by recognizing proteins expressed on the cell surface called, major histocompatibility complexes (MHC). In humans, these cell surface proteins are called human leukocyte antigens (HLA). When immune cells encounter MHCs they do not recognize, an immune response is triggered to rid the body of the foreign entity. This mechanism is important for protecting the body from pathogens, but can be a hindrance when considering cell or organ transplantation from one individual to another.

A research team led by Sunao Sugita, deputy project leader of the Laboratory for Retinal Regeneration (Masayo Takahashi, Project Leader) have now demonstrated experimentally that immune responses can be avoided when retinal pigment epithelial (RPE) cells generated from induced pluripotent cells (iPSCs) derived from a MHC/HLA homozygous donor are matched with MHC/HLA of recipient. The research team developed an *in vivo* experimental method in a primate model, in which they transplanted RPE cells derived from iPSCs from a MHC homozygote monkey into a different monkey, and an *in vitro* experimental system using RPE cells derived from human iPSCs and co-cultured them with human immune cells, including T cells. Their study was published in two separate papers in *Stem Cell Reports*.

The laboratory has been working to establish treatments for retinal diseases using iPSC-derived cells. They developed a method to generate RPE cells from human iPSCs, and in 2014, performed the first autologous RPE transplantation into a human patient with age-related macular degeneration (AMD), a disease that damages the RPE cell layer of the retina. As the original cell source for autologous transplantation is the patients themselves—iPSCs derived from patient's own cells are differentiated into RPE cells, and later transplanted back into the patient—the potential for rejection was considered minimal. However, as this method is costly and time-consuming, the group has been considering allogeneic transplantation (iPSC-derived cells generated from one person transplanted into another person) as an approach to overcome these issues. While allogeneic transplantation raises concerns for risk of immune rejection and need for use of immunosuppressant drugs, in theory, matching MHC/HLA of graft and recipient should reduce potential for immune rejection, similar to organ transplantation, but this had not been tested before for iPSC-derived cells.

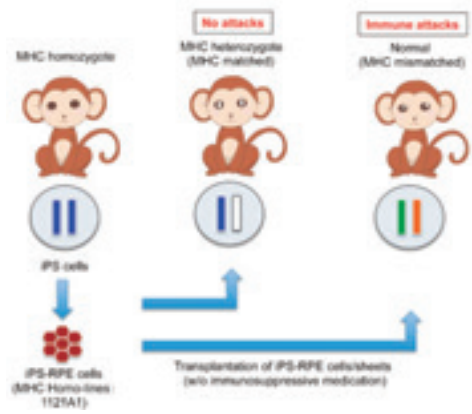
Sugita S, et al. Lack of T-cell response to iPS cell-derived retinal pigment epithelial cells from HLA homozygous donors. *Stem Cell Reports* 7(4). 619–634 (2016)
Sugita S, et al. Successful Transplantation of Retinal Pigment Epithelial Cells from MHC Homozygote iPSCs in MHC-Matched Models. *Stem Cell Reports* 7(4). 635–648 (2016)

The team generated RPE cells and sheets from monkey iPSC lines with an MHC homozygous profile, and then transplanted these RPE cells or sheets into the sub-retinal space of the eyes of MHC-matched or mismatched monkeys. In MHC-mismatched monkeys, the team observed retinal tissue damage and signs of immune rejection, such as infiltration of inflammatory cells into the subretinal space, following transplantation. On the other hand, no signs of immune rejection were observed in recipients of MHC-matched grafts, and the sheet grafts survived for at least six months post-transplantation without the need to use immunosuppressant drugs.

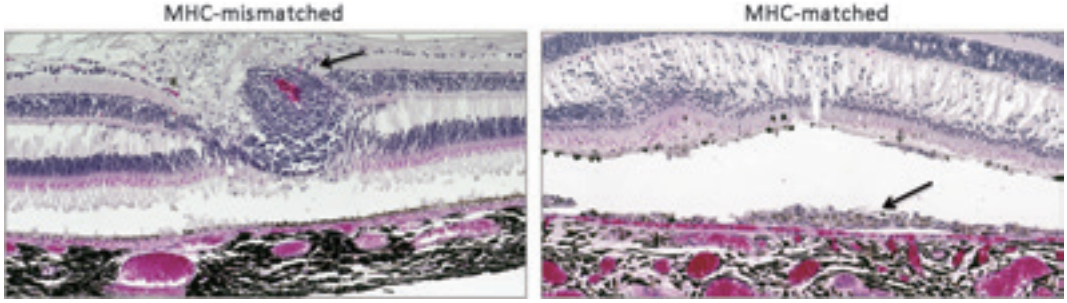
Sugita and his colleagues also examined immunogenicity using an *in vitro* experimental system with human iPSC-derived RPE cells. They generated iPSC lines from HLA homozygous donors, and then differentiated them into RPE cells, which were then co-cultured with lymphocytes isolated from a different donor. The researchers found that when HLA types were mismatched, there was an increase in inflammatory cell numbers as well as a rise in levels of IFNɣ inflammatory cytokine, similar to what is observed in early stages of an immune response. In contrast, the matching of at least three HLA gene loci (*HLA-A*, *HLA-B*, and *HLA-DRBI*) between iPSC-derived RPE cells and the donor immune cells was sufficient for preventing the trigger of an immune response. The HLA matching of these three loci have been known to be critical when considering organ transplantation or bone marrow transplants, and the present study demonstrated for the first time that the same is true for allogeneic transplantation of iPSC-derived RPE cells.

The laboratory announced the start of a new clinical research study in June 2016, which includes plans to use the iPSC bank established at the Center for iPS Cell Research and Application (CiRA) at Kyoto University to derive RPE cells for allogeneic transplantations.

“The potential risks posed to the whole body in using immunosuppressant drugs is a high price to pay when considering the size of the eye. But our current study shows that these drugs may not be needed if at least three HLA loci are matched,” explains Sugita. “While RPE cells were the focus of our studies, the *in vivo* and *in vitro* experimental systems that we developed may be adaptable and prove useful for other groups working towards iPSC-based clinical applications in other tissues or organs.”



Schematic of *in vivo* experimental model using monkeys. When RPE cells generated from monkey MHC homozygote iPSCs were transplanted into MHC-matched recipient monkey (illustrated as blue gene) no immune rejection was observed.



Left: When MHC-mismatched, an inflammatory nodule with many infiltrating inflammatory cells was observed around the graft.
Right: When MHC-matched, no signs of rejection were observed and graft survived in subretinal space.

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Disoriented axons lead to olfactory disorder after trauma

Aya MURAI

Olfaction or sense of smell, while not critical for life, can greatly influence the quality of life. The olfactory system is capable of detecting and discriminating a wide range of airborne odor molecules in the air; odorants inspired into the nasal cavity contact odorant receptors (ORs) expressed by olfactory sensory neurons (OSNs) of the olfactory epithelium, and the OSNs then relay the information via the glomeruli in the olfactory bulb (OB) to the mitral and tufted cells, the second order neurons in the olfactory system. While the olfactory neural circuits are stably maintained throughout life, damage to OSN axons due to head trauma in adults can lead to an olfactory disorder called dysosmia, where patients experience reduced odor sensitivity as well as unpleasant perception of different odors. It has yet to be shown how the neural circuitry is affected after OSN axon injury, which may yield hints for ways to treat dysosmia.

Junior research associate Aya Murai and others in the Laboratory for Sensory Circuit Formation (Takeshi Imai, Team Leader), in collaboration with Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, carried out a detailed analysis of a mouse model for dysosmia, and discovered that during the recovery process following axonal injury, OSN axonal targeting was impaired and the connectivity between axons of OSNs and dendrites of mitral cells was also markedly reduced. Their study, published in the online journal *eNeuro*, unveiled the mechanism underlying dysosmia pathogenesis.

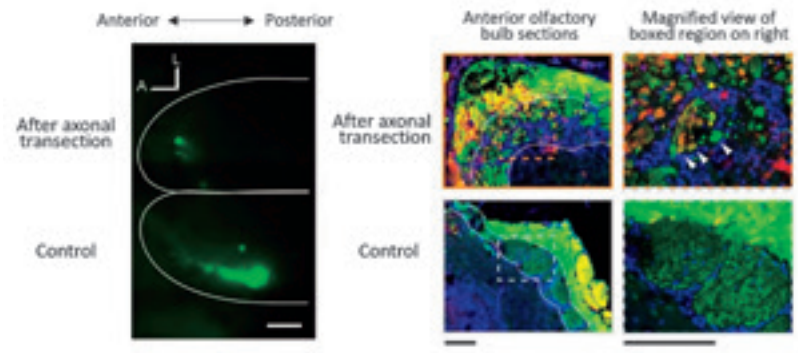
The neural circuitry of the olfactory system is elaborate and complex. Each OSN expresses a single OR type, and OSNs expressing the same ORs all converge their axons to one glomerulus in the OB. Olfactory information is interpreted from the activation patterns of over 1,000 sets of glomeruli, stimulated by over 1,000 types of OSNs. Unlike other neurons, OSNs are unique in that they can be regenerated, and undergo continuous turnover throughout life, while maintaining the original topographical circuitry. After damage to OSN axons by head trauma, newly generated OSNs often show impaired axonal connections, but why this occurred was not well understood.

The team surgically transected a portion of OSN axons projecting to the olfactory bulb to generate a mouse model simulating axonal injury following head trauma, and then examined the recovery process of the OSN, searching for the mechanism underlying dysosmia. Normal OSN turnover takes place in two phases; first, OSN roughly determine the anteroposterior course to project

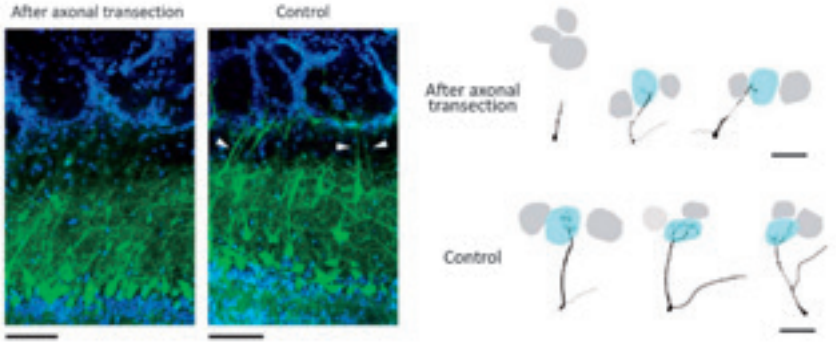
their axons, and then as axonal projection progresses, will refine their path as needed to their glomerulus target. In contrast, in the axonal injury mouse model, the initial anteroposterior course targeting of axons was often impaired, with axons extending a direction opposite to that seen in normal OSN turnover. Their experiments also revealed a concentration of mistargeted axons in more anterior region of olfactory bulb and formation of small glomerulus-like structures innervated by heterogeneous mix of OSN axons.

They also analyzed the mitral cells, which relay signals received from the glomerulus to the olfactory cortex. Mitral cells are normally connected to the glomerulus through a single primary dendrite, but after OSN axonal injury, only half of the mitral cells were found to maintain connections with OSNs, and the arborized tips of the primary dendrites showed signs of atrophy contributing to reduction in connectivity. Live imaging *in vivo* of mitral cell activity also confirmed that axonal injury model showed reduced olfactory sensitivity than normal mouse.

"When OSN axons are severed due to severe head trauma, we saw impaired targeting of axons as well as reduced connectivity to mitral cells. This suggests that in normal OSN turnover, the existing OSN axons act as part guide and part scaffold to direct new OSN axons to the correct target," explains Imai. "Thus, degeneration and eventual loss of 'scaffold' axons after injury appears to be the underlying cause of dysosmia. It may be possible to prevent dysosmia if OSN axon degeneration after injury can be delayed enough to allow newly generating OSNs axons to be guided to their correct targets. We would like to continue seeking hints to treat dysosmia."



Left: Olfactory bulb viewed from above. After axonal transection, axonal projections show a large anterior shift (green: OSNs expressing MOR29B, one type of OR; scale bar 500 µm). Right: Cross-section of olfactory bulb viewed from front. Nrp1-positive axons normally targeted to the posterior were mistargeted to anterior. Nrp1-positive and -negative axons were jumbled and formed small glomerular-like structures (blue, nucleus; green, ONS; red, Nrp1; scale bar 100 µm).



Left: Arborization of mitral cell dendrites is greatly reduced after axonal transection (blue, nucleus; green, mitral cell). Right: When the mitral cell dendrites were traced, they were found to rarely innervate the glomerular layer, and showed less arborization. Scale bar, 100µm.

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One signal, two cell fates

Guangxia MIAO

Tubular networks in the body are essential for transporting fluids such as blood through blood vessels, or promoting exchange of gases in the trachea and bronchiolar structures of the respiratory system. Formation of tubular networks are triggered by signaling factors secreted during early stages of development, which directs some cells to become so-called 'leading' tip cells to begin protruding outward from an initially spherical epithelial cyst and lead 'follower' stalk cells to specific locations to form the branching structures. In tracheal tube formation, FGF signaling is known as the major branching signal for specifying tip cells. Whereas in most animal species only one tip cell is specified, in *Drosophila,* two tip cells are in fact specified by FGF. Interestingly, these two tip cells will later differentiate into two distinct cell types. One becomes a fusion cell (FC) that connects branches of tracheal primordia from adjacent body segments together by undergoing topological change into a donut shape, and the other differentiates into a terminal cell (TC), which acquires a complex bifurcating and lumenal structure, and functions similar to bronchioles. The mechanism underlying the divergent cell fates of tip cells remained poorly understood.

A study led by research scientist Guangxia Miao of the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader) zoomed in on the *Drosophila* tracheal primordia and the mechanisms involved in sequential specification of two tip cell fates by a single inductive FGF signal. Their work, published in *Development*, revealed that whereas FGF initially specifies FC fate and directs branch migration to their destination, once there, the expression of transcription factor Escargot (Esg) suppresses FGF signaling in the FC and permits activation of FGF signaling in an adjacent cell to differentiate into a terminal cell (TC), resulting in two cells with distinct properties.

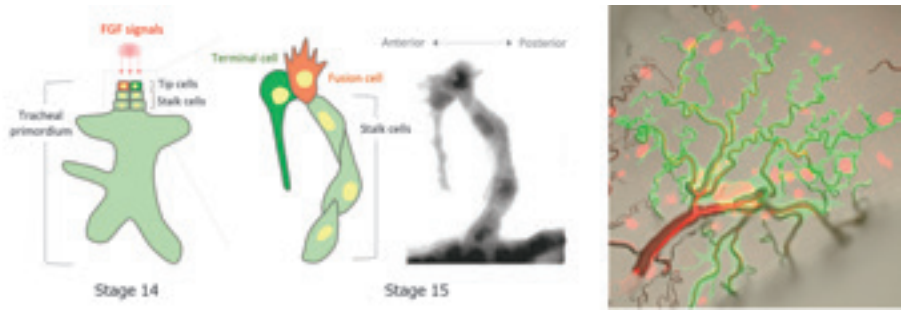
FGF signaling has been known to play multiple roles during tracheal tube formation. First, it specifies the FC-type of tip cells that will lead branch migration. Following specification, FCs will in turn inhibit FGF signaling in neighboring cells through activation of Notch-Delta, converting them into stalk cells that will trail behind tip cells. FGF signaling is also involved in guiding FC migration and differentiation of tip cells into TCs. How this single FGF signal can control multiple aspects of cell differentiation and cell motility was unclear.

The team began by analyzing the distribution of the cells in which FGF signaling was activated. During early stages of tracheal development, downstream factors of

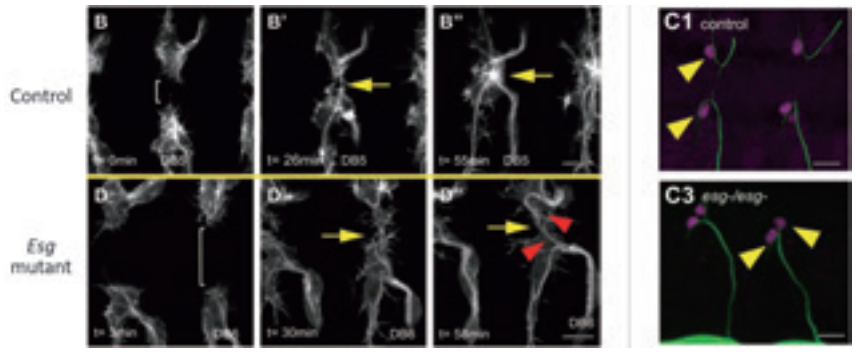
FGF signaling were activated in a small group of cells in the primordium that includes the future FC and TC, but by stages 13 to 15, FGF signaling in these cells, with the exception of the TC, was lost. The team then turned their attention to Esg, whose expression appeared to be linked with FC specification. Analysis of *Esg* mutant flies revealed that FCs in these mutants failed to fuse with its partner FC, continued to migrate and elongate, and as seen in TCs, formed bifurcations. Further, *Esg*-mutant FCs were confirmed to show TC-like properties such as activated FGF signaling and expression of TC-specific marker genes. These findings indicated that Esg is expressed specifically in FCs, functioning to suppress FGF signaling and prevent FCs from differentiating into TCs.

When the team used live-imaging to track FC and TC positions, they found that between stages 14 to 15, FCs and TCs always undergo an anterior-to-posterior positional shift. This positional shift appeared to be critical for establishing the tracheal network, but it was not clear what signal was controlling this event. Using the technique previously developed by the lab, in which ectopic FGF expression could be induced at any place and time by laser irradiation, they examined the behavior of FCs and TCs to FGF expressed ectopically in different areas around the nascent tube. FCs moved away from FGF signals, whereas TCs moved toward FGF signals and created new bifurcations. In *Esg* mutants, FCs lost its repulsive response to ectopic FGF. Thus, the positional shift is brought about by the difference in responses between FCs and TCs to FGF signals.

"Analogous to train track switching devices, Esg coordinates time-lagged cell responses of tip cells to FGF, which results in two cells with distinct properties," explains Hayashi. "There are more cell types in the body than there are signaling factors. Thus the cells must adopt strategies allowing it to reuse a single signal to generate cell type diversity, and this study gives a glimpse into one such mechanism that evolved in organisms."



Left: Diagram and microscope image of branching morphogenesis of *Drosophila* trachea system. Tip cells and stalk cells are specified at stage 14, and the tip cells differentiate into FC and TC at stage 15. Anterior is to the left, and posterior is to the right. Right: The TC is a single cell, but is capable of acquiring a complex bifurcating and lumenal formations, and functions similarly to mammalian bronchioles.



Left: In *Esg* mutants, FCs fail to fuse with their partner FCs, resulting in multiple branching. Right: Prospective FCs in *Esg* mutants become TC-like, resulting in two TCs. Scale bar, 10μm.

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In vitro model for spinocerebellar ataxia developed using patient-derived iPSCs

Keiko MUGURUMA, Yoshihito ISHIDA

The cerebellum is a vital region of the brain intimately involved in the regulation of motor coordination and some aspects of cognition. Disorders affecting the cerebellum can be debilitating, leading to loss of speech articulation and uncoordinated and unsteady gait. One group of hereditary genetic diseases linked to the cerebellum is spinocerebellar ataxia (SCA), an intractable disorder characterized by progressive loss of motor coordination, while the patients retain full mental capacity. There are numerous types of SCA and many causative genes have been identified, but much remains unknown about underlying molecular mechanisms of SCA pathogenesis and no effective treatments are currently available for this disease. Thus, there is a need for a suitable model system to study SCA pathogenesis and to screen for drug compounds that may be effective for treating SCA.

Now, a study by CDB visiting scientist Yoshihito Ishida*1 and research specialist Keiko Muguruma of the Laboratory for Cell Asymmetry has reported the successful generation of mature Purkinje cells from induced pluripotent stem cells (iPSCs) derived from SCA type 6 (SCA6) patients. They further revealed that when placed under conditions of stress, the patient-derived Purkinje cells exhibit some vulnerabilities, and demonstrated that this stress culturing method can be used as an *in vitro* model system for evaluating drug candidates for treating SCA. Their work, published in the journal *Cell Reports*, was carried out in collaboration with Hiroshima University and Kyoto University.

SCA6 is characterized by selective degeneration and loss of Purkinje cells in the cerebellum, and cerebellar atrophy. It is caused by a mutation in the *CACNA1A* gene encoding the Cav2.1, the α1-subunit of a calcium channel. The mutated gene has an expanded CAG trinucleotide repeat in the region encoding the Cav2.1 C-terminal domain, resulting in an abnormally long section of glutamine residues (or polyQ). Muguruma, who previously reported the successful generation of mature Purkinje cells from human embryonic stem cells and iPSCs, and her collaborators wondered whether it would be possible to simulate pathology of SCA6 *in vitro* by applying their protocol for Purkinje cell differentiation using patient-derived iPSCs.

The team generated iPSCs from skin or blood cells of healthy donors and of SCA6 patients with heterozygous alleles or homozygous alleles for CAG repeats, and confirmed that their established iPSC lines have the same number of CAG repeats as their respective donors.

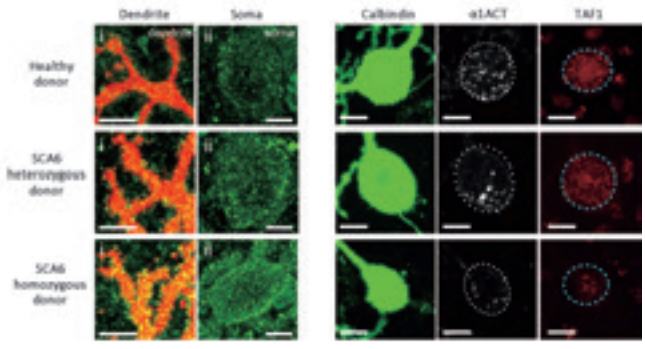
The iPSCs were then differentiated into Purkinje cells, and similar to known SCA pathology, patient-derived Purkinje cells showed high cytoplasmic levels of Cav2.1. A fragment of the C-terminal domain of Cav2.1 containing the CAG repeat, called α1ACT, also functions as a transcription factor targeting TAF1 in the nucleus, which is involved in cell growth and development. Expression levels of α1ACT and TAF1 were found be to markedly reduced in patient-derived Purkinje cells than in control Purkinje cells, which was consistent with reports from other studies indicating that α1ACT containing CAG repeat expansion loses ability to activate transcription of TAF1 and consequently inhibits neural cell growth and development.

Recapitulating *in vivo* disease pathology in a cell culture system within a relatively short period presented a challenge as SCA6 is a late-onset disease, meaning symptoms begin appearing later in life. Thyroid hormone T3 is known to be an essential factor for Purkinje cell maturation and maintenance, the group temporarily depleted T3 from their culture system to create conditions of stress and analyzed its effect on Purkinje cells. Whereas Purkinje cells from healthy donors showed no significant changes, many of the patient-derived Purkinje cells died or if they did survive, had significantly reduced dendritic arborization, indicating that T3 depletion caused vulnerability in patient-derived Purkinje cells.

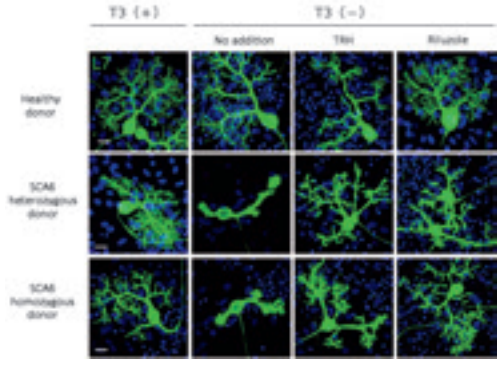
The group also explored the possibility of using their patient-derived Purkinje cells following stress for drug assays, testing different compounds on their effectiveness in reducing the vulnerabilities observed in patient-derived Purkinje cells. They found that thyroid releasing hormone (TRH), which has been used in treatments for SCA6, and a compound called Riluzole, which has been used in treatments for another neurodegenerative disorder called ALS, both displayed positive effects in improving survival and maintenance of patient-derived Purkinje cells.

"The strength of our *in vitro* model comes from combining the techniques for generating human iPSCs from patients with those for inducing self-organizing cerebellar tissues and Purkinje cells to recapitulate ontogenesis," explains Muguruma. "Other types of SCA involve degeneration of other neural cells in the cerebellum as well as in the cerebrum. With our expertise in generating cerebellar and cerebral tissues from disease-specific iPSCs, we hope to contribute to unveiling the pathology of different SCA types and other neurodegenerative diseases."

*1 Visiting scientist Yoshihito Ishida is employed by Shionogi & Co. Ltd., however the company was not directly involved in any aspect of this study.



Left: Cav2.1 accumulation levels were higher in SCA6 Purkinje cells than healthy ones, with homozygous SCA6 Purkinje cells showing highest accumulation. Right: In contrast, α1ACT and TAF1 expression levels were lower in SCA6 Purkinje cells than control Purkinje cells, with homozygous SCA6 Purkinje cells showing lowest expression levels.



Depletion of T3 from culture media created stress conditions, which led to degeneration of patient-derived Purkinje cells. Addition of TRH or Riluzole could stave off the vulnerabilities caused by T3 depletion. Scale bar, 20 μm.

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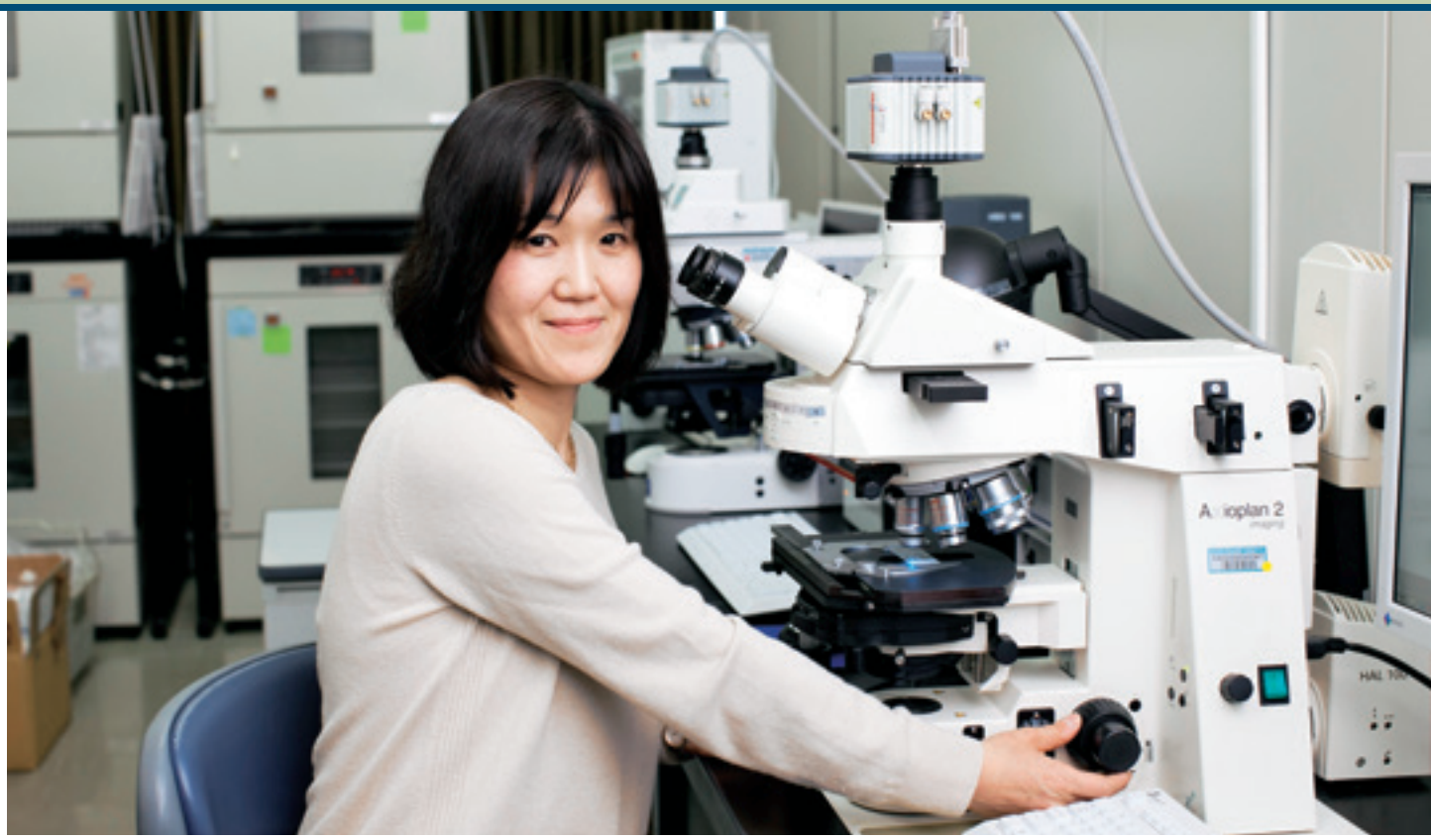
DAAM1 stabilizes adhesion between lateral membranes of epithelial cells



Tamako NISHIMURA

Epithelial cells are arranged as polarized monolayer sheets and are often found lining the surface or lumen of organs. The epithelial cell edge that comes into contact with fluids or the external environment is referred to as the apical side, while the opposite side that attaches to connective tissues is referred to as the basal side. To maintain the sheet-like configuration, epithelial cells attach to each other along their lateral membranes through a number of special junctional structures. Near the apical side is a complex of 'tight junction' and 'adherens junction,' known as the apical junctional complex (AJC), which is lined with a bundle of actin filaments (F-actin) that lies beneath the apical membrane forming a circumferential actin belt, and has been well studied to date. Below the AJC is a junctional structure called lateral membrane contacts (LCs), which spans most of the lateral membrane region and whose functions are not as well documented.

A study led by visiting researcher Tamako Nishimura in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader), published in the *Journal of Cell Biology*, took a close look at LCs of epithelial cells and uncovered a role played by DAAM1, a formin family protein associated with actin polymerization, in stabilizing the adhesion of lateral membranes of adjacent epithelial cells. They also revealed the molecular mechanism underpinning instability of LCs in the absence of DAAM1.

In a past study from the same laboratory, also led by Nishimura, in which they reported the mechanism involved in apical constriction of neural epithelial cells during neural tube formation, DAAM1 was identified as one factor recruited to the cell-cell adhesion sites to mediate constriction. Studies by other groups have reported observations of abnormal sarcomere formation and cell-cell adhesion between cardiomyocytes in *DAAM1* mutant mouse, and of reduced *DAAM1* expression possibly contributing to invasiveness of astrocytoma cells, one type of brain tumor cells; thus, suggesting DAAM1 playing another role in regulation of cell-cell adhesion (junctions).

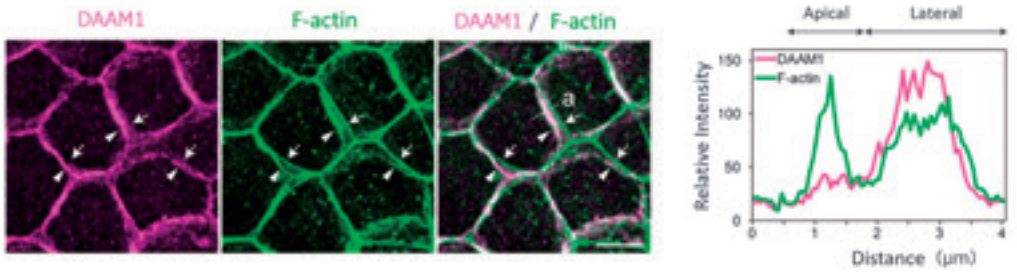
Nishimura's team used a mouse mammary gland epithelial cell line to first examine the localization of DAAM1, and found that it accumulated at epithelial junctions along the lateral membrane. F-actin was also distributed along the lateral membrane, overlapping with that of DAAM1, and differing from F-actin distribution seen in the apical actin belt. When *DAAM1* was knocked down, they observed reduced F-actin localization along

the lateral membrane, as well as larger tilt angles of LC membranes. Time-lapse imaging revealed more dynamic lateral membrane movements in DAAM1-depleted cells compared to control cells. When cells were cultured in a gel matrix for several days, control cells formed spherical cysts, with cells arranged in radial manner, whereas DAAM1-depleted cells formed disorganized aggregates, with some cells appearing to be extruded out from the aggregates. Interestingly, in co-culture of control and DAAM1-depleted cells, DAAM1-depleted cells extended long protrusions into nearby cell layers, reminiscent of behavior seen in invasive cells, when flanked by control cells. Hence, these results suggest that DAAM1 mediates stability of cell junctions through F-actin, which in turn contributes to maintenance of epithelial integrity.

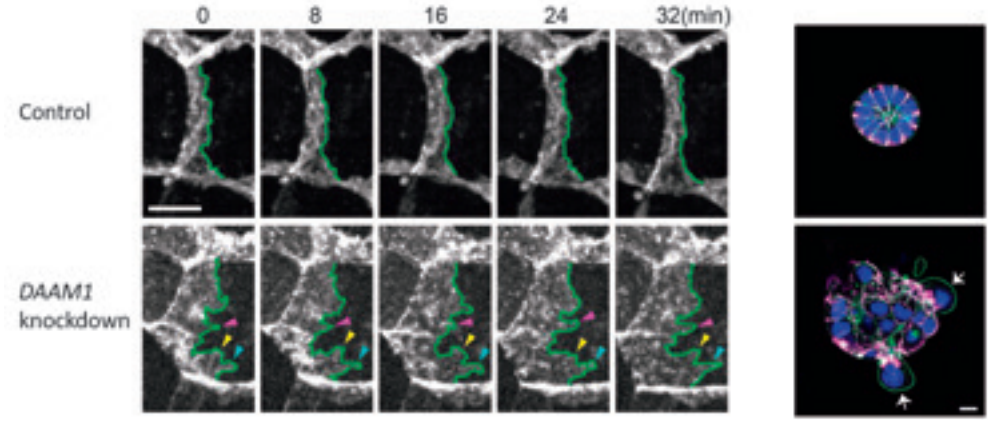
The team next investigated why the LCs become unstable after DAAM1 depletion. During normal cell migration, cell edges in the direction of migration move dynamically forming lamellipodium, and its motility is known to be mediated by a molecular complex called the WAVE complex. The team thus speculated that the WAVE complex also plays a role in LC instability following DAAM1 depletion. They analyzed the WAVE complex components in DAAM1-depleted cells and identified WAVE2 and its upstream regulator Rac as effectors of LC instability, resulting in acquired membrane motility.

"Our current study demonstrated that DAAM1 maintains stability of epithelial cell junctions by suppressing WAVE complex-mediated membrane mobility," explains Nishimura. "It was surprising to discover that epithelial cells, which are considered stable when packed together, in fact harbor the potential to acquire motility."

Takeichi adds, "The WAVE complex–mediated motility of LC membranes is likely critical for repair mechanisms involving cell migration, such as wound healing. At the same time, errors in DAAM1 regulation of the WAVE complex may lead to cancer cell-like invasive behavior. Therefore, detailed studies on DAAM1 functions *in vivo* may reveal clues into mechanisms of cancer invasion."



Left: DAAM1 was localized at LCs of epithelial cells. When cells viewed from a direction perpendicular to the cell layer, DAAM1 localization was seen to overlap with F-actin distributed in lateral membrane (arrowheads) as opposed to F-actin localized in the apical actin belt (arrows). Right: Tracings of relative signal intensity of DAAM1 and F-actin distributed along dotted line 'a' in the image to the left.



Left: *DAAM1* knockdown results in abnormal tilting of lateral membrane faces, and exhibit more dynamic motility of the lateral membrane than control cells. Right: When cells were cultured in Matrigel for several days, control cells formed cysts in which cells were arranged radially, whereas DAAM1-depleted cells showed disorganized aggregation with some cells being extruded from the cell mass.

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Lulling mice into a short hibernation-like state

Masayo TAKAHASHI, Genshiro SUNAGAWA

The approach of winter signals the start of the hibernation period for some animals, such as squirrels and bears. Hibernation, a period of days or weeks of inactivity, can be considered an extreme form of energy conservation as some animals are known to lower their metabolic rates to just a few percent of normal awake levels, thereby reducing demand for oxygen during this period. A shorter hibernation-like state (minutes to hours) called torpor is also induced in some animals, and is marked similarly by active reduction in metabolism (hypometabolism). The underlying mechanisms regulating hibernation and torpor remain largely unknown as hibernating and torpor species are few and far between, making them difficult subjects to use in research. Recently, however, there have been reports that mice, which are commonly used in research, can undergo torpor under certain conditions, hinting at possibilities of studying this phenomena in the laboratory. Understanding how active hypometabolism can be induced may benefit humans as well in clinical applications, where reducing oxygen consumption by inducing hypometabolism could slow down progress of brain damage after stroke, or help prolong storage periods for organs or tissues for transplantation.

In a study led by research scientist Genshiro Sunagawa in the Laboratory for Retinal Regeneration (Masayo Takahashi, Project Leader), published in the online journal *Scientific Reports*, they created an algorithm for detecting torpor based on actual measured values of baseline metabolism of individual mice, which can be used to stably observe and evaluate torpor states of mice. The team further analyzed the mouse thermoregulatory system between normal (awake) and torpid states to reveal a common feature between torpor and hibernation.

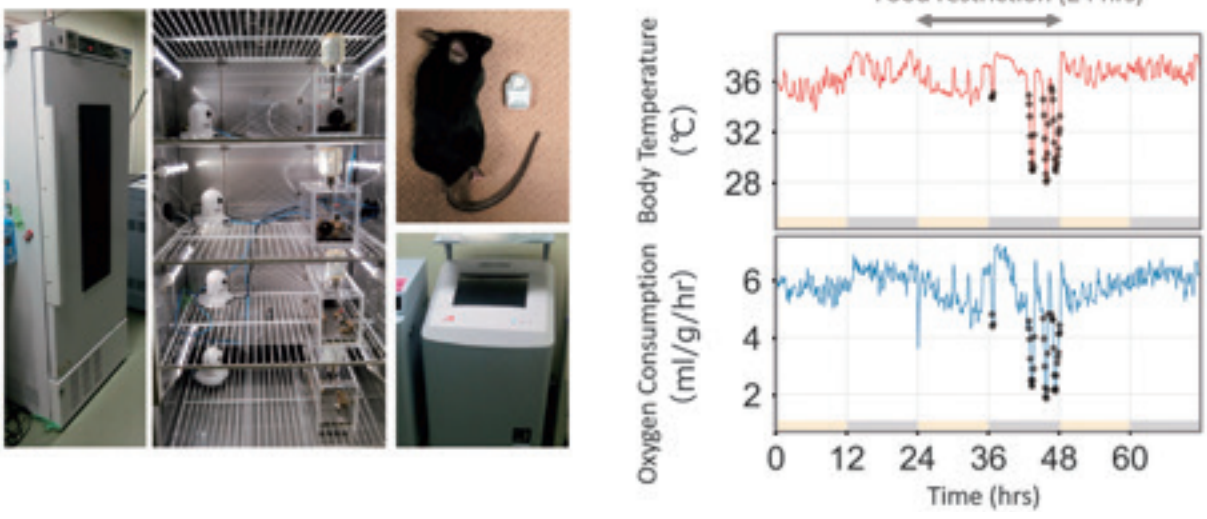
Warm-blooded animals have an internal thermoregulatory system to maintain a relatively stable body temperature. When temperatures in the outside environment drop, the body is more prone to losing heat, and consequently begins to actively generate heat to compensate for the heat loss. Normally, if this mechanism stops functioning and the body temperature falls below a certain threshold or reference temperature, the tissues and cells incur damages and eventually die, but this does not occur in hibernating or torpid animals. Instead, these animals appear to possess a mechanism that signals to the body to abandon or suppress its normally active thermoregulatory system.

Torpor takes place over a shorter period and is more unstable compared with hibernation, and even shows great variation between individual animals. This has made it

difficult to clearly define and evaluate states of torpor. The team prepared a chamber fitted with sensors to measure and record body temperature and oxygen consumption of mice under controlled ambient temperature conditions. Baseline metabolism of the individual mice were calculated from the data collected over 24 hours of observation during which time they had no physical contact with researchers, and values falling outside the range of the baseline metabolism (outliers) were defined as 'torpor.' Using their model to evaluate torpor states, they found that a constant ambient temperature between 12°C to 24°C and restricting access to food for 24 hours could stably and reliably induce torpor in mice.

The team then created a mathematical model for the body's thermoregulatory system in response to ambient temperature changes, and using the data collected from the above experiments, estimated the parameters that changed significantly. Parameters included how easily the body loses heat, the reference temperature the body is trying to maintain, the actual body temperature, and the sensitivity of the thermoregulatory system. During hibernation, both the reference temperature and sensitivity of the thermoregulatory system show a dramatic reduction. In torpid mice, they find that reference temperature shows only minimal changes, while the sensitivity of the thermoregulatory system falls to levels similar to that seen in hibernation.

"Our study demonstrates that a common feature of hibernation and torpor is that sensitivity of the thermoregulatory system is significantly reduced during these states. This suggests that to induce active hypometabolism there is either a signal relayed to the body to suppress or reduce thermogenesis, or there is a mechanism signaling to the body to ignore the default thermogenesis system. Our goal is to unveil the complete mechanism regulating this phenomena," explains Sunagawa. "This work also paves the way to use mice as a model to understand active hypometabolism, as well as seek possibilities for applying hypometabolism in clinical applications."



Left: Temperature-controlled mouse chamber. Mouse cages were placed in the temperature-controlled chamber, and oxygen consumption was recorded. A transmitter to measure body temperature was implanted into the mouse. Right: Based on actual measured values, torpor was defined as outliers from the baseline metabolism (black dots).

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HITI: An innovative *in vivo* genome-editing technology

Yuji TSUNEKAWA

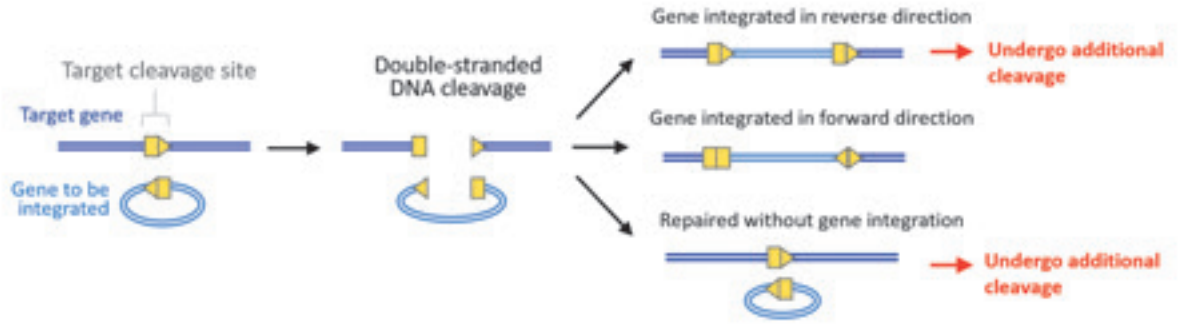
Targeted genome-editing technology has generated great interest for biomedical research fields for its potential in clinical applications to treat or improve symptoms of some genetic diseases. In particular, the development of the CRISPR/Cas9 system has revolutionized the biology field with the ease and precision in which the genome can be manipulated. This innovative system exploits the activity of CRISPR/Cas9, the bacterial immune system used to respond to pathogen invasion, and the cell's intrinsic DNA repair mechanism, either via non-homologous end joining (NHEJ), which is seen in both proliferating and non-proliferating cells, or homology-directed repair (HDR), which is used only in proliferating (dividing) cells. Using CRISPR/Cas9 in combination with NHEJ-mediated or with HDR-mediated methods have proven useful for knocking out function of genes through errors caused by small DNA sequence insertions or deletions at target sites during NHEJ repair or specific sequence modifications in gene of interest. In contrast, gene knock-ins have been more challenging, as this usually utilizes the HDR-mediated mechanism, which is unavailable in differentiated cells. Thus, if the NHEJ mechanism, which is found in both proliferating and non-proliferating cells, can be exploited for efficient targeted gene integration, it would pave the way for potential use *in vivo*, where the majority of cells are non-proliferative.

Research scientist Yuji Tsunekawa in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Team Leader), together with Keiichiro Suzuki and Juan Carlos Izpisua Belmonte at the Salk Institute of Biological Studies, USA, and other collaborators, developed a novel genome-editing technology, which exploits the NHEJ repair mechanism with the CRISPR/Cas9 system called, homology-independent targeted integration (HITI). This technology allows them to insert transgenes into both proliferating and non-proliferating cells efficiently. Their work, published in *Nature*, further demonstrates that HITI can be used for gene editing *in vivo* in the mouse and rat models.

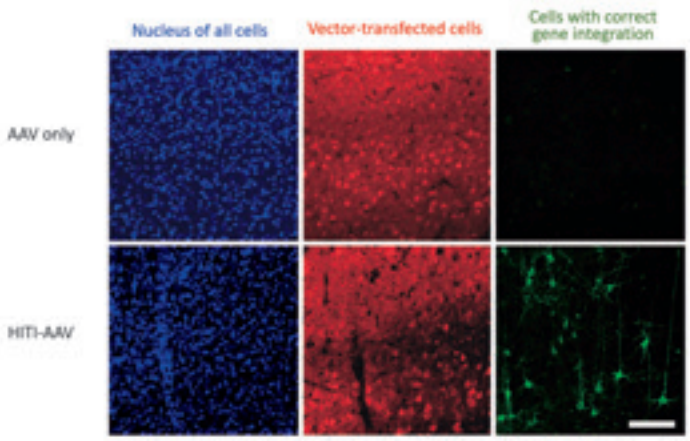
HITI donor vectors were constructed to ensure robust gene integration only when inserted in the forward direction, and if inserted in the reverse direction or is unintegrated, the DNA would undergo further cleavage by Cas9 until inserted correctly or gRNA is no longer able to bind to target sequences due to errors during NHEJ repair (see scheme). When the HITI donor vector was tested in human cell lines, the team observed gene integration at target sites at efficiencies approximately ten times higher than conventional HDR-mediated methods. Effectiveness of HITI in non-proliferating cells was also examined using mouse primary neurons, in which they found gene insertion in 60% of transfected cells.

The team next tested whether their method can be used in non-dividing cells *in vivo*. The adeno-associated virus (AAV) expressing Cas9 systems and HITI donor was injected directly into different tissues of adult mice, such as the brain and muscles, and they confirmed that transgenes were correctly knocked in some cells in the vicinity of injection sites. When HITI-AAV vectors were delivered intravenously just after birth, gene insertion was confirmed in a small percentage (3–10%) of cells within various tissues of the body. The team also injected their HITI-AAV vector in the eye of a rat model for retinitis pigmentosa (RP), after which they detected a rise in mRNA expression levels of *Merkt*, a gene implicated in RP, as well as thickening of degenerating photoreceptor layer.

"As efficiency of gene insertion *in vivo* is still low and some mistargeting and mutations of target genes do occur, there is still much work that need to be done to improve the HITI method before it can be used in clinical applications. Nevertheless, our study clearly demonstrates that *in vivo* genome editing is possible," explains Tsunekawa. "The HITI technology is sure to become a powerful tool not only for clinical applications, but also for basic research. It can be used to visualize and track fates of specific cells by inserting fluorescent marker genes, or modify genomes in animals, such as non-human primates, for which generation of transgenic models has been difficult."



Scheme of HITI method, which uses NHEJ-mediated targeted integration. If cleavage is repaired without gene integration or with integration in reverse direction, DNA undergoes additional cleavage until forward gene integration or gRNA can no longer bind to target cleavage sequence due to errors from NHEJ repair.



Targeted GFP knock-in using HITI in adult mouse neurons *in vivo*. HITI-AAV was constructed so GFP cassette would be inserted downstream of *Tubb3* gene, which is expressed in neurons. When HITI-AAV was injected directly into adult mouse brain, many neurons expressing GFP were observed, indicating GFP gene was correctly integrated (lower panels), whereas in the control, cell transfection was observed with very little integration (minimal GFP signals) was detected (top panels).

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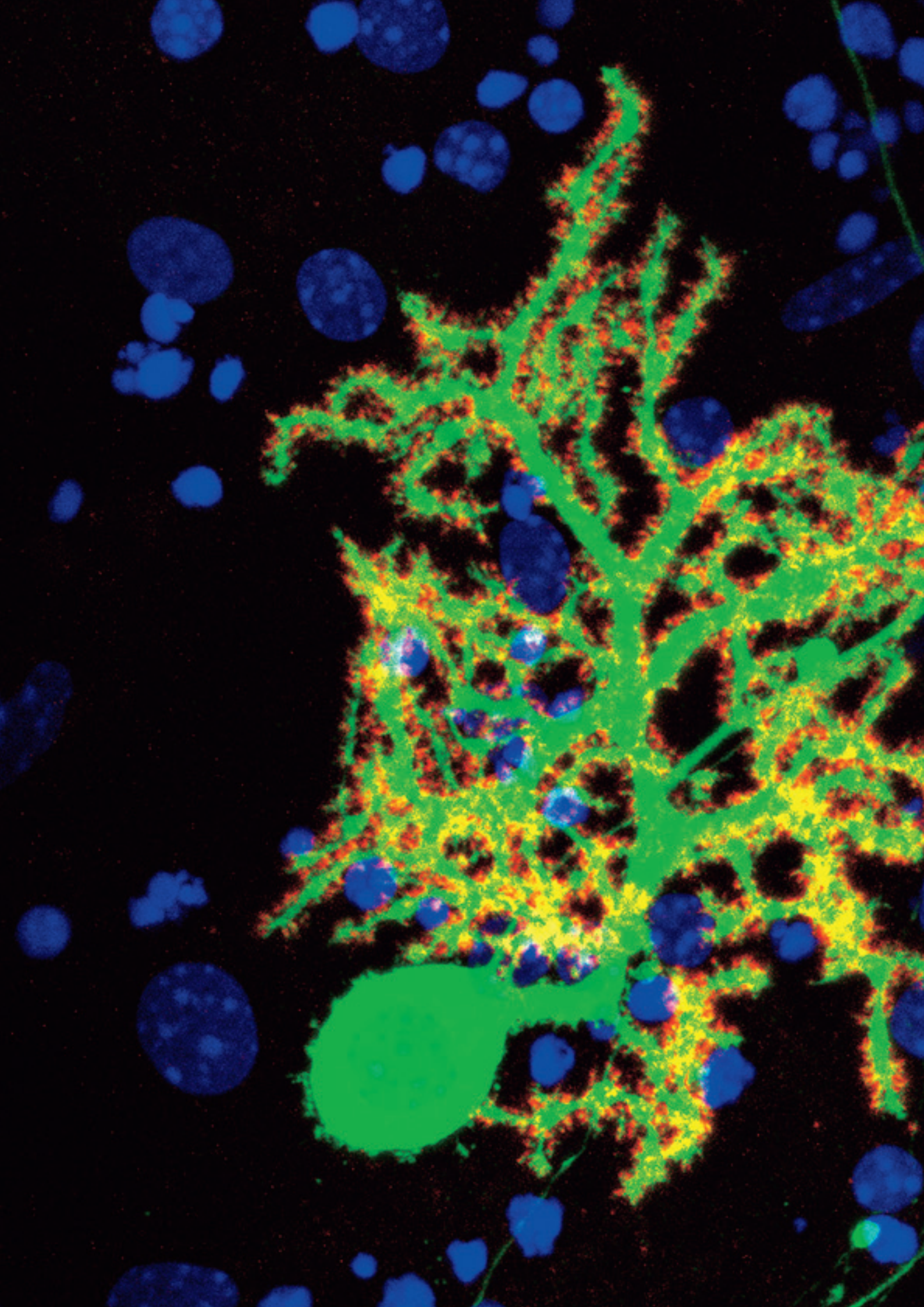

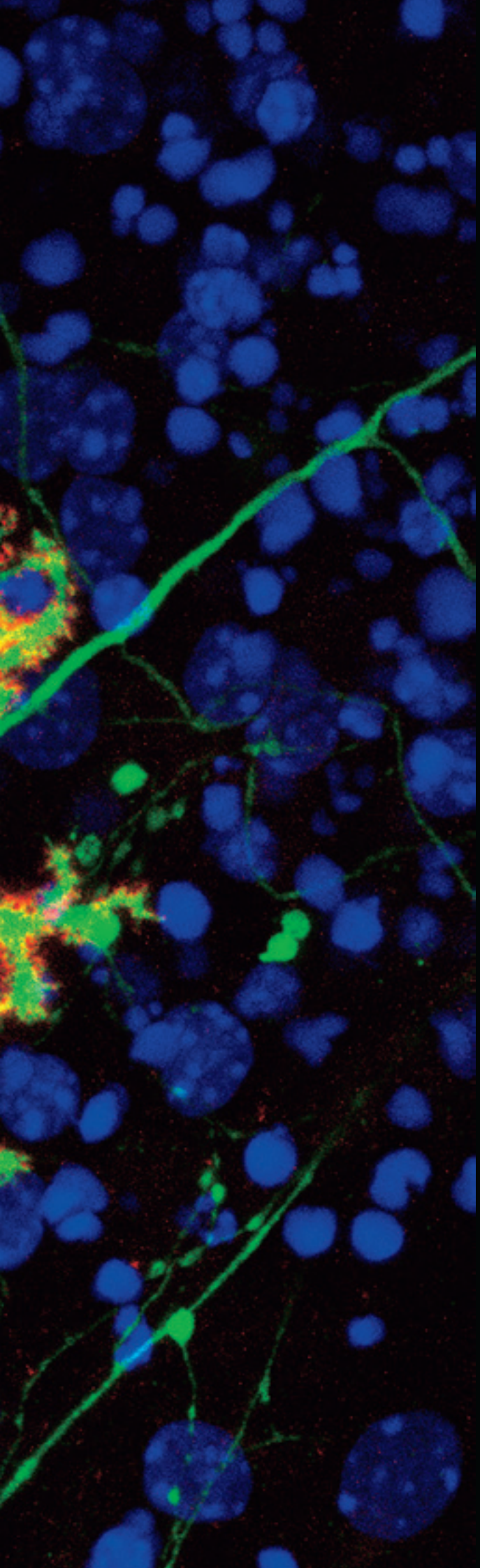

Laboratories

Cerebellar Purkinje cells generated from healthy donor-derived iPSCs. L7 (green) and GRID2 (red) are Purkinje cell-specific markers, and DAPI (blue) marks cell nuclei. Image: Keiko Muguruma, Laboratory for Cell Asymmetry

in vitro Histogenesis

http://www.cdb.riken.jp/en/research/laboratory/eiraku.html

Team Leader
Mototsugu EIRAKU Ph.D.

Mototsugu Eiraku received his Ph.D. from Kyoto University in 2005, and joined the RIKEN Brain Science Institute as a research scientist that same year. In 2006, he joined the RIKEN CDB Laboratory for Organogenesis and Neurogenesis, where he worked as a research scientist until 2010. That year, he was appointed as Deputy Unit Leader of the Four-dimensional Tissue Analysis Unit within the Division for Human Stem Cell Technology, and in 2013, was promoted to Unit Leader. From November 2014, he has served as Team Leader of the Laboratory for in vitro Histogenesis.

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Morio UENO

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Eriko SAKAKURA

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Fumi WAGAI

Recent Publications

Hasegawa Y, et al. Emergence of dorsal-ventral polarity in ESC-derived retinal tissue. *Development* 143 (21). 3895–3906 (2016)

Sakaguchi H, et al. Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. *Nat Commun* 6. 8896 (2015)

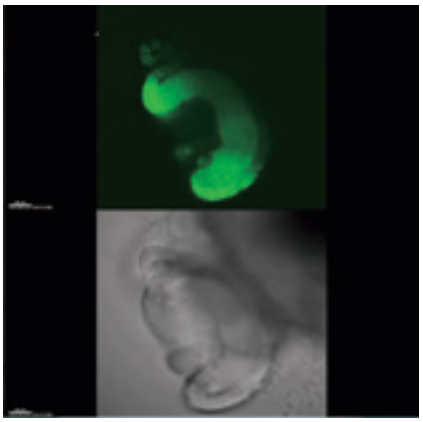
Kuwahara A, et al. Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue. *Nat Commun* 6. 6286 (2015)

Okuda S, et al. Vertex dynamics simulations of viscosity-dependent deformation during tissue morphogenesis. *Biomech Model Mechanobiol* 14. 413–25 (2015)

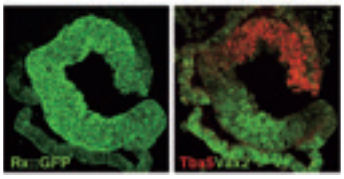
Kadoshima T, et al. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc Natl Acad Sci U S A* 110. 20284–9 (2013)

Nakano T, et al. Self-formation of optic cup and storable stratified neural retina from human ESCs. *Cell Stem Cell* 10. 771–85 (2012)

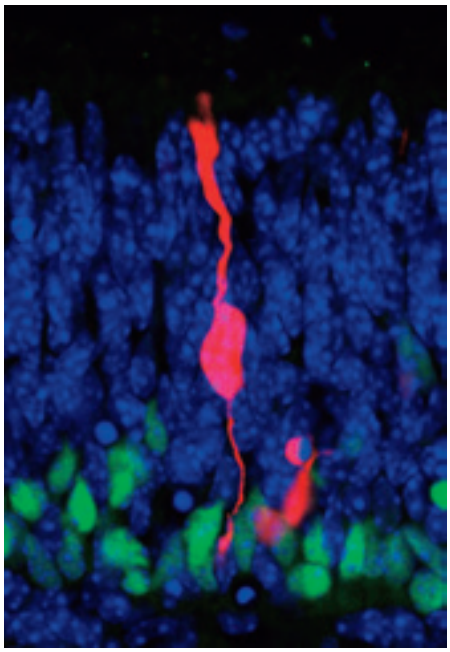
In vitro generation of a functional organ with complex structures is one major goal for the field of developmental and cell biology. To achieve this goal, it is a reasonable strategy to recapitulate the ontogeny, which is the most efficient and robust process for organogenesis that has been acquired through evolution, in an *in vitro* system. Our laboratory aims to clarify the molecular and cellular dynamics underlying organogenesis, and to develop new technologies for *in vitro* recapitulation, that is, three-dimensional functional organ formation from stem cells. These researches aim to make important contributions to the field of developmental biology, stem cell biology, and regenerative medicine.



Self-organized optic cup formation from ES cells



Emergence of dorso-ventral polarity in ES cell-derived optic cup



Photoreceptor cell (red) and bipolar cells (green) in ES cell-derived neural retina

Tissue Microenvironment

http://www.cdb.riken.jp/en/research/laboratory/fujiwara.html

Team Leader

Hironobu FUJIWARA Ph.D.

Hironobu Fujiwara attained his baccalaureate from Kyoto Pharmaceutical University, and went on to receive his M.S. and Ph.D. from Osaka University, the latter in 2003, for his work on the characterization of the human laminin-8 protein. From 2003 to 2007, he worked as a postdoctoral researcher at the Osaka University Institute for Protein Research, with support from the ERATO Sekiguchi Biomatrix Signaling Project (2003–2006). He then moved to the Cancer Research UK Cambridge Institute, where he completed a second postdoctoral fellowship before returning to Japan in 2012 to take a position as a Team Leader at the RIKEN CDB.

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Hiroki MACHIDA

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Recent Publications

Donati G, et al. Epidermal Wnt/beta-catenin signaling regulates adipocyte differentiation via secretion of adipogenic factors. *Proc Natl Acad Sci U S A* 111. E1501–9 (2014)

Fujiwara H, et al. The basement membrane of hair follicle stem cells is a muscle cell niche. *Cell* 144. 577–89 (2011)

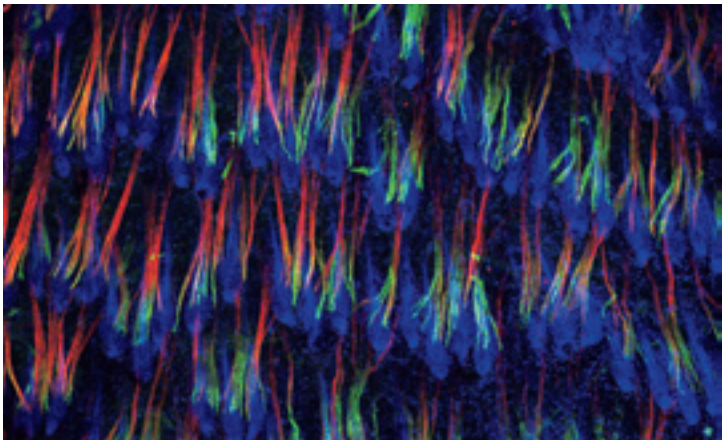
Watt F M and Fujiwara H. Cell-extracellular matrix interactions in normal and diseased skin. *Cold Spring Harb Perspect Biol* 3(4). a005124 (2011)

Ferreira M, et al. An activating beta1 integrin mutation increases the conversion of benign to malignant skin tumors. *Cancer Res* 69.1334–42 (2009)

Fujiwara H, et al. Regulation of mesodermal differentiation of mouse embryonic stem cells by basement membranes. *J Biol Chem* 282. 29701–11 (2007)

Fujiwara H, et al. Rac regulates integrin-mediated endothelial cell adhesion and migration on laminin-8. *Exp Cell Res* 292. 67–77 (2004)

In our bodies, we have millions of different environments in which cells reside, which are known as cellular or tissue microenvironments. These specialized tissue microenvironments instruct the fate and behaviors of cells. The aim of our lab is to gain a better understanding of the mechanisms underlying the ways in which tissue microenvironments are regionally specialized, and how these specialized microenvironments in turn instruct cell behavior, cell-cell communication, and organ formation. Our projects are focused on understanding 1) the extrinsic regulation of stem cells and 2) the role of extracellular matrix (ECM) heterogeneity in organogenesis, using mammalian skin as a model. A more in depth knowledge of these mutually related research focuses will provide a molecular basis to further understand how microenvironments regulate stem cells and organ formation, and for developing tailor-made microenvironments for different lineages of stem cells in the skin.



The image shows arrector pili muscles anchored to the bulge of hair follicles in a whole-mount preparation of mouse dorsal skin, viewed from the dermal side. Arrector pili muscles are visualized by staining for α-smooth muscle actin (green) and SM22α (red). The whole-mount is labeled with a nuclear counterstain (blue).

Organismal Patterning

http://www.cdb.riken.jp/en/research/laboratory/hamada.html

Team Leader

Hiroshi HAMADA M.D., Ph.D.

Hiroshi Hamada was appointed director of the RIKEN Center for Developmental Biology in April 2015, and also established the Laboratory for Organismal Patterning at the Center in October of the same year. He received his M.D. and Ph.D. from Okayama University in 1979, and worked at the National Institutes of Health (USA) and Memorial University of Newfoundland (Canada) for nine years before returning to Japan. His interest in development stems from earlier work on embryonal carcinoma cells, which he performed in Canada. His current interests are the mechanisms underlying symmetry-breaking and the origins of body axes.

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Recent Publications

Minegishi K, et al. A Wnt5 activity asymmetry and intercellular signaling polarize node cells for breaking left-right symmetry in the mouse embryo. *Dev Cell* (2017, in press)

Shinohara K, et al. Absence of radial spokes in mouse node cilia is required for rotational movement but confers ultrastructural instability as a trade-off. *Dev Cell* 35. 236–246 (2015)

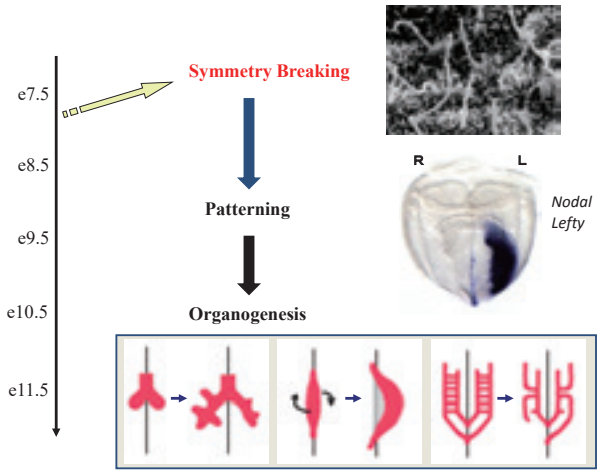
Nakamura T, et al. Fluid flow and interlinked feedback loops establish left-right asymmetric decay of *Cerl2* mRNA in the mouse embryo. *Nat Commun* 3. 1322 (2012)

Yoshiba S, et al. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science* 338. 226–231 (2012)

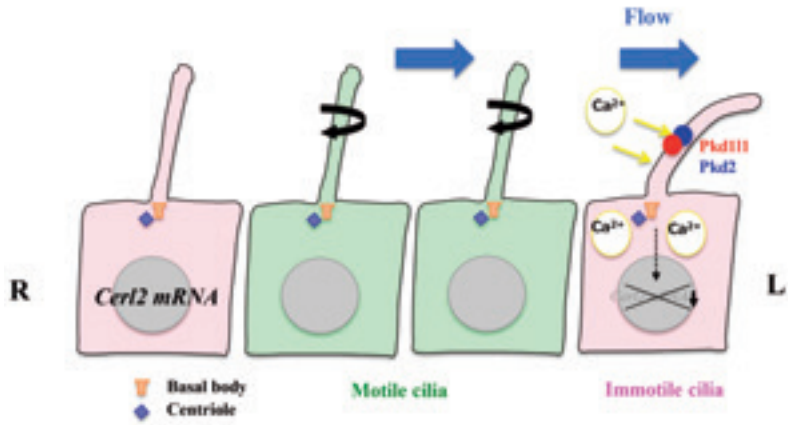
Shinohara K, et al. Two rotating cilia in the node cavity are sufficient to break left-right symmetry in the mouse embryo. *Nat Commun* 3. 622 (2012)

Takaoka K, et al. Origin and role of distal visceral endoderm, a group of cells that determines anterior-posterior polarity of the mouse embryo. *Nat Cell Biol* 13. 743–752 (2011)

My lab studies how left-right asymmetries develop in the mouse embryo. In particular, we focus on two types of cilia that are required for left-right symmetry breaking: rotating cilia that generate leftward fluid flow, and immotile cilia that sense the fluid flow. We also study the role of maternal epigenetic regulators in pre-implantation development. We address these questions by integrating live imaging, structural biology, fluid dynamics and mathematical modeling.



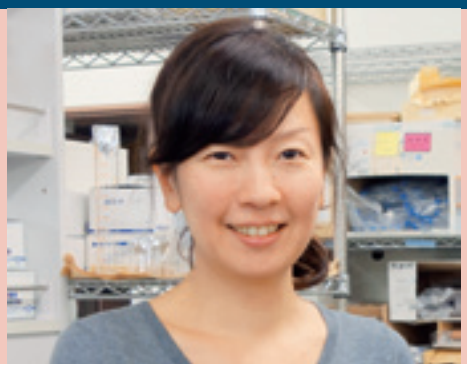
Three steps for generating left-right asymmetry



Role of motile and immotile cilia in left-right symmetry breaking

Neocortical Development

http://www.cdb.riken.jp/en/research/laboratory/hanashima.html



Team Leader

Carina HANASHIMA Ph.D.

Carina Hanashima received her Ph.D. from Waseda University School of Science and Engineering in 1999. She worked as a postdoctoral fellow in the laboratory of Eseng Lai at Memorial Sloan-Kettering Cancer Center, Cell Biology Program from 1999 to 2002, where she was engaged in research in developmental neuroscience. She moved to Skirball Institute Developmental Genetics Program in 2002, to Gord Fishell's laboratory where she extended her research to cell specification in the neocortex. She was appointed Team Leader at the CDB in September 2007.

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Recent Publications

Toma K, et al. Encoding and decoding time in neural development. *Dev Growth Differ* 58(1): 59–72 (2016)

Toma K and Hanashima C. Switching modes in corticogenesis: mechanisms of neuronal subtype transitions and integration in the cerebral cortex. *Front Neurosci* 9. 274 (2015)

Bullmann T, et al. A transportable, inexpensive electroporator for *in utero* electroporation. *Dev Growth Differ* 57(5): 369–377 (2015)

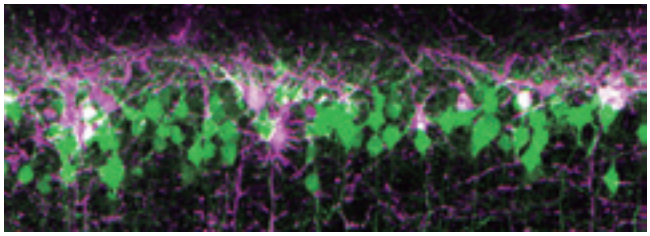
Toma K, et al. The timing of upper-layer neurogenesis is conferred by sequential derepression and negative feedback from deep-layer neurons. *J Neurosci* 34.13259–76 (2014)

Kumamoto T and Hanashima C. Neuronal subtype specification in establishing mammalian neocortical circuits. *Neurosci Res* 86. 37–49 (2014)

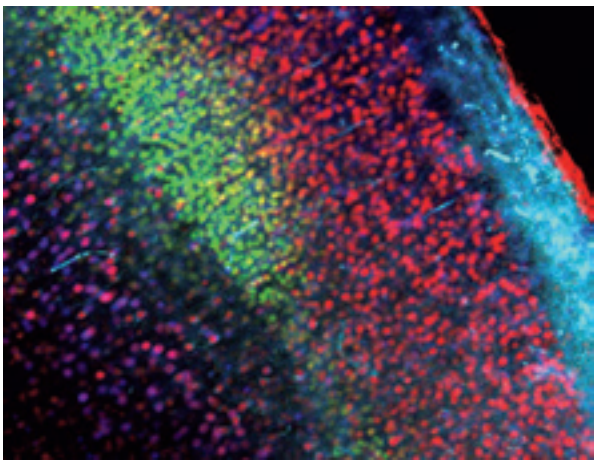
Yeh M L, et al. Robo1 modulates proliferation and neurogenesis in the developing neocortex. *J Neurosci* 34. 5717–31 (2014)

The neocortex, the most complex structure of all vertebrate brain systems, is organized into cytoarchitectonic groups of neurons that process unique sensory inputs, such as pressure, light and sound. This highly ordered structure is nonetheless generated from a relatively simple sheet of neuroepithelium during development. Research in our laboratory aims to understand how these diverse arrays of cortical neurons are specified and coordinated into high-functional territories: 1) What is the mechanism by which diverse cell fates are determined in the neocortex? 2) How are neurons precisely arranged into distinct cortical layers and areas? 3) To what extent does the establishment of functional areas rely on environmental inputs?

Recent work from the laboratory has shown that the fate of neocortical neurons is controlled through cortical intrinsic programs in which neuron fate is established by temporal changes in transcriptional regulatory network. However, in the mature cortex, cortical areas differ in their types and numbers of specific layer neurons along both the anterior-posterior (AP) and medial-lateral (ML) axes. We are exploring the extent to which intrinsic determinants control the specification of neuronal subtypes within discrete regions of the neocortex, as well as the extrinsic influences that refine the boundaries between functional areas of neocortex. To further these studies, we employ genetic manipulations in mice that will enable conditional loss of gene and cellular functions, recombination-mediated cell-lineage tracing, and systematic approaches to identify novel molecules responsible for precise areal specification. Through these studies we wish to understand the mechanistic basis by which unique sensory perceptions and functional circuitries develop in the human neocortex.



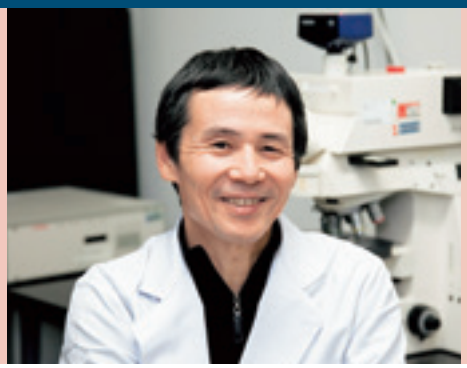
Robo1 receptor is necessary for inside-out layer formation. Upon Robo1-suppression, later-born neurons labeled with DsRed at embryonic day (E) 16 (magenta) cannot migrate past earlier-born cells (E15 GFP-labeled cells, green).



The neocortex is composed of distinct neuronal subtypes that establish six layers. Coronal section of a one-week-old mouse neocortex showing Reelin (layer I, cyan), Brn2 (layer II/III, red), RORß (layer IV, green), Ctip2 (layer V/VI, blue) expressing neurons.

Morphogenetic Signaling

http://www.cdb.riken.jp/en/research/laboratory/hayashi.html



Team Leader

Shigeo HAYASHI Ph.D.

Shigeo Hayashi received his B.Sc. in Biology from Kyoto University in 1982, and his Ph.D. in Biophysics from the same institution in 1987, for his work on lens-specific regulation of the chicken delta *crystallin* gene. Inspired by the discovery of the homeobox, he changed his research focus to the developmental genetics of *Drosophila* and spent three years as a postdoctoral research associate in Matthew Scott's lab at the University of Colorado before returning to Japan to work at the National Institute of Genetics. He became an associate professor at the same Institute in 1994, and professor in 1999. Also in 1999, he received the incentive prize of the Genetics Society of Japan for Genetic Studies on *Drosophila* Development. He was named Group Director of the Morphogenetic Signaling research group at the RIKEN CDB in May 2000, and Team Leader of the same group in 2014. His current research interests are dynamic aspects of cell adhesion, cell migration, and cell morphogenesis in *Drosophila*.

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Ikuko FUKUZYOU
Noriko MORIMITU

Assistant
Ryoko ARAKI
Mai SHIBATA

Recent Publications

Miao G and Hayashi S. Escargot controls the sequential specification of two tracheal tip cell types by suppressing FGF signaling in *Drosophila*. *Development* 143. 4261–4271 (2016)

Otani T, et al. IKKepsilon inhibits PKC to promote Fascin-dependent actin bundling. *Development* 143. 3806–3816 (2016)

Kato K, et al. Microtubule-dependent balanced cell contraction and luminal-matrix modification accelerate epithelial tube fusion. *Nat Commun* 7. 11141 (2016)

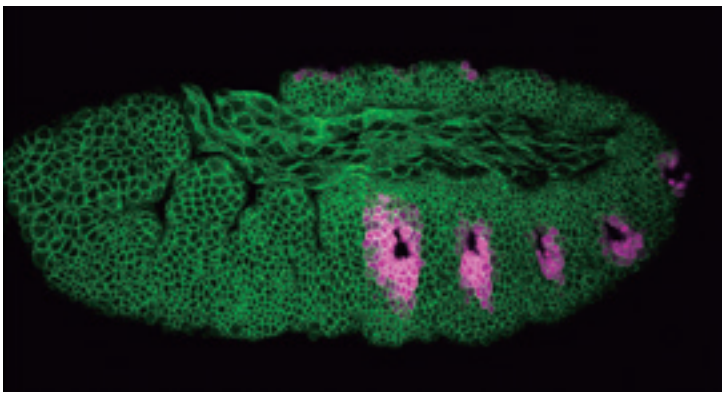
Hannezo E, et al. A cortical instability drives periodic supracellular actin pattern formation in epithelial tubes. *Proc Natl Acad Sci U S A* 112. 8620–8625 (2015)

Dong B, et al. Rab9 and retromer regulate retrograde trafficking of luminal protein required for epithelial tube length control. *Nat Commun* 4. 1358 (2013)

Kondo T and Hayashi S. Mitotic cell rounding accelerates epithelial invagination. *Nature* 494. 125–9 (2013)

Our research aim is to understand fundamental mechanisms of animal morphogenesis with particular interest in the mechanical basis of tissue movement and its interaction with the extracellular environment. Our main research focus is the tracheal system in the *Drosophila* embryo, a network of tubular epithelium used as a respiratory organ. Trachea is formed through invagination, tube formation, elongation, fusion, and final maturation into a respiratory organ. We are particularly interested in the mechanical control of epithelial architectures. Epithelium is stabilized by cell-cell adhesion and cell-matrix adhesion. Breaking this stability is essential for initiating morphogenetic movement. We found that prospective tracheal primordium is under negative tension (pressurized). Anisotropic redistribution of tissue tension and timely mitosis initiates local mechanical instability that leads to tissue invagination movement (Kondo and Hayashi, 2013). Once the tracheal network is formed, tube diameter and length are enlarged to reach the final size. Tracheal size change involves increase in cell size, especially an increase in apical cell area facing the luminal side. A key question is how individually controlled cellular growth is coordinated to form coherent tissue architecture. We found that extracellular matrix in the luminal space plays a central role by providing mechanical stability to the tubules (Dong et al., 2013, 2014). Defects in extracellular matrix components lead to destabilization of tube shape and malformation, resulting in tubule morphology seen in organs under pathological conditions.

Another research area of interest is the mechanism of cell morphogenesis. Here we ask the question to what extent single cells can autonomously organize nanometer scale cellular patterns. Our studies have uncovered the role of the cellular trafficking center as an organizer of cell elongation (Otani et al., 2011, 2015, 2016).



Drosophila embryo at the beginning of tracheal placed invagination (magenta). Cell boundaries are marked green.

Developmental Epigenetics

http://www.cdb.riken.jp/en/research/laboratory/hiratani.html

Team Leader

Ichiro HIRATANI Ph.D.

Ichiro Hiratani received his B.Sc. in Biological Sciences from the University of Tokyo in 1998, and his Ph.D. from the same institution in 2003 for his work on *Xenopus* embryonic development with Masanori Taira. In 2003, he moved to the United States for his postdoctoral training with David Gilbert at the State University of New York Upstate Medical University and studied the developmental regulation of DNA replication timing and nuclear genome organization. He later moved with Gilbert to Florida State University in 2006, and then returned to Japan in 2010 to take a position as Assistant Professor at the National Institute of Genetics. In 2011, he was awarded a grant from the JST PRESTO program in Epigenetics. He was appointed to his current position as Team Leader at the RIKEN CDB in October 2013.

Staff

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Technical Staff
Akie TANIGAWA

Student Trainee
Marc ABELLA
David MEYER

Part-Time Staff
Yoshiko KONDO

Recent Publications

Shang WH, et al. Chromosome engineering allows the efficient isolation of vertebrate neocentromeres. *Dev Cell* 24. 635–48 (2013)

Ryba T, et al. Genome-scale analysis of replication timing: from bench to bioinformatics. *Nat Protoc* 6. 870–95 (2011)

Hiratani I and Gilbert D M. Autosomal lyonization of replication domains during early Mammalian development. *Adv Exp Med Biol* 695. 41–58 (2010)

Ryba T, et al. Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. *Genome Res* 20. 761–70 (2010)

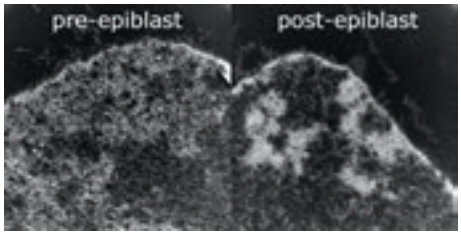
Hiratani I, et al. Genome-wide dynamics of replication timing revealed by in vitro models of mouse embryogenesis. *Genome Res* 20.155–69 (2010)

Hiratani I, et al. Global reorganization of replication domains during embryonic stem cell differentiation. *PLoS Biol* 6. e245 (2008)

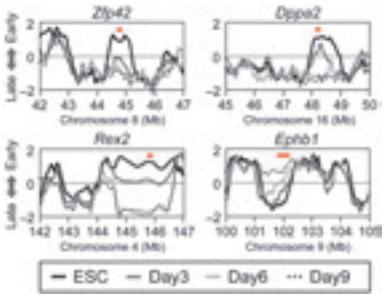
We wish to clarify the molecular mechanisms underlying global facultative heterochromatin formation during early mouse embryogenesis, with the belief that understanding the developmental regulation of higher-order chromosome organization will lead to a deeper understanding of cell differentiation.

The term facultative heterochromatin refers to chromosomal regions that condense, become inactivated, and are stably maintained in this manner after a certain developmental stage. A classic example is the inactive X chromosome in mammals, which becomes detectable immediately prior to the formation of germ layers and is stably maintained thereafter in all downstream lineages. Intriguingly, we recently discovered that many autosomal domains also undergo a similar process of facultative heterochromatin formation at the same developmental stage, which accounts for more than 6% of the genome. This suggests that facultative heterochromatin formation at this stage is not specific to the inactive X, but is rather a more widespread phenomenon affecting the entire genome. Recent studies have also revealed low reprogramming efficiency of cells immediately after this developmental stage, already as low as downstream somatic cell types. Thus, this facultative heterochromatin is a common epigenetic feature of all somatic cells beyond the germ layer formation stage, and the reprogramming experiments imply a potential link to the cell's differentiated state.

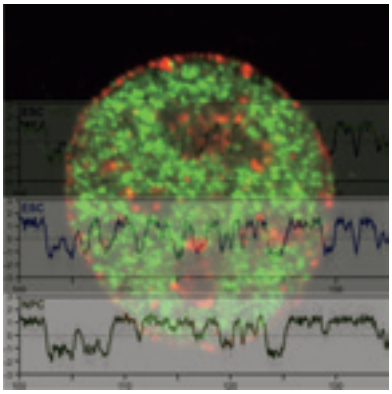
For these reasons, we combine genome-wide approaches with molecular and cell biology and imaging techniques to elucidate the molecular mechanisms underlying the facultative heterochromatin formation process. In the future, we will address the biological significance of this phenomenon and eventually wish to understand the fundamental implications of higher-order chromosome organization.



Electron microscopy reveals a large-scale genome reorganization during pre- (left) to post-epiblast (right) transition, consistent with predictions made by DNA replication profiling.



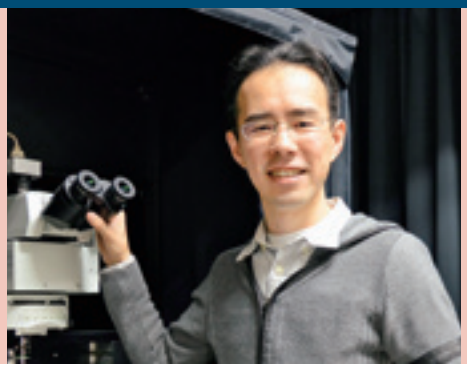
Genome-wide DNA replication profiling during ES cell differentiation can reveal domains that show large-scale changes in nuclear organization.



Early- and late-replicating DNA localize to the interior (green) and periphery (red) of the nucleus, respectively. Because of this relationship, genome-wide DNA replication profiling (graphs) can be used to deduce the 3D genome organization at the sequence level.

Sensory Circuit Formation

http://www.cdb.riken.jp/en/research/laboratory/imai.html



Team Leader

Takeshi IMAI Ph.D.

Takeshi Imai completed his Ph.D. in the laboratory of Prof. Hitoshi Sakano at the University of Tokyo Graduate School of Science in 2006. He did consecutive postdoctoral fellowships under the CREST Program and The University of Tokyo in 2006 and 2007, before his appointment as Assistant Professor at the same university in 2009. He received independent funding under the JST PRESTO program that year, before joining the RIKEN CDB as Team Leader in 2010. He also holds an adjunct position as Associate Professor at the Kyoto University Graduate School of Biostudies.

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Eri YAMASHITA

Recent Publications

Murai A, et al. Distorted Coarse Axon Targeting and Reduced Dendrite Connectivity Underlie Dysosmia after Olfactory Axon Injury. *eNeuro* 3. e0242-16 (2016)

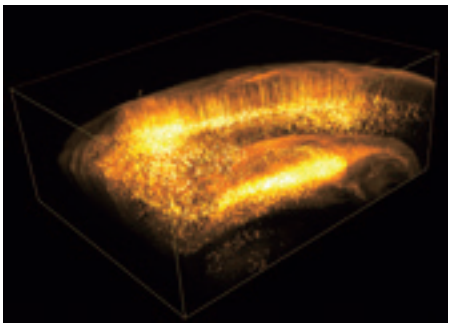
Ke M T, et al. Super-Resolution Mapping of Neuronal Circuitry with an Index-Optimized Clearing Agent. *Cell Rep* 14. 2718–2732 (2016)

Imai T. Construction of functional neuronal circuitry in the olfactory bulb. *Semin Cell Dev Biol* 35.180–88 (2014)

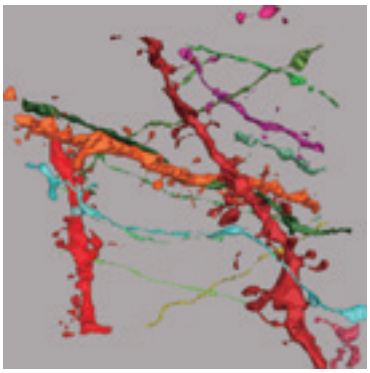
Nakashima A, et al. Agonist-independent GPCR activity regulates anterior-posterior targeting of olfactory sensory neurons. *Cell* 154.1314–25 (2013)

Ke M T, et al. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nat Neurosci* 16.1154–61 (2013)

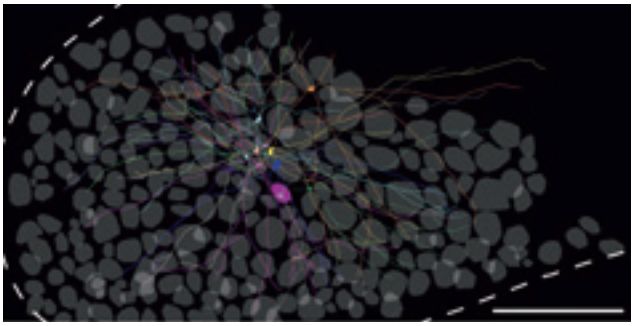
The mammalian central nervous system is composed of enormous numbers of neurons. How do these neurons establish their identity and form functional neuronal circuitries? To address this question, we are studying the mouse olfactory system as a model system. In the mouse olfactory system, odorants are detected by ~1,000 types of olfactory sensory neurons, each expressing a single type of odorant receptor. The olfactory bulb, the primary olfactory area of the brain, receives inputs from olfactory sensory neurons through 1,000 sets of glomeruli. These inputs are then processed in the olfactory bulb circuits and are then sent out to the olfactory cortex. These complex neuronal circuits emerge in an autonomous fashion based on cell-cell interactions and neuronal activity after birth, rather than by strict genetic programs. Using the olfactory bulb as a model system, we are trying to understand simple rules behind the formation of complex neuronal circuitry.



Adult Thy1-YFP-H mouse brain was cleared with an optical clearing agent SeeDB and imaged using two-photon microscopy. 3D rendering image of a volume of 4 mm x 5 mm x 2 mm, encompassing cerebral cortex and hippocampus, is shown.



Super-resolution mapping of neuronal circuitry. Brain slices of a Thy1-YFP-H transgenic mouse were cleared with SeeDB2, imaged with super-resolution microscopy, and reconstructed in 3D.



Tracing of sister mitral cells associated with a common glomerulus in the olfactory bulb. Neurons connecting to a single glomerulus was labeled by neuronal tracer and the sample was cleared with SeeDB.

Axial Pattern Dynamics

http://www.cdb.riken.jp/en/research/laboratory/inomata.html

Team Leader

Hidehiko INOMATA Ph.D.

Hidehiko Inomata received his doctorate from the Tokyo Institute of Technology in 2003, and moved to take a research scientist position at the Foundation for Biomedical Research and Innovation in Kobe, Japan that same year. From 2004 to 2012, he worked as a research scientist in the RIKEN CDB Laboratory for Organogenesis and Neurogenesis, and in 2012, was appointed a Senior Scientist in the same lab. In 2011, he was named a researcher in the Japan Science and Technology Agency (JST) PRESTO program. He was appointed Team Leader of the Laboratory for Axial Pattern Dynamics in 2014. In 2015, he was also named a researcher in the Japan Science and Technology Agency (JST) PRESTO program.

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Recent Publications

Inomata H, et al. Scaling of dorsal-ventral patterning by embryo size-dependent degradation of Spemann's organizer signals. *Cell* 153.1296–311 (2013)

Takai A, et al. Anterior neural development requires Del1, a matrix-associated protein that attenuates canonical Wnt signaling via the Ror2 pathway. *Development* 137. 3293–302 (2010)

Inomata H, et al. Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. *Cell* 134. 854–65 (2008)

Arakawa A, et al. The secreted EGF-Discoidin factor xDel1 is essential for dorsal development of the *Xenopus* embryo. *Dev Biol* 306. 160–9 (2007)

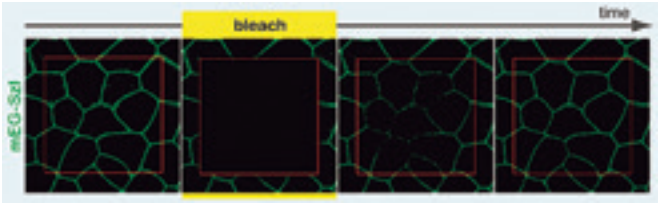
Onai T, et al. XTsh3 is an essential enhancing factor of canonical Wnt signaling in *Xenopus* axial determination. *EMBO J* 26. 2350–60 (2007)

Inomata H, et al. A scaffold protein JIP-1b enhances amyloid precursor protein phosphorylation by JNK and its association with kinesin light chain 1. *J Biol Chem* 278. 22946–55 (2003)

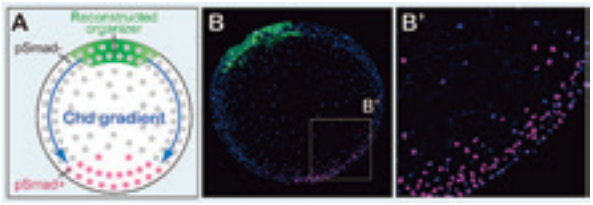
Developmental processes take place through the exchange of information by cells within the constrained spatial environment of the embryo. Such intercellular communication is essential for the formation of a well-ordered body; in its absence, our individual cells would behave in an uncoordinated fashion, and fail to follow the patterns needed for the development of the head, limbs, or other body parts. Factors that play central roles in such developmental signaling are known as morphogens.

In our research we will seek to gain a deeper understanding into processes informed by positional information in a spatial context (developmental fields), using vertebrate (mainly frog and zebrafish) axis formation as a model. The establishment of the frog dorsoventral axis depends on gradients of morphogens secreted by the organizer region. In order to ensure that development based on simple concentration gradients is stably reproducible, cell-cell communications mediated by morphogens need to be robust against perturbations. One example of such robustness can be seen in the response of a frog embryo when bisected; such embryos follow normal developmental patterns, despite being half the ordinary size, a phenomenon known as 'scaling.' Our team has previously shown how scaling is maintained through morphogen-mediated intercellular communication when the spatial size of the embryo is perturbed. In our lab, we address visualization of morphogen gradients and *in vivo* imaging along with biochemical approaches to study how developmental robustness is maintained.

We are also working to develop methods for controlling the shape of morphogen gradients. Gradients are primarily regulated by production, diffusion, and degradation, which indicates that by controlling these factors, it should be possible to arbitrarily design gradients that reconstruct tissue patterns in the embryo. By using such methods, we hope to gain a deeper understanding of developmental systems.



FRAP assays of mEGFP-tagged Sizzled shown by snapshots. From left to right; before bleaching, at the end of bleaching, 50 s after bleaching, and 230 s after bleaching.



Graded DV patterning by the reconstructed organizer. Substantial pSmad accumulation was observed on the side opposite to the reconstructed organizer, indicating that the influence of chordin was distance-sensitive.

Chromosome Segregation

http://www.cdb.riken.jp/en/research/laboratory/kitajima.html

Team Leader

Tomoya KITAJIMA Ph.D.

Tomoya Kitajima received his Master's and doctoral degrees from the University of Tokyo, for his thesis on identification of Shugoshin as a conserved protector of chromosome cohesion at centromeres. After receiving his Ph.D. in 2004, he served as research associate at the Institute of Molecular and Cellular Biosciences at the same university, before moving to the European Molecular Biology Laboratory in Heidelberg, Germany as a postdoctoral researcher. He was appointed Team Leader at the CDB in 2012.

Staff

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Assistant
Kaori HAMADA

Recent Publications

Sakakibara Y, et al. Bivalent separation into univalents precedes age-related meiosis I errors in oocytes. *Nat Commun* 6. 7550 (2015)

Yoshida S, et al. Inherent instability of correct kinetochore-microtubule attachments during meiosis I in oocytes. *Dev Cell* 33(5): 589–602 (2015)

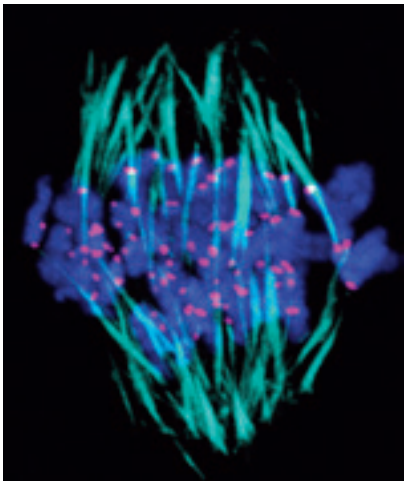
Kim J, et al. Meikin is a conserved regulator of meiosis-I-specific kinetochore function. *Nature* 517(7535): 466–471 (2015)

Solc P, et al. Multiple requirements of PLK1 during mouse oocyte maturation. *PLOS ONE* 10(2): e0116783 (2015)

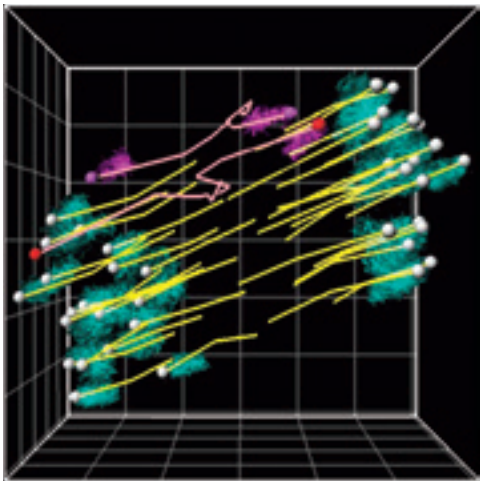
Kyogoku H, et al. Nucleolus Precursor Body (NPB): A Distinct Structure in Mammalian Oocytes and Zygotes. *Nucleus* 5(6): 493–498 (2014)

Kitajima TS, et al. Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. *Cell* 146. 568–81 (2011)

The oocyte becomes an egg through meiosis. The egg fertilizes with a sperm and undergoes repeated cell divisions to give rise to an entire body. We study chromosome segregation during meiosis in oocytes and during mitosis in fertilized eggs, taking advantage of techniques for high-throughput and high-resolution live imaging of mouse oocytes combined with micromanipulation and genetic engineering methods. The first cell division that oocytes undergo is meiosis I. Chromosome segregation in this division is error-prone and the rate of errors increases with maternal age. Subsequently, chromosomes are segregated in meiosis II upon fertilization, and then segregated again in mitosis after DNA replication. We will reveal distinct mechanisms for chromosome segregation during these subsequent but fundamentally different cell divisions. By uncovering the mechanism of chromosome segregation during meiosis I in oocytes, we understand why oocyte meiosis I is error-prone and related to age. Comparing the mechanisms in meiosis I with those found in meiosis II and mitosis may provide insights into the capacity of cells to flexibly use different strategies for chromosome segregation. The findings will be exploited to collaborative studies with reproductive medicine.



Kinetochore-microtubule attachments



Chromosome segregation error

Histogenetic Dynamics

http://www.cdb.riken.jp/en/research/laboratory/kuranaga.html

Team Leader

Erina KURANAGA Ph.D.

Erina Kuranaga received her doctorate in medical science from the Osaka University Graduate School of Medicine in 2004, after which she moved to the University of Tokyo Graduate School of Pharmaceutical Sciences as assistant professor in the Department of Genetics. In 2006, she was promoted to associate professor in the same department, where she remained until she joined the RIKEN Center for Developmental Biology as Team leader in 2011. She was appointed as professor at Tohoku University in 2016.

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Recent Publications

Kawamoto Y, et al. Apoptosis in Cellular Society: Communication between Apoptotic Cells and Their Neighbors. *Int J Mol Sci* 17(12): 2144. (2016)

Sato K, et al. Left-right asymmetric cell intercalation drives directional collective cell movement in epithelial morphogenesis. *Nat Commun* 6. 10074 (2015)

Obata F, et al. Necrosis-driven systemic immune response alters SAM metabolism through the FOXO-GNMT axis. *Cell Rep* 7. 821–833 (2014)

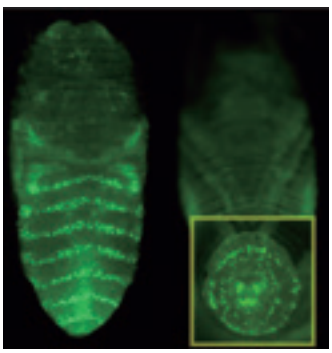
Takeishi A, et al. Homeostatic epithelial renewal in the gut is required for dampening a fatal systemic wound response in *Drosophila*. *Cell Rep* 3. 919–930 (2013)

Kuranaga E, et al. Apoptosis controls the speed of looping morphogenesis in *Drosophila* male terminalia. *Development* 138. 1493–9 (2011)

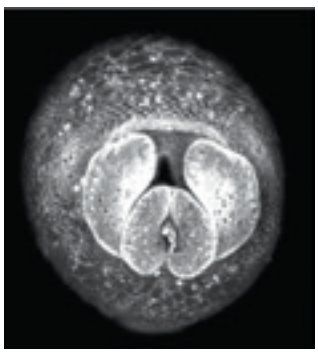
Kuranaga E, et al. *Drosophila* IKK-related kinase regulates nonapoptotic function of caspases via degradation of IAPs. *Cell* 126. 583–96 (2006)

The development of multicellular organisms involves the collective effect of multiple events at the level of the individual cell, such as proliferation, differentiation, adhesion, and migration. Programmed cell death, for example, is a process by which cells are selected for death at set times in development, allowing for the sculpting of tissue, and is used in the adult organism to maintain homeostasis by eliminating cells that have developed abnormalities. Perturbations in cell death signaling can thus affect an organism's physiological stability, and result in developmental defects, tumorigenesis, or neurodegenerative diseases. Cell death plays an important role in maintaining the cellular society not only by eliminating unneeded cells at given sites and stages, but in other functions, such as regulating the proliferation and migration of neighboring cells, as well. Such cellular behaviors give rise to cell networks capable of organizing into tissues, the study of which requires an experimental approach to spatiotemporal information in living systems, such as can be obtained through the real-time live imaging of biological phenomena.

We have chosen the fruit fly *Drosophila melanogaster* as our primary research model, seeking to take advantage of its utility in developmental studies and wealth of genetic data in studying the coordination of histogenesis through live imaging and genetic screens. To elucidate the role of cell death in histogenetic processes, we will analyze caspase mutant phenotypes in which the exterior male genitalia (terminalia) develops abnormally. In normal *Drosophila* development, the terminalia rotates 360° as it forms, but in caspase mutants, this revolution is incomplete. Image analysis reveals that in wildtype, the speed of this rotation is variable, with distinct initiation, acceleration, deceleration, and termination stages; caspase inhibition results in loss of the acceleration phase, and failure in terminalia development. We will seek to identify how caspases function and how cell death controls acceleration of the rotation by searching for associated genes and live imaging analysis. It has further been predicted that cell death alone cannot account for rotation that maintains the tissue area, suggesting other mechanisms are also at work. We will conduct single-cell analyses to determine whether other behaviors such as proliferation or migration are also altered. Through the use of the extensive *Drosophila* genetics toolset and live imaging technologies, we hope to be able to address questions that have proven technically challenging in the past, and by visualizing the activities of individual cells, develop a better understanding of how cellular network systems work in histogenesis.



Dorsal (left) and ventral (right) views of *Drosophila* pupae that express fluorescent protein in cells located posterior component of each segment. Yellow box indicates location of male genitalia.



Caudal view of DE-Cadherin:GFP-expressing *Drosophila*. This image was taken before rotation.

Laboratory for Cell Asymmetry

http://www.cdb.riken.jp/en/research/laboratory/matsuzaki.html

Team Leader

Fumio MATSUZAKI Ph.D.

Fumio Matsuzaki is engaged in the study of the genetic programs underlying neural development, with a focus on the asymmetric division of neural stem cells. His laboratory has discovered several key mechanisms controlling asymmetric divisions in neural progenitor cells using *Drosophila* and mouse as model systems. He has also recently found a novel type of self-renewing progenitor in the developing cerebral cortex in rodents, providing new insights into the enormous increase in brain size during mammalian evolution.

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Recent Publications

Suzuki K, et al. *In vivo* genome editing via CRISPR-Cas9 mediated homology-independent targeted integration. *Nature* 540.144–149 (2016)

Tsunekawa Y, et al. Developing a *de novo* targeted knock-in method based on *in utero* electroporation into the mammalian brain. *Development* 143. 3216–22 (2016)

Okamoto M, et al. Cell cycle–independent transitions in temporal identity of mammalian neural progenitor cells. *Nat Commun* 7. 11349 (2016)

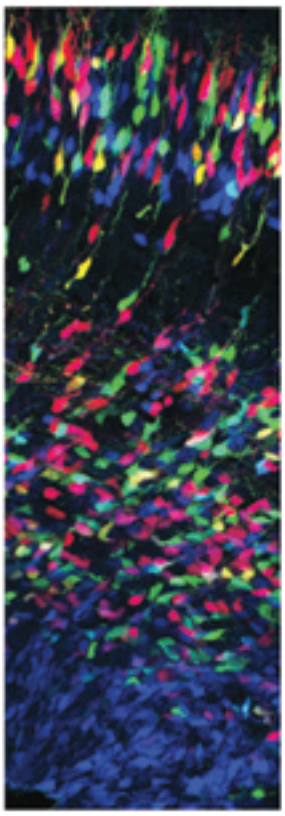
Matsuzaki F and Shitamukai A. Cell division modes and cleavage planes of neural progenitors during mammalian cortical development. *Cold Spring Harb Perspect Biol* 7. a015719 (2015)

Pilz G. A, et al. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. *Nat Commun* 4. 2125 (2013)

Yoshiura S, et al. Tre1 GPCR signaling orients stem cell divisions in the *Drosophila* central nervous system. *Dev Cell* 22. 79–91 (2012)

Our group explores the mechanisms underlying the organization of cells into highly ordered structures in the developing brain. During brain development, neural stem cells generate a large number of neurons and glia of different fates at appropriate points in time; the framework and size of the brain depend on the spatiotemporal behavior of neural stem cells, which are highly dynamic in their modes of division and gene expression. Using invertebrate (*Drosophila*) and vertebrate (mouse) model systems, we focus our study on genetic and epigenetic programs, by which behaviors of neural stem cells are controlled and brain development is governed.

Drosophila neural stem cells, called neuroblasts, provide an excellent model system for the investigation of fundamental aspects of asymmetric division, a process essential to the generation of cells of divergent type during proliferation. We have been investigating mechanisms controlling asymmetric divisions, including the cell polarity and spindle orientation. We also extend our research scope to understand how neurogenesis is controlled in tissue space depending on the environments that surround the nervous system. We recently identified an extrinsic mechanism that controls the orientation of division (cell polarity) in neuroblasts relative to the overriding ectoderm (Yoshiura et al., 2012), which determines the orientation of neural tissue growth.



The vertebrate brain evolved rapidly, resulting in an expansion of the size of the brain, which comprises a larger number of neurons arranged in a vastly more complex functional network than that in invertebrates. Neural stem cells typically adopt three states—proliferative (symmetrically dividing), neurogenic (asymmetrically dividing), and resting—and undergo transitions among the states, on which the basic organization of the brain depend. We are investigating mechanisms that determine the individual states of neural stem cells, and control transitions between states in mouse as well as mechanisms for generating neural progenitor cell diversity. We recently discovered a novel transition in the division mode in the developing mouse cortex from radial glia (typical neural stem cells with the epithelial structure) to translocating neural stem cells, basal radial glia (Shitamukai et al., 2011), which become a major population of neural stem cells in mammals with gyrencephalic brains, such as primates and ferrets. We are investigating the mechanisms that underlie the formation, maintenance, and expansion of these neural stem cells, by using model mice that produce large numbers of basal radial glia as well as ferrets.

We developed a novel method based on the CRISPR/Cas9 tool and *in utero* electroporation to knock-in genes into the developing brain. This method enables us to distinguish homozygous knock-in cells as yellow-colored cells by using two different colored fluorescence genes as donors (EGFP and mCherry). The image shows an embryonic brain where two colored donors are knocked-in in β-tubulin genes to produce fusion proteins.

Lung Development

http://www.cdb.riken.jp/en/research/laboratory/morimoto.html

Team Leader

Mitsuru MORIMOTO Ph.D.

Mitsuru Morimoto received his Ph.D. in life sciences in 2003 from Tokyo University of Pharmacy and Life Sciences. From 2003 to 2006, he studied the molecular mechanisms of somitogenesis using mouse genetics at the National Institute of Genetics. He then moved to the Washington University School of Medicine in St. Louis to work with Dr. Raphael Kopan, where he extended his research to lung organogenesis. He returned to the National Institute of Genetics in 2010, and was appointed Team Leader at the RIKEN CDB in 2012.

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Assistant
Yuka NODA

Recent Publications

Tsao P, et al. Epithelial Notch signaling regulates lung alveolar morphogenesis and airway epithelial integrity. *Proc Natl Acad Sci U S A* 113. 8242–7 (2016)

Noguchi M, et al. Directed migration of pulmonary neuroendocrine cells toward airway branches organizes the stereotypic location of neuroepithelial bodies. *Cell Rep*13. 2679–2686 (2015)

Morimoto M, et al. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. *Development* 139. 4365–73 (2012)

Morimoto M, et al. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. *J Cell Sci* 123. 213–24 (2010)

Morimoto M and Kopan R. rtTA toxicity limits the usefulness of the SP-C-rtTA transgenic mouse. *Dev Biol* 325. 171–8 (2009)

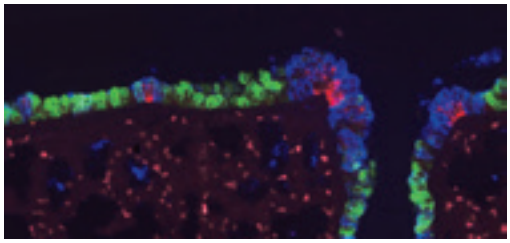
Morimoto M, et al. The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity. *Nature* 435. 354–9 (2005)

The organs of the body all play critical functional roles, which are made possible by the arrangement of differentiated cells into the structures specific to that organ. Such structures are formed throughout development, with the late embryonic and immediate postnatal periods being particularly important for the functional maturation of organ systems. Defects that arise during these organogenetic processes are closely linked to a wide range of diseases, while after birth, the body is constantly exposed to potentially damaging environmental stresses. The adult body does manifest a certain degree of regenerative ability, although this is by no means complete. To study organ formation, repair and regeneration, we have focused our research on the respiratory system in mouse.

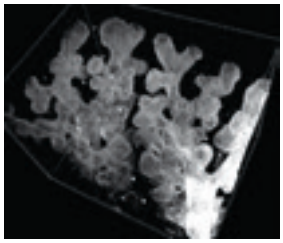
Respiratory organs in higher mammals are characterized by their efficient gas exchange, enabled by the functions of specialized cells. The development of such organs relies on the coordinated activities of both epithelial and mesenchymal tissue types, which arise from tissue-specific populations of stem cells in the developing embryo. The epithelial tissues of the conducting airways serve as the channel for the intake and exhalation of gases in the respiratory cycle, and are composed mainly of Club, ciliated, and neuroendocrine cells. In our work to date, we have shown how these various cells interact and exchange information to maintain the appropriate balance in their respective cell numbers and distributions.

Despite its location in the body's interior, the respiratory tract is constantly exposed to environmental factors, such as infections by viruses and bacteria, smoke, and chemical toxins that may damage the airway epithelium. This damage is rapidly repaired by regenerating epithelial cells supplied by somatic stem cells in the adult tissue, and as in development, the numbers and distribution of cells in the epithelium must be maintained at levels appropriate to each region of the airway.

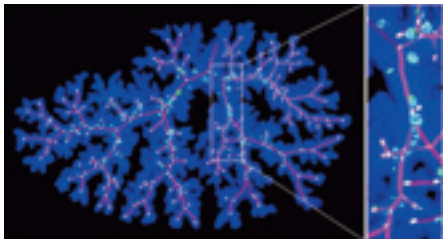
We focus on issues of how tissue morphology influences the formation of stem cell/niche tissue complexes in the development, repair and regeneration of respiratory organs, as well as mechanisms regulating cell proliferation and differentiation in developmental and regenerative processes.



Distribution of Club (green), neuroendocrine (red) and SPNC (blue) cells



3D computer reconstruction of branching bronchiole



Position of neuroendocrine cells within the entire geometric architecture of the airways

Growth Control Signaling

http://www.cdb.riken.jp/en/research/laboratory/nishimura.html

Team Leader

Takashi NISHIMURA Ph.D.

Takashi Nishimura obtained his Ph.D. in Kozo Kaibuchi's lab at the Nagoya University Graduate School of Medicine for his work on the regulation of neuronal polarization. He did postdoctoral research in the same laboratory before moving to the Institute of Molecular Biotechnology in Vienna, Austria in 2006 to pursue further postdoctoral work in Jürgen Knoblich's group. He returned to Japan in 2009, working briefly as a visiting scientist at the RIKEN Center for Developmental Biology prior to his appointment as Team Leader in July of the same year.

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Masako MINO

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Ken-ichi HIRONAKA

Technical Staff
Takayuki YAMADA

Student Trainee
Ryota MATSUSHITA

Part-Time Staff
Okiko HABARA
Noriko NISHIMURA
Junko SHINNO

Recent Publications

Yoshida M, et al. Molecular characterization of *Tps1* and *Treh* genes in *Drosophila* and their role in body water homeostasis. *Sci Rep* 6. 30582 (2016)

Okamoto N and Nishimura T. Signaling from glia and cholinergic neurons controls nutrient-dependent production of an insulin-like peptide for *Drosophila* body growth. *Dev Cell* 35. 295–310 (2015)

Matsuda H, et al. Flies without Trehalose. *J Biol Chem* 290. 1244–55 (2015)

Okamoto N, et al. A secreted decoy of InR antagonizes insulin/IGF signaling to restrict body growth in *Drosophila*. *Genes Dev* 27. 87–97 (2013)

Okamoto N, et al. Conserved role for the Dachshund protein with *Drosophila* Pax6 homolog Eyeless in insulin expression. *Proc Natl Acad Sci U S A* 109. 2406–11 (2012)

Wirtz-Peitz F, et al. Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. *Cell* 135. 161–73 (2008)

The processes of animal development, including organ size and body size, are genetically predetermined, but these processes are also influenced by environmental factors such as nutrition and temperature. The close link between cell and tissue growth control and environmental cues ensures that developmental transitions occur at the appropriate time during animal development.

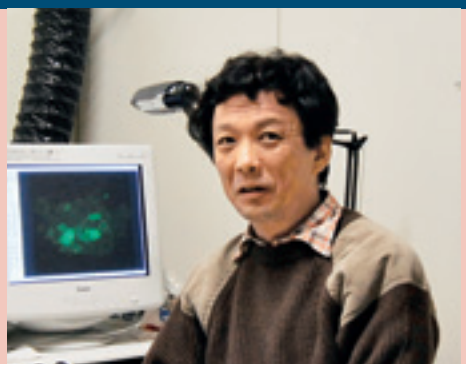
Cell proliferation and differentiation in each tissue and organ are kept under strict regulation, both spatially and temporally. Research has revealed the nature of spatial signals, such as growth factors and morphogens, but the way in which these signals direct cell and tissue growth over time remains poorly understood. In addition, growth and developmental timing are also governed by nutrient availability. Most species have a standard body size, but developing organisms are also capable of adapting their growth to fluctuating nutritional states through metabolic regulation. Therefore, linking the nutrient-sensing system to an endocrine signaling network allows organisms to control the timing of cell proliferation and differentiation.

Our team's research aims to shed light on the molecular basis for growth control and developmental timing at the cellular and tissue/organ level using *Drosophila* as a model system. In particular, we are interested in addressing the following questions: 1) How do organisms adapt their growth program to changes in energy needs and states, 2) what are the molecular mechanisms that sense nutrient availability and regulate body size, and 3) how do endocrine signals interact with metabolic and growth regulators?

To better understand the interface between nutrient availability and growth regulation, we are focusing on how nutrition controls systemic growth through *Drosophila* insulin-like peptides (Dilps). Members of the insulin family peptides have conserved roles in the regulation of growth and metabolism in a wide variety of metazoans. We have demonstrated the molecular mechanism underlying the nutrient-dependent expression of a Dilp gene. We have also conducted *in vivo* RNAi screening to identify new players regulating growth and developmental timing at the organismal level. We described the first demonstration of the glia-derived endocrine factor regulating systemic body growth. Because Dilp regulates both growth and metabolism during development, we are analyzing the physiological significance of the regulation of sugar metabolism by insulin/IGF signaling. Our work focusing on the blood sugar trehalose revealed that metabolism of hemolymph sugar plays a critical role for body growth under poor dietary conditions. Dietary condition-specific phenotype in *Drosophila* provides new insights into the significance of gene-environment interactions.

A wild-type female fly (right) and an insulin-like receptor mutant female (left)

Pluripotent Stem Cell Studies



Team Leader
Hitoshi NIWA M.D., Ph.D.

Hitoshi Niwa received his M.D. from Nara Medical University in 1989, and his Ph.D. in medical physiology in 1993 from the Kumamoto University Graduate School of Medicine for his work in gene trap methods. From 1993 to 1994, he worked as a research associate in the Department of Developmental Genetics at the same university, before taking a postdoctoral fellowship with Austin Smith at the University of Edinburgh Centre for Genome Research. He returned to Japan in 1996 as a research associate in the Department of Nutrition and Physiological Chemistry at the Osaka University Graduate School of Medicine, where he remained until taking a position as Team Leader at the RIKEN CDB. He was appointed Project Leader of the Laboratory for Pluripotent Stem Cell Studies in October 2009.

The Laboratory for Pluripotent Stem Cell Studies closed in March 2016.
Dr. Niwa is now at Kumamoto University.

Staff

Research Scientist
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Satoshi OHTSUKA
Robert Odell STEPHENSON

Research Associate
Mariko YAMANE

Technical Staff
Kumi MATSUURA

Part-Time Staff
Sachiko HASHIMOTO
Yayoi NAKAI

Recent Publications

Niwa H, et al. The evolutionally-conserved function of group B1 Sox family members confers the unique role of Sox2 in mouse ES cells. *BMC Evol Biol* 16. 173 (2016)

Nakai-Futatsugi Y and Niwa H. *Zscan4* is activated after telomere shortening in mouse embryonic stem cells. *Stem Cell Reports* 6. 1–13 (2016)

Yamane M, et al. Zscan10 is dispensable for maintenance of pluripotency in mouse embryonic stem cells. *Biochem Biophys Res Commun* 468. 826–831 (2015)

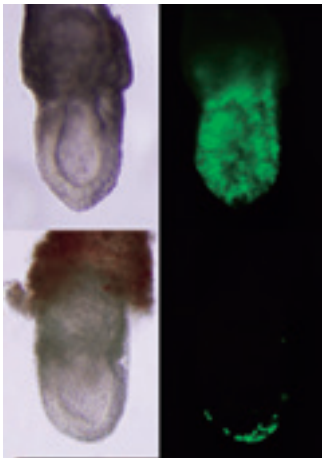
Kinoshita M, et al. *Sox7* is dispensable for primitive endoderm differentiation from mouse ES cells *BMC Dev Biol* 15. 37 (2015)

Fujii S, et al. Nr0b1 is a negative regulator of Zscan4c in mouse embryonic stem cells. *Sci Rep* 5. 9146 (2015)

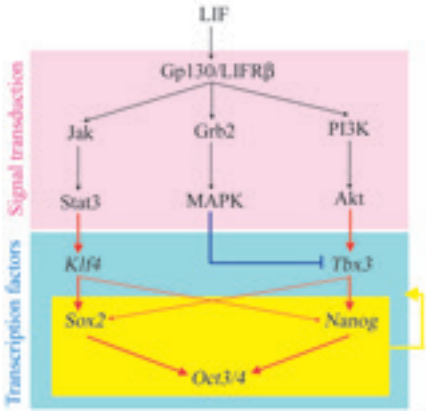
Ohtsuka S and Niwa H. The differential activation of intracellular signaling pathways confers the permissiveness of embryonic stem cell derivation from different mouse strains. *Development* 142. 431–437 (2015)

Pluripotency is a term used to describe the ability of certain types of stem cells that are capable of giving rise to at least one type of cell from all three germ layers—endoderm, mesoderm and ectoderm. Stem cells are also characterized by their ability to produce copies of themselves with similar differentiative potential, as well as more specialized cells with different properties through differentiation. Embryonic stem (ES) cells are the best known example of a type of stem cell possessing these properties of pluripotency and self-renewal. In our lab, we use ES cells as a model system for studying the molecular mechanisms that determine and enable the mysterious properties of pluripotent cells.

Pluripotency can be primarily determined by a particular set of transcription factors, for as we now know, pluripotency can be induced by four transcription factors. These transcription factors should form a self-organizing network that is able to stabilize their expression and maintain pluripotency. At the same time, the network should have the sensitivity to signals that induce differentiation. How can the transcription factor network satisfy these contradictory requirements? What is the principle behind the structure of the transcription factor networks governing developmental processes? We are now trying to answer these questions by studying ES cells and the changes they undergo in their differentiation toward trophoblast stem cells, extraembryonic endoderm cells, and primitive ectoderm cells.



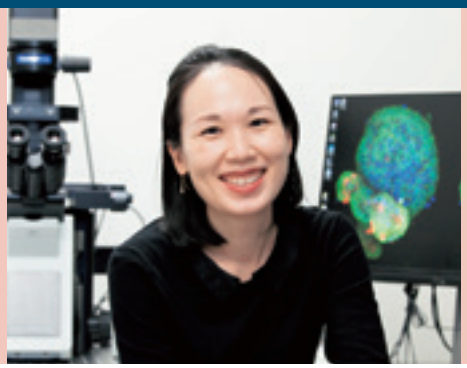
Chimeric embryos generated by injection of Gata6GR ES cells into blastocysts. These ES cells carry the constitutively-active Egfp transgene and contribute to primitive ectoderm (upper panels), whereas they contribute to parietal endoderm after induction of Gata6 activity with dexamethasone (lower panels).



Schematic model of transcription factor networks for pluripotent stem cells, trophectoderm and primitive endoderm.

Vascular Morphogenesis

http://www.cdb.riken.jp/en/research/laboratory/phng.html



Team Leader

Li-Kun Phng Ph.D.

Li-Kun Phng received her B.Sc. in Pharmacology from the University of Bristol in 2002, her M.Sc. for Research in Life Science from the University of Edinburgh in 2004, and her Ph.D. from University College London in 2009, for her work on blood vessel development in the laboratory of Holger Gerhardt at the Cancer Research UK London Research Institute. She was awarded the EMBO and HFSP Long-term Fellowships to perform postdoctoral research at the European Molecular Biology Laboratory (EMBL), Heidelberg in 2009, and in VIB/KU Leuven, Belgium in 2011. She next moved to the National Cerebral and Cardiovascular Center Research Institute, Osaka, in 2014, after receiving the JSPS Postdoctoral Fellowship for Foreign Researchers. She was appointed to her current position as Team Leader at RIKEN CDB in October 2016.

Staff

Technical Staff
Akane NOMORI

Assistant
Emi TANIGUCHI

Recent Publications

Gebala V, et al. Blood flow drives lumen formation by inverse membrane blebbing during angiogenesis *in vivo*. *Nat Cell Biol* 18(4): 443–451 (2016)

Phng LK, et al. Formin-mediated actin polymerization at endothelial junctions is required for vessel lumen formation and stabilization. *Dev Cell* 32. 123–132 (2015)

Phng LK, et al. Filopodia are dispensable for endothelial tip cell guidance. *Development* 140. 4031–4040 (2013)

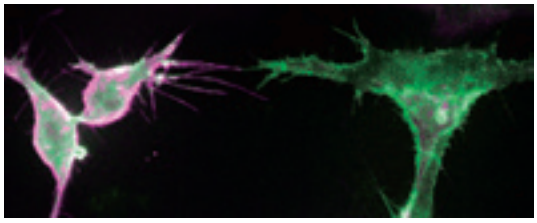
Phng LK, et al. Nrarp coordinates endothelial Notch and Wnt signalling to control vessel density in angiogenesis. *Dev Cell* 16. 70–82 (2009)

Hellström M, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature* 445. 776–770 (2007)

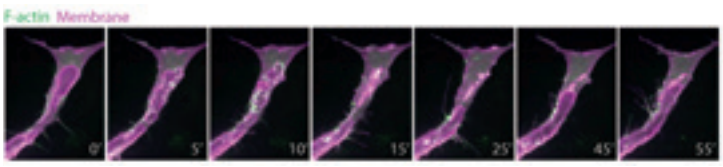
The establishment of an intricate network of interconnected blood vessels is essential for the development of many tissues and organs. Tissue vascularization frequently occurs through sprouting angiogenesis, where new blood vessels arise from pre-existing ones, and encompasses a multitude of cellular processes including polarized collective cell migration, proliferation, anastomosis and lumen formation. While many key molecules and signaling pathways have been identified to regulate endothelial tip/stalk cell specification, blood vessel guidance and arterial-venous differentiation, there is still a poor understanding of how angiogenic signals are relayed to the cell's machinery to drive changes in endothelial cell morphology, behavior and consequently, the final pattern of the vasculature.

My laboratory aims to unravel fundamental mechanisms that regulate endothelial cell dynamics and coordination during blood vessel morphogenesis. Previous studies on actin cytoskeleton revealed that specialized F-actin of different dynamics and subcellular localization drive distinct steps of vessel morphogenesis. For example, the transient polymerization of F-actin at the apical membrane controls lumen expansion while a stable pool of F-actin at endothelial cell junctions stabilizes nascent lumens to produce a functional vascular network. In the future, we aim to investigate the role of force generation in regulating endothelial cell dynamics and vessel morphogenesis, the mechanisms controlling actomyosin activity in endothelial cells and the molecular regulation of vessel lumen formation. Our long-term goal is to understand how upstream angiogenic signals as well as hemodynamic forces regulate force generation and endothelial cell behavior.

To achieve our research goals, we employ live imaging at high temporal and spatial resolution, advanced fluorescent microscopy techniques, genetics and chemical biology to elucidate the mechanical and molecular mechanisms of blood vessel morphogenesis using the zebrafish as our model organism.



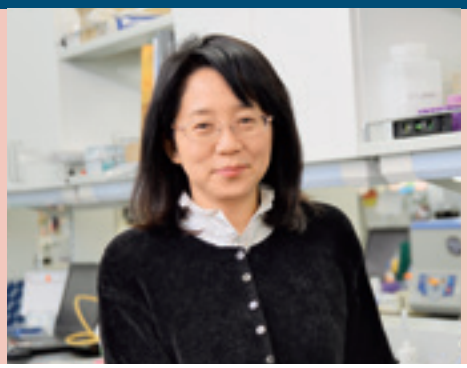
Endothelial cells are highly plastic and undergo extensive cell shape changes during blood vessel morphogenesis. Green, F-actin. Magenta, membrane.



F-actin and apical membrane dynamics during lumen invagination. Local and transient actin polymerisation (5', 10') and myosin activity at apical membranes retract inverse blebs and can cause lumen collapse (15'). Remnants of membranes eventually fuse and integrate with the apical membrane of invaginating lumen (25' to 55').

Retinal Regeneration

http://www.cdb.riken.jp/en/research/laboratory/takahashi.html



Project Leader

Masayo TAKAHASHI M.D., Ph.D.

Masayo Takahashi received her M.D. in 1986, and her Ph.D. in 1992 from Kyoto University. After an assistant professorship in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1996, where she discovered the potential of stem cells as a tool for retinal therapy. She returned to Kyoto University Hospital in 1998, and was appointed associate professor at the Translational Research Center in the same hospital in 2001. She joined the CDB as a Team Leader of the Lab for Retinal Regeneration in 2006. In 2013, her team launched a pilot clinical study of autologous iPS cell-derived RPE cell sheets for exudative aged-related macular degeneration (AMD), and performed the first RPE cell sheet graft transplantation in September 2014. Her clinical specialty is retinal disease–specifically, macular diseases and retinal hereditary diseases. Her aim is to understand these diseases at a fundamental level and develop retinal regeneration therapies.

Staff

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Junki SHO
Motoki TERADA
Kazuko TSUJIMOTO
Chikako YAMADA

Part-Time Staff
Shoko FUJINO

Recent Publications

Mandai M, et al. iPSC-derived retinal transplants improve vision in *rd1* end-stage retinal degeneration mice. *Stem Cell Reports* 8. 69–83 (2017)

Sugita S, et al. Successful transplantation of retinal pigment epithelial cells from MHC homozygote iPS cells in MHC-matched models. *Stem Cell Reports* 7(4): 635–648 (2016)

Sugita S, et al. Lack of T-cell response to iPS cell-derived retinal pigment epithelial cells from HLA homozygous donors. *Stem Cell Reports* 7(4): 619–634 (2016)

Kamao H, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Reports* 2. 205–18 (2014)

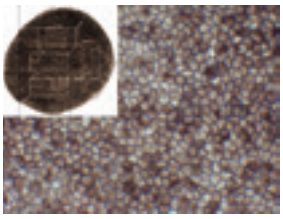
Jin Z B, et al. Modeling retinal degeneration using patient-specific induced pluripotent stem cells. *PLoS One* 6. e17084 (2011)

Osakada F, et al. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nat Biotechnol* 26. 215–24 (2008)

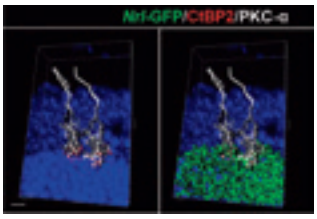
The retina has been called the “approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that in adult mammals the retina was entirely incapable of regenerating, but we now know that at least new retinal neurons can be generated after being damaged. This has opened up new hope that the ability to regenerate neurons and even to reconstitute the neural network may be retained in the adult retina. We are now exploring the exciting prospect that, by transplanting cells from outside of the retina or by regeneration from intrinsic progenitor cells, it may one day be possible to restore lost function to damaged retinas.

Our research into retinal regeneration seeks to achieve clinical applications by developing methods for inducing stem cells or embryonic stem cells to differentiate into retinal neurons and pigment epithelial cells in sufficient quantities for use in the treatment of patients suffering from conditions in which such cells have been damaged or lost. We must also ensure that such cells establish viable grafts upon transplantation and induce the reconstitution of functional neural networks. We also hope to develop means of promoting true regeneration by activating endogenous stem cells to replace cells lost to trauma or disease and thus repair damaged tissues. Access to a broad spectrum of developmental biological research information will be key to the achievement of these goals, and we appreciate the opportunities for exchange that working in the environment provided by the RIKEN CDB.

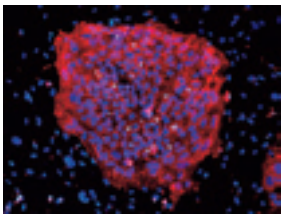
Therapeutic applications cannot be developed from basic research alone; the clinical approach—a thorough understanding of the medical condition to be treated—is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily be translatable to a human clinical therapy without an understanding of the underlying genetics and possible immunological involvement. Our goal is to study retinal regeneration based on both a strong foundation in basic research and solid clinical evidence.



iPSC-derived retinal pigment epithelium cells and cell sheet (generated for clinical use)



Mouse iPSC-derived photoreceptors are morphologically able to form synapses after transplantation into host model animals.



Human iPSCs (immunostained red, SSEA-4; blue, DAPI)

Human Organogenesis

http://www.cdb.riken.jp/en/research/laboratory/takasato.html

Team Leader

Minoru TAKASATO Ph.D.

Minoru Takasato is Team Leader of the Laboratory for Human Organogenesis at the RIKEN CDB, where he heads the kidney regeneration project. He received his B.Sc. in anthropology from the University of Tokyo, Japan in 2002, and completed his Ph.D. focusing on the study of mouse kidney development in the same university in 2008 before joining Professor Little's laboratory as a postdoctoral fellow at Institute for Molecular Bioscience, University of Queensland, Australia, in 2009. He is an expert in the directed differentiation of human pluripotent stem cells to kidney with his most recent work being published in *Nature Cell Biology* (2014) and *Nature* (2015). His most recent studies describe the generation of mini-kidneys from induced pluripotent stem cells for use in drug screening and disease modelling. His work has drawn a great deal of international interest in kidney development and stem cell research fields.

Staff

Research Scientist
Wei ZHAO

Visiting Researcher
Yoshiki SAHARA

Technical Staff
Kisa KAKIGUCHI
Kazuhiro OFUJI

Recent Publications

Takasato M, et al. Generation of kidney organoids from human pluripotent stem cells. *Nat Protoc* 11. 1681–1692 (2016)

Takasato M, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 526. 564–568 (2015)

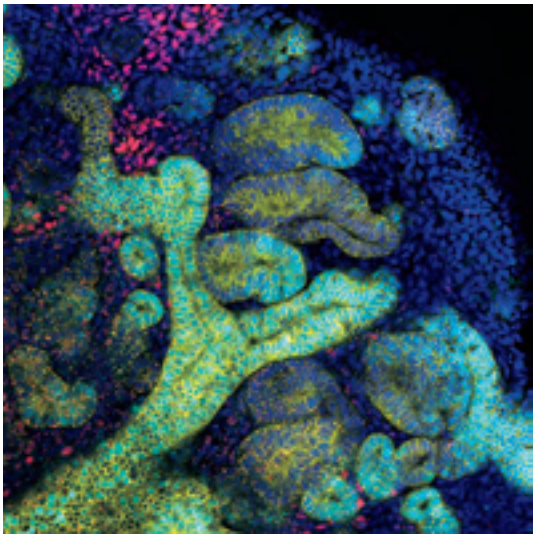
Takasato M and Little M H. The origin of the mammalian kidney: implications for recreating the kidney *in vitro*. *Development* 142. 1937–1947 (2015)

Takasato M, et al. Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat Cell Biol* 16. 118–126 (2014)

Hendry C E, et al. Direct transcriptional reprogramming of adult cells to embryonic nephron progenitors. *J Am Soc Nephrol* 24. 1424–1434 (2013)

Takasato M, et al. Trb2, a mouse homolog of tribbles, is dispensable for kidney and mouse development. *Biochem Biophys Res Commun* 373. 648–652 (2008)

What is the ultimate goal of regenerative research using human pluripotent stem cells? We think this is to recreate a whole replaceable organ *in vitro* via directed differentiation. Due to the continuous rise in the incidence of end-stage renal disease around the world (approximately 7% per annum), there is an urgent demand for regenerative strategies to compensate for the loss of renal function in these patients. In our previous study, we developed a protocol by which human pluripotent stem cells can be differentiated into the intermediate mesoderm that can self-organize into kidney organoids. While these kidney organoids comprise all anticipated renal tissues, including nephrons, collecting duct, blood vessels and renal interstitium, they are still far from the real human kidney in terms of their size, tissue complexity, maturity and functionality. By precisely recapitulating the developmental processes of the human kidney in directed differentiation of human pluripotent stem cells, we are trying to achieve the ultimate goal of generating a three-dimensional kidney that is functional and that can also be transplanted into patients. We appreciate knowledge from basic developmental biology that is essential for such regenerative studies; therefore, we are also highly interested in studies of human embryology. Utilizing our unique technology that generates hPSC-derived kidney organoids from pluripotent stages *in vitro*, we are focusing particularly on uncovering the developmental mechanisms of the human mesoderm and kidney.



Kidney organoids derived from human iPS cells. The organoid contains two kidney progenitors, the ureteric tree (yellow with cyan) and nephron progenitor (red), as well as developing nephrons (yellow).

Cell Adhesion and Tissue Patterning

http://www.cdb.riken.jp/en/research/laboratory/takeichi.html

Team Leader

Masatoshi TAKEICHI Ph.D.

Masatoshi Takeichi is Team Leader of the Laboratory for Cell Adhesion and Tissue Patterning. He completed the B.Sc. and M.S. programs in biology at Nagoya University before receiving a doctorate in biophysics from Kyoto University in 1973. After attaining his Ph.D., he took a research fellowship at the Carnegie Institution Department of Embryology under Richard Pagano. He then returned to Kyoto University, attaining a professorship in the Department of Biophysics (1986–1999), before becoming professor in the Department of Cell and Developmental Biology in the Graduate School of Biostudies at the same university. He assumed the directorship of the CDB in 2000, and resigned from this position in 2014.

Staff

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Technical Staff
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Yoko INOUE
Miwa KAWASAKI
Hiroko SAITO
Vassil VASSILEV

Assistant
Mutsuko AISO-WATANABE

Recent Publications

Nishimura T, et al. DAAM1 stabilizes epithelial junctions by restraining WAVE complex-dependent lateral membrane motility *J Cell Biol* 215. 559–573 (2016)

Toya M, et al. CAMSAP3 orients the apical-to-basal polarity of microtubule arrays in epithelial cells. *Proc Natl Acad Sci U S A* 113. 332–337 (2016)

Tsukasaki Y, et al. Giant cadherins Fat and Dachsous self-bend to organize properly spaced intercellular junctions. *Proc Natl Acad Sci U S A* 111. 1601–6 (2014)

Hayashi S, et al. Protocadherin-17 mediates collective axon extension by recruiting actin regulator complexes to interaxonal contacts. *Dev Cell* 30. 673–87 (2014)

Takeichi M. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. *Nat Rev Mol Cell Biol* 15. 397–410 (2014)

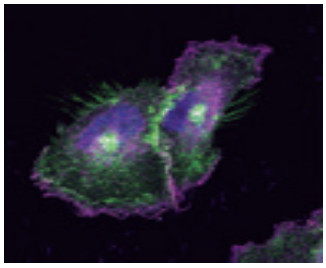
Tanaka N, et al. Nezha/CAMSAP3 and CAMSAP2 cooperate in epithelial-specific organization of noncentrosomal microtubules. *Proc Natl Acad Sci U S A* 109. 20029–34 (2012)

Animal cells organize into tissues with complex architecture. Our lab is exploring the molecular mechanisms by which individual cells assemble into a tissue-specific multicellular structure, such as epithelial sheets and neural networks. We are also interested in the molecular basis of how tissue architecture is disrupted during carcinogenesis, a process that is thought to accelerate the metastasis of cancer cells. For these studies, we are focusing on the roles played by cell-cell adhesion and recognition molecules, the cadherin family of adhesion molecules in particular, as these are known to be indispensable for tissue construction. Our current studies are divided into three categories:

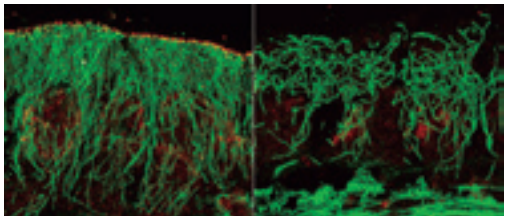
1) Cell-cell adhesion is a dynamic process, and this nature of cell-cell adhesion is implicated in various cell behaviors, such as contact-dependent regulation of cell movement and cancer metastasis. A growing body of evidence suggests that cadherins cooperate with cytoskeletal and/or motility machineries, such as actin regulators, non-muscle myosins, and Rho GTPases, in modulating cell assembly. We are therefore studying the molecular mechanisms underlying the crosstalk between cadherins and such cytoskeletal systems, and their roles in epithelial junction formation.

2) A second area of interest to our lab is to gain a better understanding of how the cell-cell adhesion machinery contributes to animal morphogenesis. Using mouse embryos, we are analyzing the roles of cadherins and associated proteins in various morphogenetic processes, including neural crest migration. We are also investigating the roles of members of the cadherin superfamily known as protocadherins, deficiencies of which have been implicated in human brain disorders. Through these studies, we expect to gain deeper mechanistic insights into the ways by which cells build the elaborate structures of the animal body.

3) In addition, we have been analyzing the functions of microtubule minus end-associated proteins, Nezha/CAMSAPs. These proteins regulate microtubule assembly patterns, centrosomal function, and organelle positioning. We are exploring the roles of these molecules in cellular morphogenesis, such as polarized epithelial formation and axon growth, with the aim of uncovering novel functions of non-centrosomal microtubules.



A pair of U251 cells in contact with one another, triple-immunostained for Protocadherin17-EGFP (green), the WAVE complex protein Abi-1 (magenta) and DNA (blue). Protocadherin17 recruits the WAVE complex to cell-cell contact sites, and convert them into a motile structure. Cell edges with this structure actively move, contributing to collective cell migration.



Microtubules (green) and CAMSAP3 (red) in intestinal epithelial cells. CAMSAP3 localizes at the apical cortex of the cells, and tether microtubules to this site through its binding to their minus ends. When CAMSAP3 is knocked out, the longitudinal arrays of microtubules are disrupted.

Organ Regeneration

http://www.cdb.riken.jp/en/research/laboratory/tsuji.html

Team Leader

Takashi TSUJI Ph.D.

Takashi Tsuji received his Master's degree from Niigata University in 1986, and after working in the pharmaceuticals industry for three years, returned to complete his doctorate at Kyushu University, and he received his doctorate in 1992 from Niigata University. He conducted research at Niigata University from 1992 to 1994 before moving to serve as researcher and then senior scientist at JT Inc. From 2001, he moved to Tokyo University of Science, and in 2007 he was appointed full professor at the same university. During his academic career, he received numerous prestigious grants and awards, and participated in numerous industry collaborations. He joined the CDB as a Group Director in 2014, and has served as Team Leader since the restructuring of the Center in November 2014.

Staff

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Mamoru ISHII
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Ryohei MINAMIDE
Makoto TAKEO

Visiting Scientist
Miho OGAWA
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Aya SHIOKAWA
Miki TAKASE
Yuko TAMAI
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Assistant
Mayumi MUROFUSHI
Sayaka NAKAMURA

Recent Publications

Takagi R, et al. Bioengineering a 3D integumentary organ system from iPS cells using an *in vivo* transplantation model. *Science Advances* 2(4): e1500887 (2016)

Ozone C, et al. Functional anterior pituitary generated in self-organizing culture of human embryonic stem cells. *Nat Commun* 7. 10351 (2016)

Ogawa M, et al. Functional salivary gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 4. 2498 (2013)

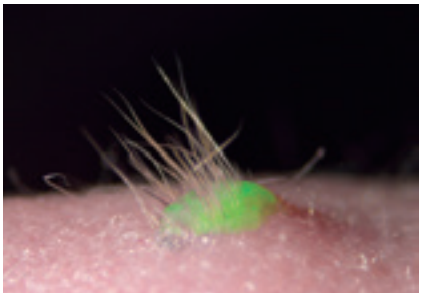
Hirayama M, et al. Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 4. 2497 (2013)

Toyoshima K E, et al. Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. *Nat Commun* 3. 784 (2012)

Ikeda E, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci U S A* 106. 13475–80 (2009)

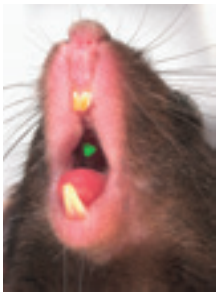
Organogenesis begins with the formation of patterned developmental fields during early embryogenesis, which provide environments appropriate for the induction of specific organs. Most organs emerge from primordia induced by interactions between epithelial and mesenchymal tissue, and following organ-specific morphological changes, develop into functional structures.

Our group is working to gain a more complete understanding of the roles of epithelial-mesenchymal interactions in organ induction, development, and morphogenesis. Using technologies developed in our group for the three dimensional (3D) control of epithelial stem cells and mesenchymal stem cells, we have generated regenerative primordia for teeth, hair follicles and endocrine tissue, such as salivary glands, and shown that these functionally integrate with surrounding tissue following transplantation into adult mice. By recapitulating organogenetic fields as seen in the early embryo to steer the self-organized formation of 3D tissue-like structures from pluripotent stem cells, such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), we seek both to elucidate the mechanisms by which such fields induce organogenesis and to develop new technologies for use in regenerative medicine. We are also interested in using 4D cell dynamics imaging techniques to quantitatively analyze organ-specific morphogenesis, and to analyze, model, and simulate molecular pathways involved in their control, in the hopes of gaining new insights into the fundamental principles underlying organogenetic processes. Building on these fundamental studies, we further seek to conduct applications research towards the development of technologies for use in therapeutic organ regeneration.



Mouse iPS cell-derived hair

Bioengineered hair follicle



Bioengineered tooth

Epithelial Morphogenesis

http://www.cdb.riken.jp/en/research/laboratory/wang.html

Team Leader

Yu-Chiun WANG Ph.D.

Yu-Chiun Wang completed his B.Sc. (1996) and M.S. (1998) from the National Taiwan University, Taiwan, and received his doctorate in 2006 from the University of Chicago, U.S.A. under the supervision of Prof. Edwin Ferguson for his work on the regulation of BMP signaling during dorsal-ventral patterning in the *Drosophila* for which he was awarded the 2007 Larry Sandler Memorial Award for best dissertation of *Drosophila* research. In 2007, he moved to Princeton University to work as a postdoctoral fellow in the laboratory of Eric Wieschaus and was also awarded a postdoctoral research fellowship from the Helen Hay Whitney Foundation for his work on understanding the mechanisms of the formation of the cephalic furrow and dorsal transverse folds during *Drosophila* gastrulation. He was appointed Team Leader of the Laboratory for Epithelial Morphogenesis at the CDB in October 2013.

Staff

Technical Staff
Mustafa SAMI
Michiko TAKEDA

International Program Associate
Anthony ERITANO

Assistant
Yuko FUJIYAMA

Recent Publications

Khan Z, et al. Quantitative 4D analyses of epithelial folding during *Drosophila* gastrulation. *Development* 141. 2895–900 (2014)

Gavin-Smyth J, et al. A genetic network conferring canalization to a bistable patterning system in *Drosophila*. *Curr Biol* 23. 2296–302 (2013)

Wang Y C, et al. Distinct Rap1 activity states control the extent of epithelial invagination via alpha-catenin. *Dev Cell* 25. 299–309 (2013)

Wang Y C, et al. Differential positioning of adherens junctions is associated with initiation of epithelial folding. *Nature* 484. 390–3 (2012)

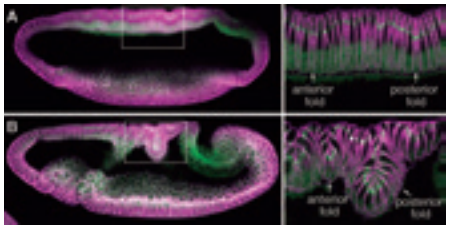
Wang Y C and Ferguson E L Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. *Nature* 434. 229–34 (2005)

Podos S D, et al. The DSmurf ubiquitin-protein ligase restricts BMP signaling spatially and temporally during *Drosophila* embryogenesis. *Dev Cell* 1. 567–78 (2001)

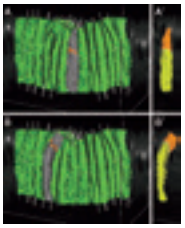
The central question in developmental biology is how cells, tissues and organs acquire their specific functions and shapes. A large body of work over the past several decades has yielded a broad understanding of how functional specialization is achieved through differential gene expression. In contrast, far less is known about how cell shapes and tissue structures are controlled and remodeled. Although a general theme has emerged whereby cytoskeletal elements control the cell shapes, while alteration of individual cell shapes collectively organizes the tissue architecture, the underlying molecular and mechanical mechanisms remain poorly understood. My lab aims at identifying novel mechanisms that orchestrate the formation of three-dimensional epithelial structures. Our long-term goal is to comprehensively understand the mechanistic principles of tissue morphogenesis in order to conceptualize the origin of morphological diversity both within an organism and among evolutionary lineages.

We are currently focusing on how modifications of epithelial cell polarity control cell shapes using gastrulating *Drosophila* embryos as the model system. Our previous work identified a novel mechanism for cell shape changes whereby cell shortening is induced upon a basal repositioning of the apical-basal polarity and cell-cell adhesive apparatus adherens junctions. The cell shortening occurs in two narrow strips of cells, producing heterogeneities in cell height within the tissue, thereby allowing it to bend. The polarity-based mechanism represents the first instance wherein the initiation of epithelial folding does not involve the canonical myosin-dependent apical constriction. Since cell-cell adhesion and apical-basal polarization are two fundamental features of epithelial tissues, our work potentially heralds a general mechanism for cell shape changes and epithelial folding. In addition, we found that after initiation, the depths of epithelial folds differ depending on the degrees of neighboring cell invagination. Genetic evidence suggests that the strength of mechanical coupling between adherens junctions and their underlying actin cytoskeleton determines the extent of cell invagination. Our ongoing work promises to identify genes and forces that sculpt distinct morphological features.

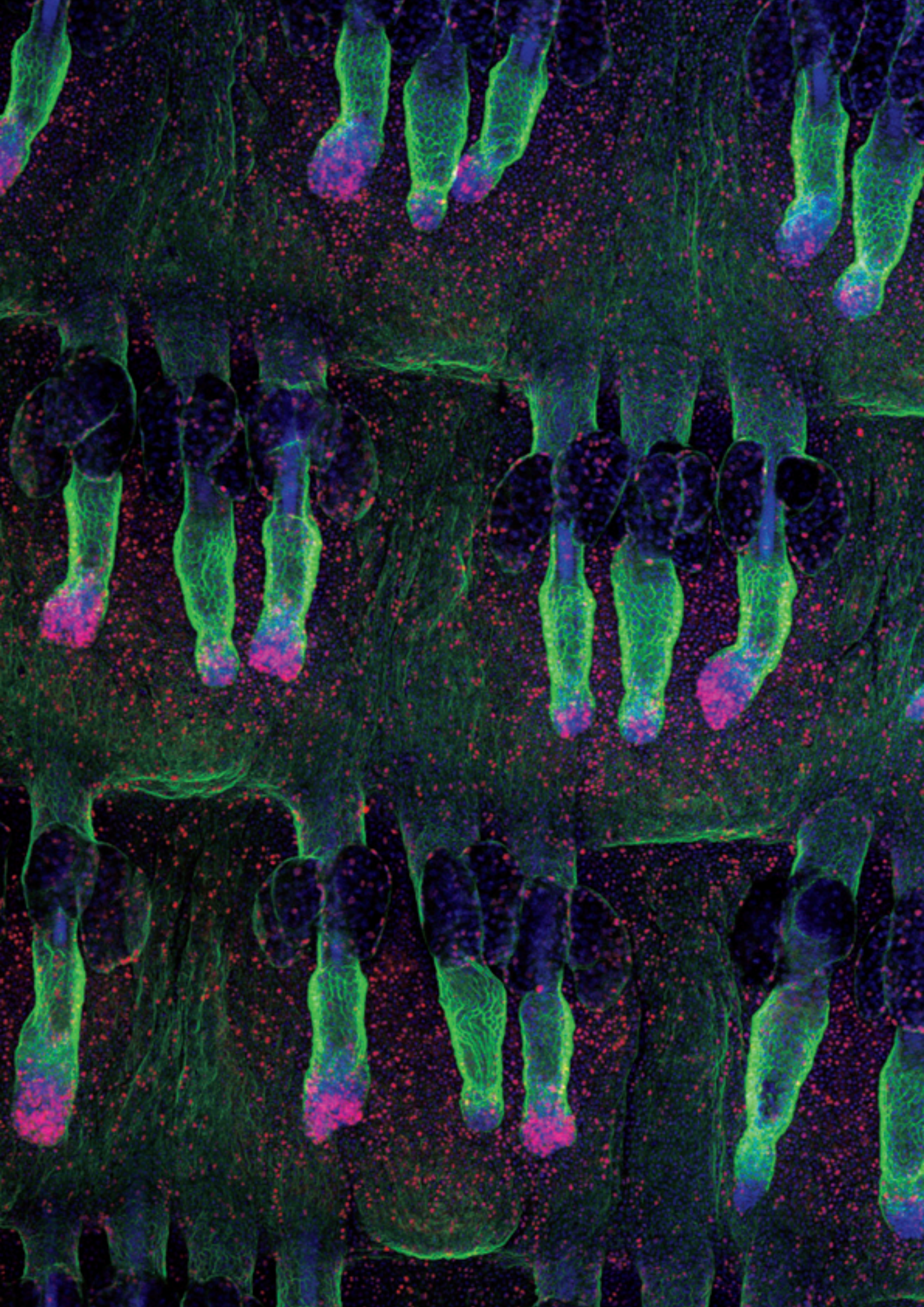
We employ an integrated approach that combines genetic manipulation, two-photon deep tissue live imaging and computational cell shape reconstruction. We are also in the process of designing novel imaging strategies that could be used to visualize mechanical forces and computational algorithms that reconstruct and quantify 4D cell shapes. Furthermore, we will launch a multidisciplinary, international collaboration that combines genetics, computational and evolutionary approaches to analyze the history and function of transiently formed epithelial structures that do not eventually contribute to a body part or organ.

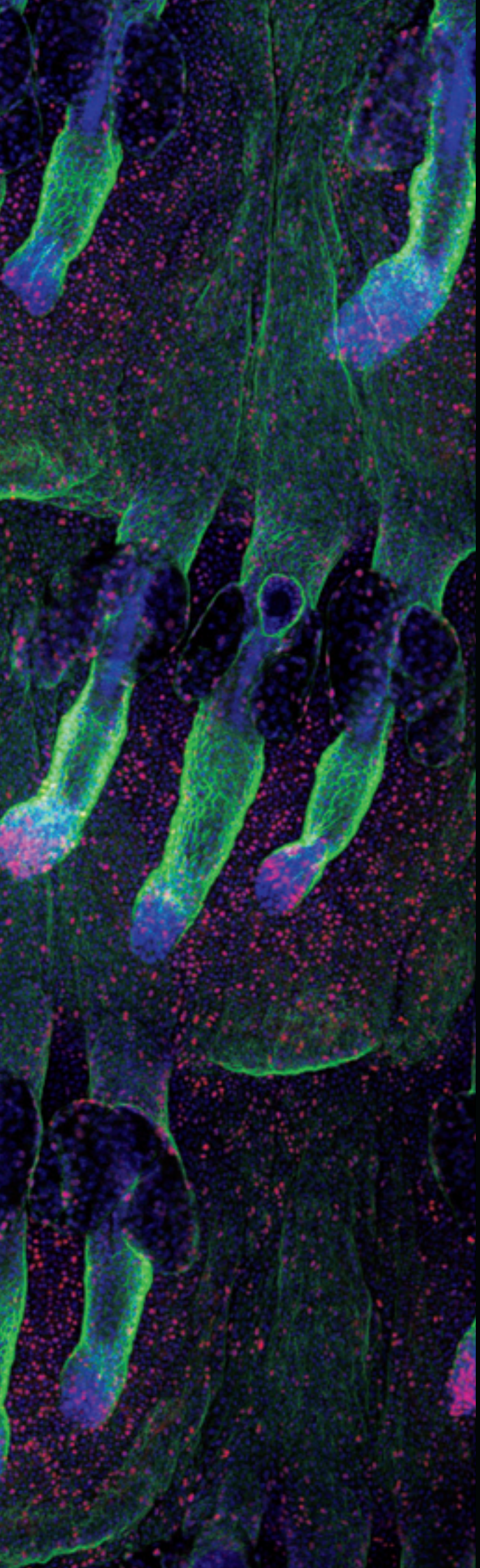


Dorsal fold formation during *Drosophila* gastrulation. The dorsal epithelium of the *Drosophila* gastrula forms two epithelial (anterior and posterior) folds, shown here with the mid-sagittal optical sections at an early (A) and a late (B) stage. Adherens junctions are labeled in green; plasma membrane in magenta.



Reconstruction of 3D cell shape during dorsal fold formation. EDGE4D software was developed in collaboration with Dr. Zia Khan (University of Maryland). 3D reconstruction of cell shape was based on cell membrane labeling. Immunolabeling of adherens junctions (orange in A and B) defines the boundaries between the apical and basal-lateral compartments (A' and B').





Activities

3D image of skin from talk of adult mouse stained with antibodies for Integrin-α6 (green), and Ki67 (red), and DAPI (blue). Quiescent skin stem cells of hair follicles are stained green. Proliferating stem cells are stained red.
Image: Hironobu Fujiwara, Laboratory for Tissue Microenvironment

Size in Development: Growth, Shape and Allometry

March 28–30, 2016

The RIKEN CDB hosted its fourteenth annual symposium on March 28 to 30, in the CDB auditorium. The theme of this year's symposium was entitled, "Size in Development: Growth, Shape and Allometry," and an audience of 176 scientists and students from over 18 different countries convened at the Center to take part in a program of talks and discussions exploring the question of organismal size control.

The three-day program featured over 30 invited and selected talks and poster sessions in which more than 60 presenters discussed their research that covered a range of topics including growth control, organ size and shape, scaling and morphogenesis, and evolution of allometry. The co-organizers of this year's symposium were Shigeo Hayashi, Hidehiko Inomata and Mitsuru Morimoto from the CDB, and Stefano Piccolo from University of Padua Medical School, Italy.

The CDB symposium series was launched in 2003 as a forum to encourage the timely exchange and discussion of recent findings, progress and trends in developmental biology, regeneration and other related fields.

Session 1 Organ Size and Shape Control

Yingzi Yang
(National Human Genome Research Institute, NIH and Harvard School of Dental Medicine, USA)
Mary Baylies
(Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, USA)
Alberto Rosello-Diez (Sloan Kettering Institute, USA)
Shigeo Hayashi (RIKEN Center for Developmental Biology, Japan)
Mitsuru Morimoto (RIKEN Center for Developmental Biology, Japan)
Justin Crest (University of California, Berkeley, USA)
Mototsugu Eiraku (RIKEN Center for Developmental Biology, Japan)
James Wells
(Cincinnati Children's Hospital Medical Center and University of Cincinnati, USA)
Richard A. Schneider (University of California, San Francisco, USA)

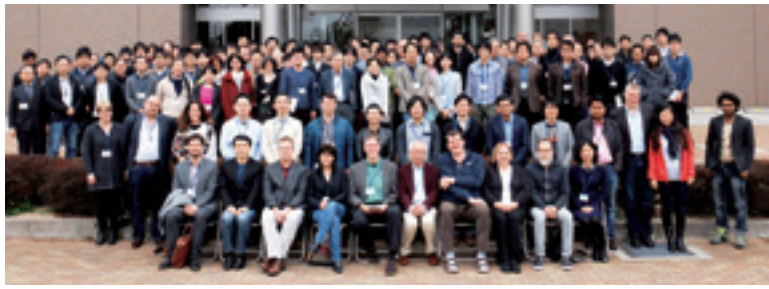
Session 2 Growth Control

Hiroshi Sasaki (Osaka University, Japan)
Kazuo Yamamoto (Nagasaki University School of Medicine, Japan)
Stefano Piccolo (University of Padua Medical School, Italy)
Makoto Furutani-Seiki
(Yamaguchi University School of Medicine, Japan and University of Bath, UK)
Georg Halder
(VIB Center for the Biology of Disease, and KU Leuven Center for Human Genetics, University of Leuven, Belgium)
Yusuke Mii (National Institute for Basic Biology, Japan)
Iswar K. Hariharan (University of California, Berkeley, USA)
Duojia Pan
(Howard Hughes Medical Institute and Johns Hopkins University School of Medicine, USA)
Igor Adameyko (Karolinska Institutet, Sweden)











Session 3 Scaling and Morphogenetic Control

Hidehiko Inomata (RIKEN Center for Developmental Biology, Japan)
Kazutaka Hosoda (Kyoto University, Japan)
Anna Kicheva (IST Austria, Austria)
Rebecca Heald (University of California, Berkeley, USA)
Hirohisa Kyogoku (RIKEN Center for Developmental Biology, Japan)
Timothy E. Saunders
(Mechanobiology Institute, National University of Singapore, Singapore)
Alexander Aulehla (EMBL Heidelberg, Germany)
Kana Ishimatsu (Harvard Medical School, USA)
Nadia Rosenthal
(The Jackson Laboratory, USA and Imperial College London, UK)

Session 4 Allometry in Evolution

Ehab Abouheif (McGill University, Canada)
Toru Miura (Hokkaido University, Japan)
Alejandro Burga (University of California, Los Angeles, USA)
Mitsuyasu Hasebe (National Institute for Basic Biology, Japan)
David M. Kingsley
(Howard Hughes Medical Institute and Stanford University, USA)
Elaine A. Ostrander
(National Human Genome Research Institute, National Institutes of Health, USA)






2017 CDB Symposium

Towards Understanding Human Development, Heredity, and Evolution

March 27–29, 2017

The fifteenth annual symposium, "Towards Understanding Human Development, Heredity, and Evolution," will be held on March 27–29, 2017, at the RIKEN CDB.

Recent advances in technologies, including stem cell culture, live imaging, single-cell approaches, next-generation sequencing, and genome editing, have led to an unprecedented expansion in our knowledge on human development and heredity. Comparative studies between humans and other mammals have also shed light on evolutionary processes, and in turn provided critical insights into the mechanisms for key diseased states. Given this exciting progress and promising prospects in the relevant fields, it is important and also timely to discuss current understandings of human development, heredity, and evolution, in addition to exploring the possibilities that have opened up to reconstitute such processes *in vitro* using pluripotent and other stem cells. The CDB Symposium 2017 will cover a broad range of topics, including human germline development, early embryogenesis, organogenesis from stem cells and relevant disease models, as well as human genetics and evolution.

Invited Speakers

Déborah Bourc'his
(Institut Curie, France)
Guillaume Bourque
(McGill University and Genome Quebec Innovation Center, Canada)
Patrick F. Chinnery
(University of Cambridge and MRC Mitochondrial Biology Unit, UK)
Mototsugu Eiraku
(RIKEN CDB, Japan)
Anne Ferguson-Smith
(University of Cambridge, UK)
Zev J. Gartner
(University of California, San Francisco, USA)
Edith Heard
(Institut Curie, France)
Agnar Helgason
(deCODE Genetics, Iceland)
Rudolf Jaenisch
(MIT, Whitehead Institute, USA)
Yoshiya Kawaguchi
(Center for iPS Cell Research and Application, Kyoto University, Japan)
Tomoya Kitajima
(RIKEN CDB, Japan)
Arnold Kriegstein
(University of California, San Francisco, USA)
Rasmus Nielsen
(University of California, Berkeley, USA)
Ryuichi Nishinakamura
(Kumamoto University, Japan)
Molly Przeworski
(Columbia University, USA)
Lluis Quintana-Murci
(Institut Pasteur-CNRS, France)
Mitinori Saitou
(Kyoto University, JST ERATO, Japan)
Rickard Sandberg
(Karolinska Institutet, Sweden)
Austin Smith
(Cambridge Stem Cell Institute, UK)
Michael Snyder
(Stanford University, USA)
Azim Surani
(Wellcome Trust/Cancer Research UK Gurdon Institute, UK)
Gen Suwa
(The University Museum, The University of Tokyo, Japan)
Takashi Tsuji
(RIKEN CDB, Japan)

2016 Seminars

The RIKEN CDB makes special efforts to provide a full and diverse series of invited seminars by scientists from around the world. To date, the Center has hosted close to 800 such talks, in addition to numerous meetings, internal forums, and colloquia. The following speakers were invited to the CDB to give a seminar in the period from January to December 2016.

Date	Title	Speaker
01-14	Integrated genomics approaches for the identification of causative variants and genes in GWAS-identified risk loci: Application to inflammatory bowel disease	Michel GEORGES
01-22	Evolution and development of the bifurcated caudal axial skeleton in the twin-tail goldfish	Kinya G. OTA
02-26	Rocaglates convert DEAD-box protein eIF4A into a sequence-selective translational repressor	Shintaro IWASAKI
03-09	Nature Publishing Group - Scientific data seminar	Varsha KHODIYAR
03-15	Biochemical analysis of genome functions using the locus-specific chromatin immunoprecipitation technologies: Key tools to elucidate 4D Nucleome	Hodaka FUJII
03-24	Antisense therapy for Fukuyama type congenital muscular dystrophy	Mariko TANIGUCHI-IKEDA
03-31	Dynamics of bilaterian genome architecture evolution: Insights from the octopus and hemichordate genomes	Oleg SIMAKOV
04-20	The human ciliopathy protein JBTS17 is required for basal body docking and intraflagellar transport for ciliogenesis	Michinori TORIYAMA
04-22	Biomedical and Engineering Seminar	Shunji YAMANAKA
04-28	Development of new cell-free regeneration therapies based on the novel protein complex inducing tissue-regenerating microenvironment	Akihito YAMAMOTO
05-10	Controlling contractile instabilities in the actomyosin cortex	Stephan GRILL
06-06	Warburg-like metabolism regulates vertebrate somitogenesis	Masayuki OGINUMA
06-07	Mechanism of the asymmetry of signaling endosomes during asymmetric division	Marcos GONZALEZ-GAITAN
06-08	Dissecting the regulatory circuitry of microRNAs in neutrophils	Qing DENG
06-13	Molecular mechanisms underlying faithful chromosome segregation-- Inner centromere-shugoshin network	Yoshinori WATANABE
06-27	Defining the stem cell lineages in adult skin epidermis	Aiko SADA
06-30	Versatile protein tagging with split fluorescence protein: A scalable strategy for GFP tagging of endogenous human proteins	Sayaka SEKINE
07-12	Formation of the vertebrate gut	Clifford J. TABIN
07-13	Cell lineages in the early mammalian blastocyst	Janet ROSSANT
08-02	Enhancer control of transcriptional bursting	Takashi FUKAYA
09-09	The evodevo & physics of skin appendage and skin colour patterning in vertebrates	Michel C. MILINKOVITCH

Date	Title	Speaker
09-14	Developmental genetics of mammalian reproductive organs	Richard BEHRINGER
09-26	From nerve growth to synapse formation and plasticity	Mu-ming POO
10-03	In for the long haul: Maintaining chromosome cohesion in mammalian oocytes	Mary HERBERT
10-03	The final cut: control of progression through meiosis II	Wolfgang ZACHARIAE
10-18	Human naïve pluripotent stem cells and early embryogenesis	Yasuhiro TAKASHIMA
10-19	Pervasive translational regulation of the cell signaling circuitry underlies mammalian development	Kotaro FUJII
10-31	Stem cell heterogeneity and function in development and regeneration	Shahragim TAJBAKHSH
11-11	Developmental control of replication timing and chromosome architecture	David M. GILBERT
11-21	The developmental basis for the recurrent evolution of deuterostomy and protostomy	José M. MARTIN-DURAN
11-24	Mechanical forces influence three-dimensional cell behaviours during mouse mandibular arch development	Hirotaka TAO
11-25	Order from chaos: Rules and self-organizing properties of division patterns in plants	Alexis MAIZEL
11-28	Shaping cells and tissues: From embryonic development to synthetic biology	Stefano DE RENZIS
11-30	Homology and diversity in animal genomes	Peter W. H. HOLLAND
12-05	Investigating the interplay between obesity and cancer using *Drosophila*	Susumu HIRABAYASHI
12-07	The regulatory mechanism of gastric stem cell and its malignant transformation	Yoku HAYAKAWA
12-08	Decoding cellular immortality using genetically engineered human pluripotent stem cells	Kunitoshi CHIBA
12-12	Assembly of neural circuits	Liqun LUO
12-14	An extensive HLH transcription factor network promoting longevity in response to signals from the gonad	Shuhei NAKAMURA
12-19	Mouse embryo keeps paternal chromosomes away from maternal spindle to maintain the ploidy during fertilization	Masashi MORI
12-20	X chromosome inactivation initiated by dysfunctional Xist RNA	Takashi SADO

2016 Events

The RIKEN Center for Developmental Biology strives to engage with the public through a variety of media, including its website, printed materials and media coverage, and through direct interactions such as guided tours, open house events, and other public outreach activities. The CDB also organizes events for the scientific community to facilitate the exchange of information between scientists from around the world in a setting outside the laboratory environment.

CDB Retreat in Awaji

The Center's research staff gathered on Awaji Island for the annual CDB Retreat from September 29–30. Held in a new location this year on the southern end of Awaji Island, the laboratory heads, research scientists, student trainees, and technical staff took part in the closed meeting to share and openly discuss the latest developments in their respective projects outside normal lab settings. The program included short talks by students and young scientists, and lectures by Team Leaders Minoru Takasato and Erina Kuranaga, QBiC Unit Leader Yuchi Taniguchi, and CDB alumnus Hiroshi Sawa (National Institute of Genetics).

KAIST-CDB-CAS Joint Meeting

The 3rd joint meeting between RIKEN CDB, KAIST's Department of Biological Sciences (Korea), and Chinese Academy of Sciences' Institute of Genetics and Developmental Biology (China) was held at the KAIST Institute, in Daegu, Korea from October 27 to 28. Five team leaders from the CDB attended the meeting which is held every two years, rotating locations amongst the three institutions with the aim to strengthen institutional ties as well as share and discuss recent findings in the area of developmental biology.

Open House 2016

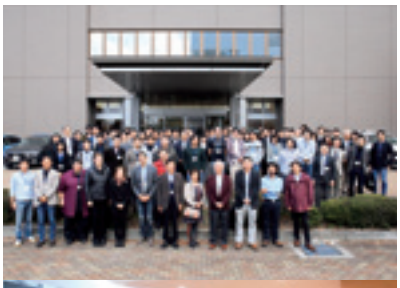
The CDB, along with the other RIKEN Kobe Campus research centers held its annual Open House on Saturday, November 5. Over 1,700 people of all ages dropped by the CDB to learn about the research being carried out at the Center. One of the highlights of the event is the Open Labs where visitors are permitted to walk through the laboratories, see samples of cells and tissues under microscopes, and talk to scientists about their research. Other events included an exhibit introducing different model organisms used in research, a DNA experiment corner where visitors could try out techniques used by scientists for DNA analysis, and a talk show and Q&A session with scientists working at the CDB campus. There was also a book lounge featuring books recommended by CDB scientists, and a handicraft corner for kids making animal masks and color-your-own bags. This is the Center's largest annual event geared for the general public, allowing scientists to engage with the public face-to-face.

The 27th CDB Meeting “Body Surface Tactics: Cellular crosstalk for the generation of super-biointerfaces”

The 27th CDB Meeting entitled, “Body Surface Tactics: Cellular crosstalk for the generation of super-biointerfaces,” co-hosted by the Japan Skin Research Club and the RIKEN Symposium, was held November 14 and 15. Over 130 students and scientists from both academia and industry studying the body surface in different disciplines, from cell and developmental biology, regenerative biology, vascular and neurobiology, to immunology, evolution, mathematics and medicine, were in attendance. The talks and discussions covered a broad range of topics including epithelial formation, functional unit formation, maintenance and regeneration, evo-devo, new technologies, and dysfunction and therapeutics. Keynote lectures were given by Yann Barrandon of EPFL in Switzerland and Singapore A-STAR, and Cheng-Ming Chuong of the University of Southern California, USA.

The 28th CDB Meeting “Cilia and Centrosomes: Current Advances and Future Directions”

The 28th CDB Meeting was held from November 27 to 29. There were 101 participants from within Japan and abroad attended the meeting, which was co-hosted by the Cilia Club and the MEXT Grant-in-Aid for Scientific Research on Innovative Areas “Cilium-centrosome system regulating biosignal flows.” Research on cilia and centrosomes have progressed along parallel paths, and only recently have researchers begun to realize the importance of tying these two fields together to gain a more comprehensive perspective of the common molecular mechanisms regulating the function of these organelles and their association with various diseases. Twenty-two talks and over 30 posters were presented at the meeting, on a range of topics including roles of the cilia and centrosomes in cell cycle, embryogenesis, and disease pathogenesis.



2016 Educational Programs

As part of its commitment to promoting awareness and understanding of development, regeneration, evolution, stem cells, regenerative medicine and related fields, and its interests in maintaining close ties with the academic and research communities, the RIKEN CDB dedicates itself to conducting training and educational programs for a wide range of audiences. Although not an academic institution, the Center works with students, educators, and scientists, as well as other organizations, to develop, implement, and promote a variety of instructional courses throughout the year.

Intensive lecture program for graduate students

The CDB has formed partnerships with a number of graduate schools in the Kansai region, and organizes a two-day lecture program every year for graduate students enrolled in partnering institutions. This year's lecture program was held August 3 to 4, and included talks by selected laboratory heads and research scientists on their research and tours of labs and some of the technical facilities. Some graduate students attending the lecture program can also receive credit towards their degree program.

Summer school for high school students

The tenth annual one-day summer school program for high school students was held on August 18 and 23. This program features a talk by a CDB scientist, a tour inside a working laboratory, and a hands-on scientific experiment. The theme of this year was genes and their functions, and the students analyzed the DNA extracted from their own cheek epithelial cells to determine whether or not they carry a single nucleotide polymorphism in their gene encoding an enzyme involved in metabolizing alcohol. Two scientists in the Laboratory for Organismal Patterning contributed to the program by giving a talk on their research as well as showing the students actual specimen samples and some of the state-of-art equipment used in their lab.

Internship for undergraduate students

Thirty-four undergraduate students from universities around Japan were invited to spend a week, from August 22 to 26, as interns in the participating laboratories on the CDB campus. The internship program offers the students the chance to work on small-scale projects under the supervision of scientists in the hosting labs, as well as listen to lectures by laboratory heads and visit different labs. The activity-filled week concluded with a presentation session on the final day, where the interns presented the findings from their projects to the other participants.

High school teachers' workshop

A one-day practical workshop for high school biology teachers was held at the CDB on October 2. The program was co-organized by the CDB, the Japanese Society for Developmental Biologists (JSDB), and the Hyogo Prefectural High School Educational Committee for Biology. Twenty-eight teachers (18 participants and ten observers), mainly from around the Kansai area, took part in the workshop. This year's theme was genetics using the fruit fly, and the program was led by Team Leader Shigeo Hayashi, who also oversaw its development. The aim was for the teachers to gain a firsthand understanding of how genes and environmental factors affect phenotypes through examining *Drosophila* mutants caused by sex-linked inheritance or epigenetic factors. The program was designed to facilitate the teachers to take what they learned back to the classroom. Several of the teachers who participated in this workshop also had the chance to teach their own students about what they learned by serving as TAs in a tutorial program modified for high school students in December.

2016 Awards

As one of the world's leading institutes in the fields of developmental biology and stem cell research, the RIKEN CDB strives to maintain the highest levels of originality, impact and quality in its scientific contributions. The individual achievements and careers of many of its scientific staff have been recognized with awards from numerous organizations, including government ministries and agencies, private foundations and academic societies. The CDB takes great pride in the achievements of its researchers, as individuals and as indispensable members of their laboratories.

Awardee	Position	Laboratory	Award	Organization
Tomoya Kitajima	Team Leader	Chromosome Segregation	MEXT Prize (The Young Scientist's Prize)	The Ministry of Education, Culture, Sports, Science and Technology (MEXT)
Hiroshi Hamada	Director	Organismal Patterning	EMBO Associate Member	The European Molecular Biology Organization (EMBO)
Minoru Takasato	Team Leader	Human Organogenesis	The 2016 Australian Museum Eureka Prize for Scientific Research	The Australian Museum
Shuhei Yoshida	Research Scientist	Chromosome Segregation	MBSJ 39th Annual Meeting Excellent Poster Presentation Award	The Molecular Biology Society of Japan

Kobe sits at the heart of the Kansai region of western Japan, close to both the bright lights of Osaka and Kyoto's tranquility. The local climate is temperate, making it possible to enjoy a wide range of seasonal activities, all within a short train ride or drive from the center of the city. Japan's renowned public transport system allows rapid and convenient access to both local and national destinations, and the many area airports, including the Kobe Airport located less than 10 minutes from the CDB, and the Kansai International Airport, provide immediate gateways to any destination in the world. Costs of living are comparable to those in many major Western cities, and comfortable modern homes and apartments are available to suit all budgets.

The bustling heart of Kobe includes a variety of distinct neighborhoods. The downtown area of Sannomiya sits square in the city's center, and its range of international restaurants and late-night bars promise a great evening out any night of the week. The neighboring Motomachi district offers a mix of upscale department stores and funky shopping arcades, standing in contrast to the colorful Chinatown that's right next door. A short walk north from city center lies the old foreign settlement of Kitano, whose clapboard houses and well-kept parks are a perfect retreat from the dynamism downtown.

RIKEN in Kobe
Budget and Staff
About RIKEN
RIKEN Campuses

Kobe occupies a splendid location between mountains and seas, with easy access to many natural spots. A short drive or bus ride takes you to the island of Awaji, with its beaches and first-rate seafood. Hiking trails crisscross the forested mountains that span the entire length of the city, beckoning hikers and picnickers to enjoy a day in the fresh air. The city is also dotted with parks that come into bloom at the start of cherry blossom season, and its many rivers, beaches and scenic areas make it easy to take enjoyable day trips, inside the city or out.

Its central location in Japan puts Kobe within close reach of many of the country's most popular destinations. Kyoto, the ancient capital, is only an hour away by train, making it possible to enjoy some of Japan's most popular historic sites and cultural assets. Nara, another early city, features herds of tame deer in the precincts of some of the world's oldest wooden buildings. Complementing the old-world style of these two cities is Japan's second city of Osaka, which offers a hip and modern take on urban living.

Photography: Shigekazu SHINYA

RIKEN in Kobe

The RIKEN Center for Developmental Biology was the first research center established by RIKEN in the city of Kobe, only the second in all of Western Japan. In 2013, RIKEN made sweeping changes to its organizational structure, which eliminated regional administrative organizations referred to as Institutes (including the former RIKEN Kobe Institute, of which CDB was a member), and conferred greater autonomy to the individual research centers. These are now grouped by proximity into a number of Branches around the country.

The RIKEN Kobe Branch is now home to several other life science research centers, including the RIKEN Center for Life Science Technologies (CLST), and the Osaka-based RIKEN Quantitative Biology Center (QBiC). The CLST was established in 2013 through the fusion of research programs at the former RIKEN Yokohama and Kobe Institutes, and focuses on technology research and development to support the medical and pharmaceutical sectors, while QBiC focuses on measurement, analysis, and modeling technologies and techniques to model cell dynamics.

Kobe is also home to the RIKEN Advanced Institute for Computational Science (AICS), which is associated with the national K Supercomputer project.

Administrative support at RIKEN Kobe and CDB

- Kobe Branch
  - RIKEN Kobe Administrative Division
    - General Affairs
    - Human Resources
    - Finance
    - Osaka Administrative Section
  - Information Networks Office – Kobe Branch
  - RIKEN Kobe Safety Center
  - Planning Office for each Center

Center for Life Science Technologies (CLST)

The RIKEN Center for Life Science Technologies (CLST) works to develop key technologies for breakthroughs in medical and pharmaceutical applications, and to conduct groundbreaking R&D for the next-generation of life sciences. The CLST aims to promote research on biomolecules and into life science technologies, focusing on designing molecular structures at the atomic level, manipulating molecular function at the cellular level, and tracing molecular dynamics at the whole-body level.

Quantitative Biology Center (QBiC)

The RIKEN Quantitative Biology Center (QBiC) focuses on the complex spatiotemporal relationships between components of biological systems through innovative measurement, analysis, and modeling technologies and techniques. These will be used to predict, design, simulate, and manipulate cellular phenomena, which may help to revolutionize research and applications in the life sciences, including such fields as regenerative medicine and diagnostics.

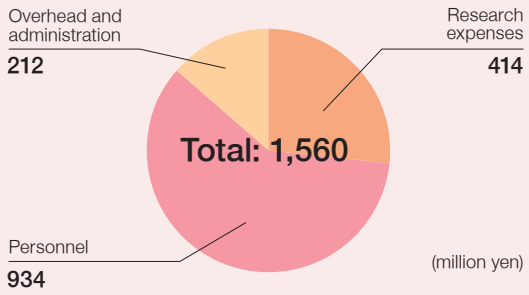
Advanced Institute for Computational Science (AICS)

The RIKEN Advanced Institute for Computational Science (AICS) houses the K supercomputer, which is used to generate cutting-edge scientific results and technological breakthroughs through collaboration and integration of computational and computer sciences, to assist and advance the science of forecasting in areas that are linked directly to our daily lives, from predicting weather patterns and effects of natural disasters, to drug design and development of new devices and materials. More recently, they are involved in the development of a supercomputer to succeed the K computer.

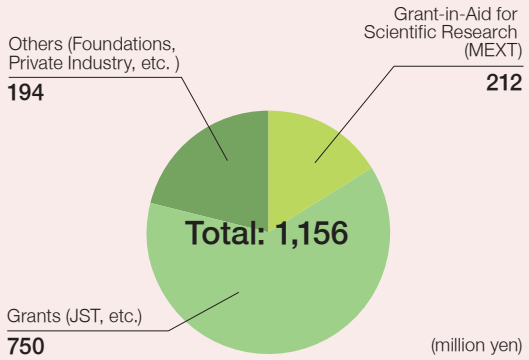
Kobe Administrative Services

The Kobe Administrative Division dedicated to providing core administrative services to the entire RIKEN Kobe Branch. Its main areas of responsibility are general affairs, facilities management, human resources, contracts, finances and accounting. The Information Networks Office maintains both network access and multiple intranet services of the Kobe Branch. The Safety Center provides training and supervision for lab and workplace safety, implements and monitors disaster prevention measures, and ensures compliances with national and institutional regulations governing laboratory practice and technology. Within the CDB, the Developmental Biology Planning Office coordinates important activities including budget and funding management, and administrative support for laboratory performance reviews, as well as provides support for domestic and international communications and for organizing scientific meetings. The Library Office manages the CDB research literature collections and interlibrary loans.

2016 CDB Budget



In addition to the dedicated funds outlined above, individual labs and investigators are encouraged to compete for external funding from sources such as the Japan Society for the Promotion of Science (JSPS), the Japan Science and Technology Agency (JST), and other governmental and private sources. These external funds represent a significant component of the Center's total funding every year.



2016 CDB Staff

Laboratory heads	19
Deputy leaders	2
Research scientists	54
Research associates	4
Technical staff	61
Assistants	16
Visiting scientists	121
Student trainees	27
Part-time staff	25
Other	10
Total	339
Kobe Branch Administration	84

About RIKEN

The mission of RIKEN is to conduct comprehensive research in science and technology (excluding only the humanities and social sciences) as provided for under the “RIKEN Law,” and to publicly disseminate the results of its scientific research and technological developments. RIKEN carries out high level experimental and research work in a wide range of fields, including physics, chemistry, medical science, biology, and engineering, covering the entire range from basic research to practical application.

RIKEN was first organized in 1917 as a private research foundation, and reorganized in 2003 as an independent administrative institution under the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and designated in 2015 as a National Research and Development Institute. RIKEN will be celebrating its centennial in 2017.

RIKEN Website

The RIKEN website provides an in-depth resource for online visitors to the institute. The site contains important links to the latest research achievements, all materials as well as databases and other electronic resources developed by RIKEN laboratories. For those with an interest in learning more about RIKEN’s organization, activities, and history, visit:

http://www.riken.jp

RIKEN Research

RIKEN publishes the quarterly print and online magazine *RIKEN Research* to draw the world’s attention to some of RIKEN’s best research in a timely and easy-to-understand fashion. This magazine serves as a central resource for up-to-date information on key achievements of the numerous RIKEN research centers, along with insights into the people, programs and facilities. The core component of *RIKEN Research* is the short, easy-to-read ‘Research Highlight’ articles explaining for a broad scientific audience a sampling of the latest research articles published by RIKEN scientists.

http://www.riken.jp/en/research/rikenresearch/

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Center for Advanced Photonics
Center for Sustainable Resource Science
Brain Science Institute
Nishina Center for Accelerator-Based Science
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Cluster for Industry Partnerships
Cluster for Science and Technology Hub
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RIKEN

http://www.riken.jp/

RIKEN is a publicly funded research organization established to conduct comprehensive research in science and technology, and to disseminate the results of its scientific research and technological development. RIKEN carried out basic and applied research in a wide range of fields, including physics, chemistry, biology, medical science, and engineering.

RIKEN was founded in 1917 as a private research organization, *Rikagaku kenkyusho* (The Institute of Physical and Chemical Research). In 2003, RIKEN was reorganized as an Independent Administrative Institution under the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), and in 2015, was designated as a National Research and Development Institute. RIKEN continues to engage in wide-ranging research activities spanning the basic and applied sciences.

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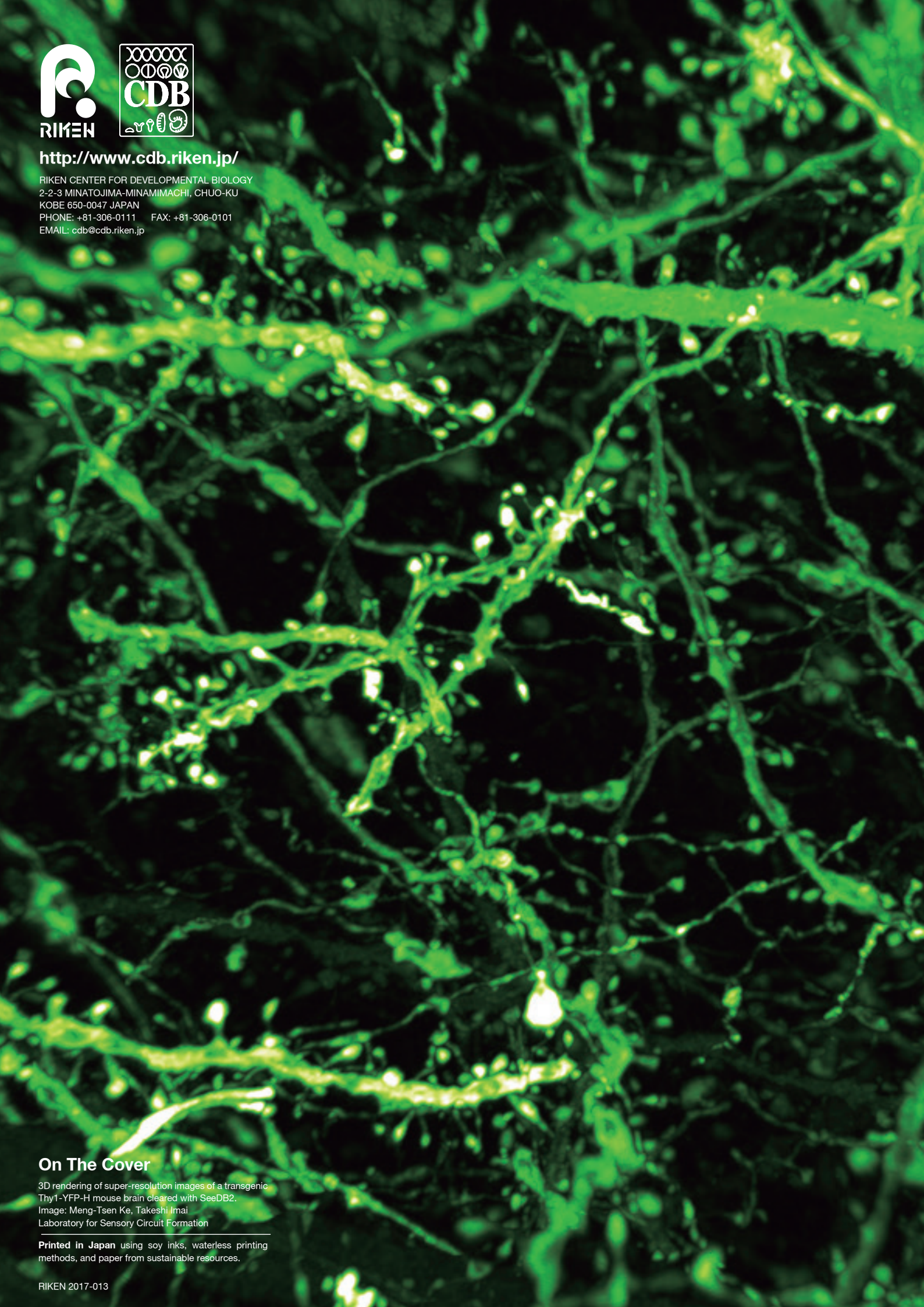

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On The Cover

3D rendering of super-resolution images of a transgenic Thy1-YFP-H mouse brain cleared with SeeDB2.
Image: Meng-Tsen Ke, Takeshi Imai
Laboratory for Sensory Circuit Formation

Printed in Japan using soy inks, waterless printing methods, and paper from sustainable resources.

RIKEN 2017-013