RIKEN Center for Developmental Biology

2017 Annual Report

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RIKEN Center for Developmental Biology

Contents

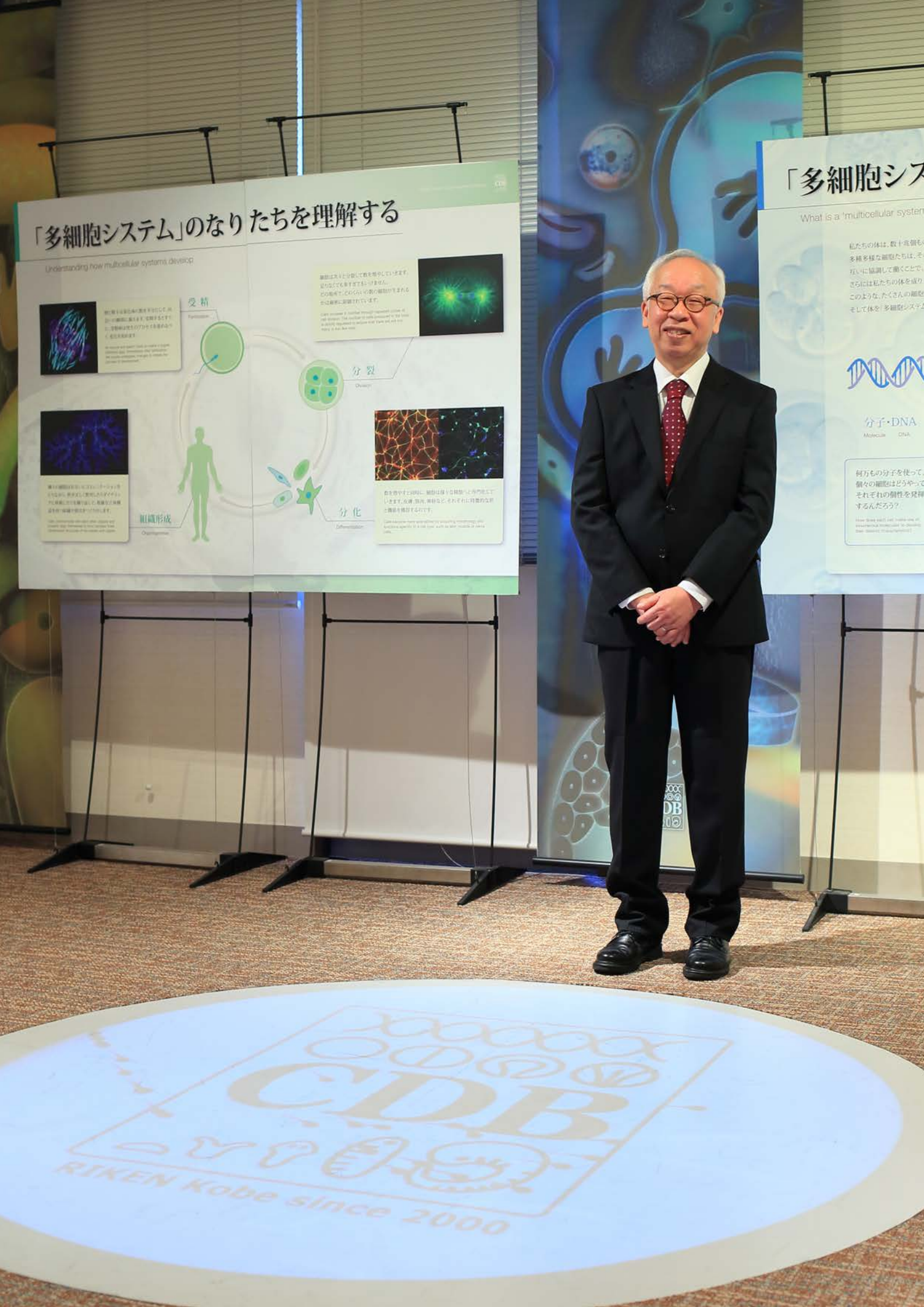
The RIKEN Center for Developmental Biology was launched in April 2000 under the auspices of the Millennium Project Research Initiative, which was established to drive research in areas of vital importance to both Japan and the world in the 21st century. The challenges facing an aging society were among 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 and cell biological level, the more complex phenomena involved in organogenesis, as well as the biology of stem cells and

Laboratories

Activities

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.


「多細胞システム」のなりたちを理解する
Understanding how multicellular systems develop
受精
Fertilization
分裂
Division
組織形成
Organogenesis
分化
Differentiation
「多細胞シス
What is a 'multicellular system
分子・DNA
Molecule DNA
CDB
RIKEN Kobe since 2000

Message from the Director

The year 2017 marked an important milestone for RIKEN—one hundred years of groundbreaking scientific research since its establishment in 1917 as a private research foundation. This legacy was celebrated throughout the year with numerous public events held around Japan in addition to a special centennial ceremony held in April in the presence of Their Majesties the Emperor and Empress of Japan, serving as an opportunity for RIKEN to renew its pledge to continue to contribute to the advancement of science and technology in Japan during the next hundred years.

2017 was also significant for the RIKEN Center for Developmental Biology (CDB), signaling a turning point in our Center's history. The final year as the CDB. For starting in April 2018, in line with the new five-year mid-term plan laid out by RIKEN, the CDB will merge with two other RIKEN centers, the Center for Life Science Technologies (CLST) and the Quantitative Biology Center (QBiC), to establish a new life science research center, the RIKEN Center for Biosystems Dynamics Research (BDR). Over the past year, the scientists and administrative staff from the three centers have dedicated countless hours discussing and preparing for the large-scale organizational change. As the first step, the three centers organized a successful two-day joint retreat to facilitate and encourage the scientific exchange among their members to find new opportunities for collaboration and discoveries.

Despite the imminent challenges ahead, the laboratories at the CDB have continued to turn out research findings that seek to broaden our scope of understanding of the genetic, molecular, and cell biological mechanisms involved in development and regeneration as well as developing scientific foundations for applications in regenerative medicine. These include revealing mechanisms implicated in epithelial cell folding, cell migration, establishment of body axes, and odor discrimination, among others. New advancements were also made in the iPS cell-based clinical research for age-related macular degeneration, which we hope will yield promising results as a new approach for treating the disorder.

One of the driving features of CDB that has allowed us to stay at the forefront of developmental and regenerative biology fields has been to encourage the turnover of principal investigators, to make way for new and innovative ideas that a new generation brings. In the latter half of the year, as part of this positive turnover, we welcomed the arrival of three new principle investigators; Itoshi Nikaido, unit leader of the Single-cell Omics Research Unit, and two team leaders, Wataru Kimura heading the Laboratory for Heart Regeneration and Kazunari Miyamichi leading the Laboratory for Comparative Connectomics. These new laboratories are sure to enhance our research programs as they settle into our community. We have also continued our efforts to foster the next-generation of scientists through our internship program for undergraduate students, our intensive summer lecture program for graduate students enrolled in affiliated universities, and through hosting and supervising graduate students hailing from around Japan as well as abroad.

The CDB remains committed to working closely and maintaining strong ties with academic and scientific communities as well as with industry to unearth and foster innovative areas of collaborative research to keep pace with the rapidly changing times. Our partnership with Otsuka Pharmaceutical, Ltd., through the RIKEN CDB–Otsuka Pharmaceutical Collaboration Center, and the joint symposiums organized with the clinicians working at Kobe Children's Hospital are a few examples to this end.

In closing, I would like to express my deepest gratitude to all of members of the CDB, to RIKEN, and the scientific community, who have not only provided their unfailing support, encouragement and cooperation during my tenure as director of the CDB, but also since the Center was first established in 2000. Although the CDB will transition to a new stage over the next year, we pledge to continue carrying out high quality groundbreaking research that we are known for, and I ask for your continued support as we confront the new challenges and opportunities ahead.

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 Collaboration 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

Center Director Hiroshi Hamada

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.
- **Heart Regeneration**
  Wataru KIMURA Ph.D.
- **Chromosome Segregation**
  Tomoya KITAJIMA Ph.D.
- **Growth Control Signaling**
  Takashi NISHIMURA Ph.D.
- **Vascular Morphogenesis**
  Li-Kun PHNG Ph.D.

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.
- **Human Organogenesis**
  Minoru TAKASATO Ph.D.
- **Organ Regeneration**
  Takashi TSUJI 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**
  The 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

Office of the Director

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.
- **Comparative Connectomics**
  Kazunari MIYAMICHI Ph.D.
- **Cell Adhesion and Tissue Patterning**
  Masatoshi TAKEICHI Ph.D.
- **Epithelial Morphogenesis**
  Yu-Chiun WANG 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 Ethics Education Officer

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.

Technical Development and Supporting Program

This program aims to develop new technologies that meet the research needs of CDB and provide technical support to labs using these technologies.

- **Single-cell Omics Research Unit**
  Itoshi NIKAIDO 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.

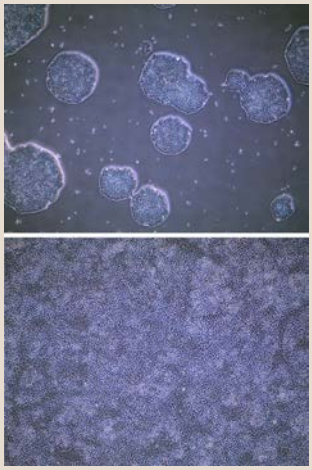
Management Committee

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
- **Hiroshi Fukai**
  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

Highlights 2017

Start of clinical research using allogeneic iPSCs



On February 6, RIKEN CDB's Masayo Takahashi, project leader of the Laboratory for Retinal Regeneration together with her collaborators from the Kobe City Medical Center General Hospital, Osaka University Hospital, and Kyoto University's CiRA, announced the start of a new clinical research study targeting age-related macular degeneration (AMD) using donor-derived (allogeneic) induced pluripotent stem cells (iPSCs). And on March 28, a man in his 60s was the first AMD patient to receive the experimental transplant of allogeneic iPSC-derived retinal pigment epithelial cells.

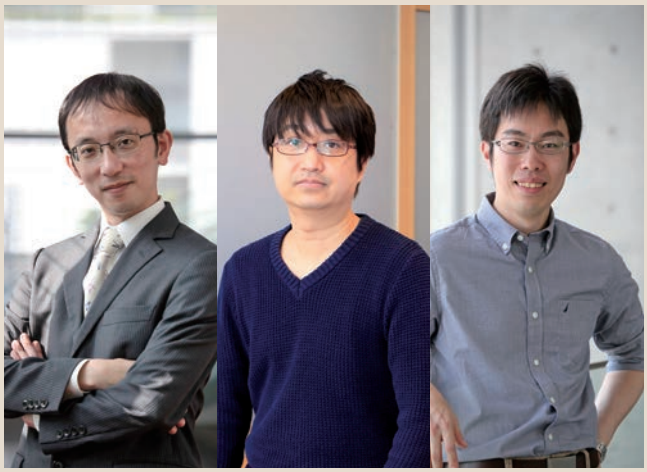
2017 CDB Symposium

The 2017 CDB Symposium entitled, "Towards Understanding Human Developmeta, Heredity, and Evolution," was held March 27 to 29 at the RIKEN CDB. Approximately 170 students and scientists from around the world convened at the CDB to take part in scientific exchanges on a wide range of topic related to human development, human genetics as well as evolution.

Japan's Prime Minister visits RIKEN CDB

Prime Minister Shinzo Abe visited the RIKEN CDB during a recent trip to Kobe on June 24. This was his second visit to the CDB, his last being in 2013. The Prime Minister heard from Director Hiroshi Hamada about the CDB's research activities, and also from Masayo Takahashi and one of her collaborators Yasuo Kurimoto, director of the Department of Ophthalmology, Kobe City Medical Center General Hospital, about the progress of the clinical research to establish an iPSC-based treatment for age-related macular degeneration. Professor Eiji Eto, deputy director of CiRA, Kyoto University was also present to introduce iPSC research activities at CiRA.

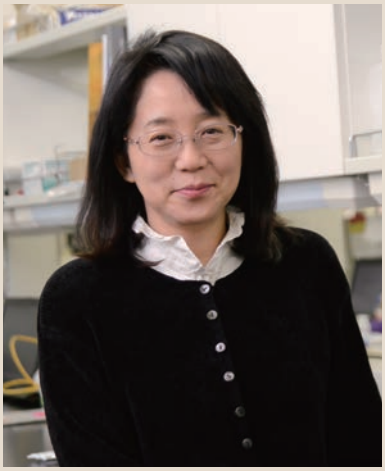
New faces at the CDB



The RIKEN CDB welcomed the arrival of three new principal investigators to its research programs. Itoshi Nikaido was appointed Unit Leader of the Single-cell Omics Research Unit in July, and Wataru Kimura and Kazunari Miyamichi joined the CDB in September as Team Leaders of the Laboratory for Heart Regeneration and of the Laboratory for Comparative Connectomics, respectively.

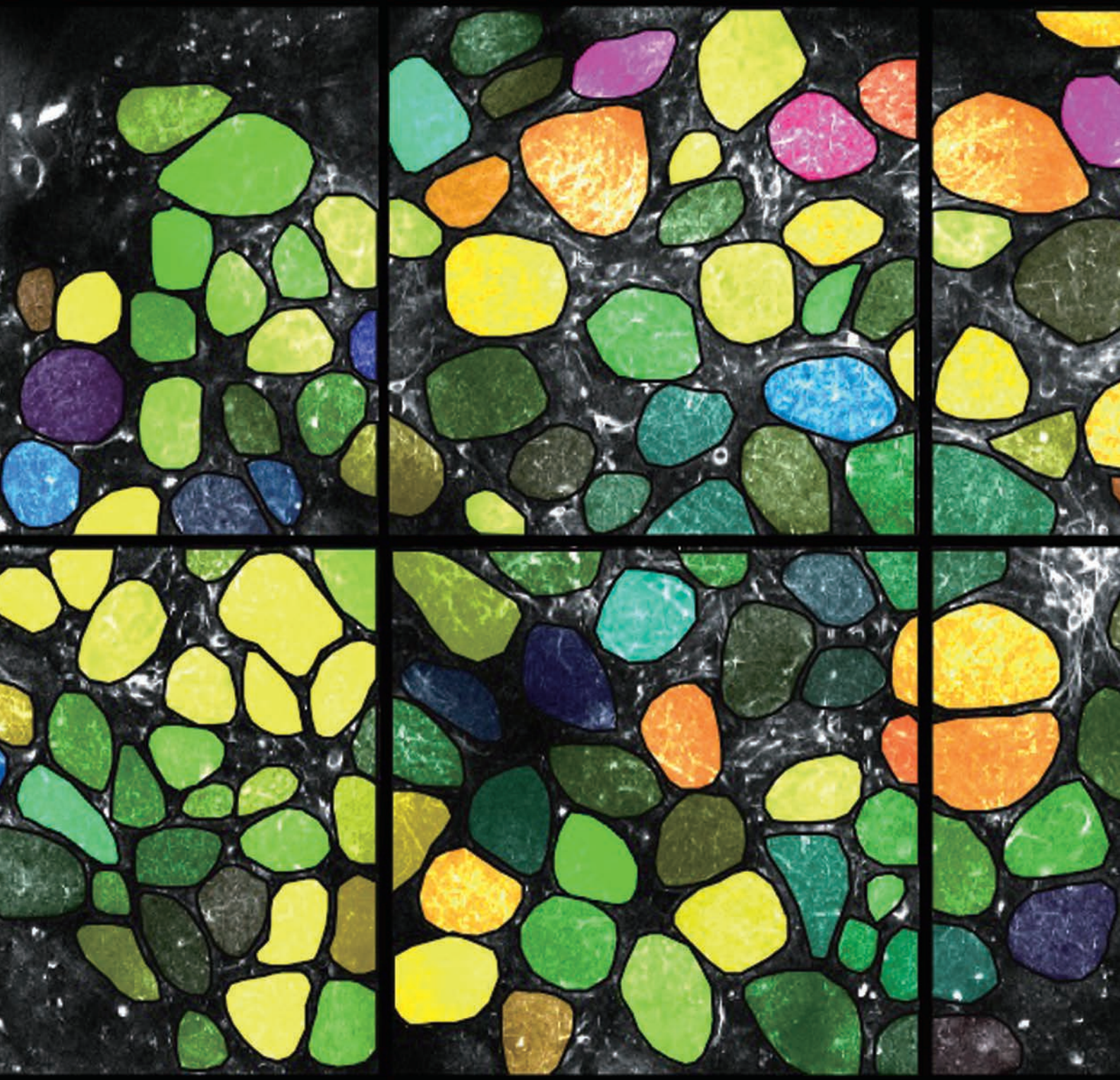
CDB Project Leader elected International Member of NAM

Masayo Takahashi, project leader of the Laboratory for Retinal Regeneration was elected as an International Member of the U.S. National Academy of Medicine (NAM) in October. Membership in the NAM is considered one of the highest honors in the fields of health and medicine, and members are elected to the NAM by their peers in recognition for their outstanding achievements and commitment to service.

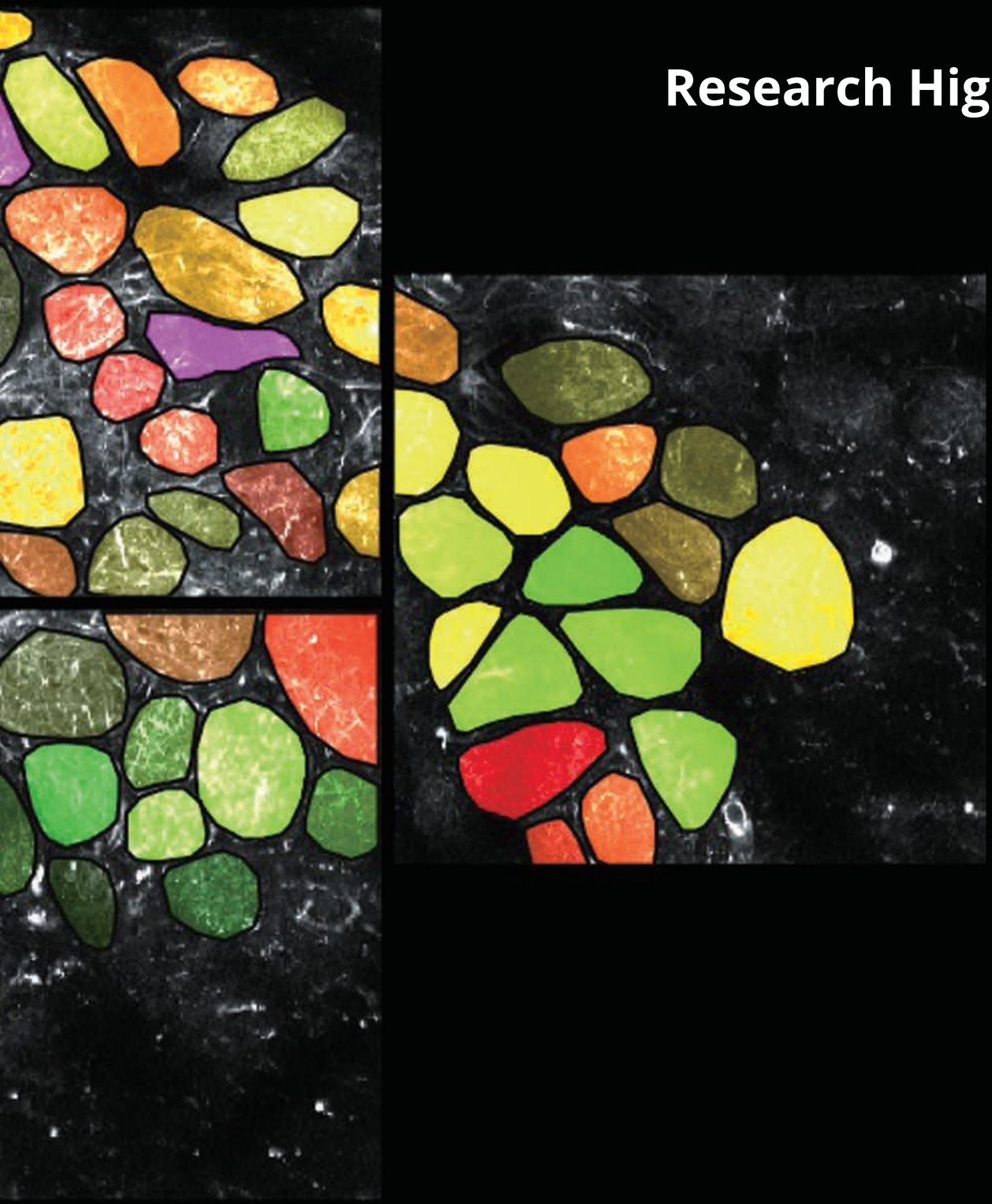


Mavericks in developmental biology

The 29th CDB meeting entitled, "Mavericks, New Models in Developmental Biology," was held on October 19 to 20 at the RIKEN CDB. Over 80 participants attended the meeting, which focused on novel model organisms used in research. The meeting was organized by Carina Hanashima (CDB Laboratory for Neocortical Development), Hiroshi Kiyonari (CLST Animal Resource Development Unit), Shigeru Kuratani (RIKEN Evolutionary Morphology Laboratory), and Richard R. Behringer from the MD Anderson Cancer Center (USA).

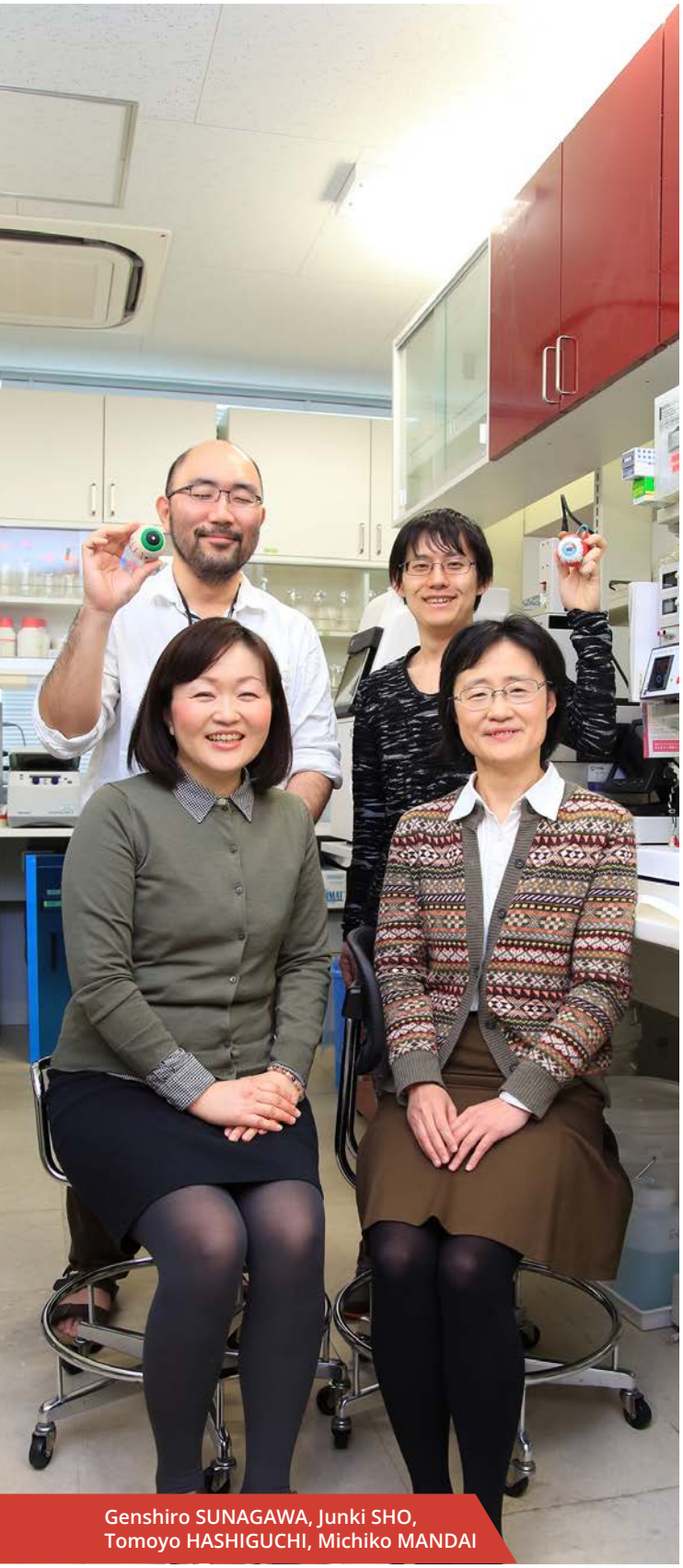


Research Highlights



Phase coding in the olfactory bulb. Mechanosensation in olfactory sensory neurons produce airflow-coupled oscillatory activity in mitral/tufted cells in the olfactory bulb. Oscillation phases in glomeruli are color coded here based on Ca^{2+} imaging experiments (red = 0, green = π/2, cyan = π, purple = 3/2π). Each glomerulus has a unique oscillation phase, forming a foundation for the phase coding in olfaction.
Image: Laboratory for Sensory Circuit Formation

Improved vision in retinal degeneration mice after iPSC-derived retina transplants



Genshiro SUNAGAWA, Junki SHO,
Tomoyo HASHIGUCHI, Michiko MANDAI

The retina is a layered neural structure at the back of the eye that plays an important role in sensing and translating light information entering the eye into neural signals sent to the brain. Light is sensed by photoreceptors (rods and cones) in the outer nuclear layer, the outermost layer of the neural retina, and this information is then transmitted in turn through the bipolar cell layer, ganglion cell layer, and finally to the optic nerve that will send neural signals to the brain to be interpreted into what we "see." Disruption to any segment of this neural transmission can affect vision. Retinitis pigmentosa is a group of genetic eye disorders characterized by progressive degeneration of retinal photoreceptors resulting in severe visual impairment, and there is currently no effective treatment to restore visual function in affected patients. Some groups have reported that transplantation of retinal tissue or stem cell-derived photoreceptors into the eye shows slight restoration of visual function, but it remained unclear whether transplanted tissues or photoreceptors actually integrated with the surrounding host environment.

A study led by Deputy Project Leader Michiko Mandai of the Laboratory for Retinal Regeneration (Masayo Takahashi, Project Leader) has demonstrated that following transplantation of induced pluripotent stem cell (iPSC)-derived retinal tissue into end-stage retinal degeneration (*rd1*) model mice, the photoreceptors in the transplanted tissue can form functional synapses with synaptic partners in the host eye using cell labeling, behavioral analyses and electrophysiological recordings. Their work was published in the journal *Stem Cell Reports*.

A series of studies by the former CDB Laboratory for Organogenesis and Neurogenesis led by the late Yoshiki Sasai established a cell culture method for directing embryonic stem cells (ESCs) to self-organize into 3D tissue structures and also reported the successful generation of 3D-retinal tissue from mouse and later human embryonic stem cells (ESCs). Mandai and her team expanded on this work to examine feasibility of using this stem cell-derived retinal tissue for transplantation to restore vision in retinal degenerative diseases. They reported that mouse ESC/iPSC-derived retinal tissue could mature and survive when transplanted into mouse eye, and observed similar results transplanting human ESC-derived retinal tissue in monkey models. Although the retinal grafts appeared to integrate with surrounding host retina in both studies, they could not ascertain whether the graft had fully integrated with the host to sense light and transmit neural signals.

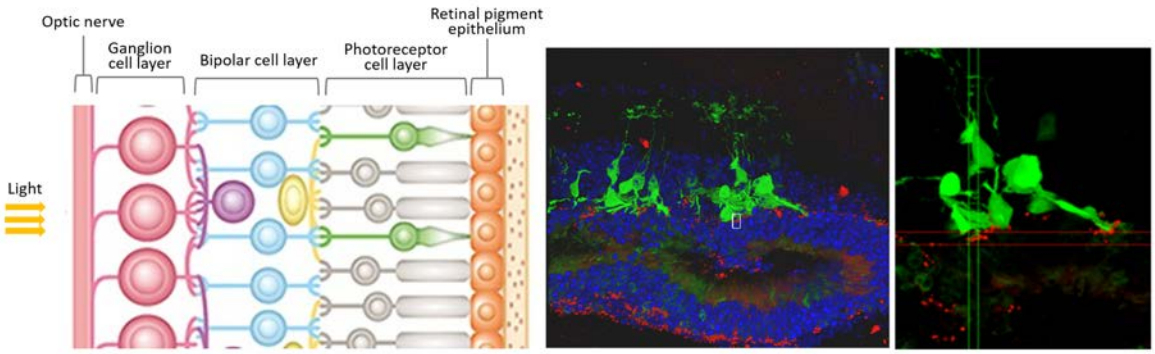
The group first examined the synaptic connections between the photoreceptors in transplanted tissue and the surrounding host cells, specifically with bipolar cells. They generated and transplanted iPSC-derived retina expressing red fluorescent markers at the synaptic terminal ends of photoreceptors into an *rd1* mouse line that expressed green fluorescent markers in dendrites of bipolar cells. They were able to visually confirm that the red photoreceptor synaptic terminal

ends of their transplanted retinal tissue were in contact with green host bipolar cell dendrites.

Next, they used a behavioral learning experiment, called shuttle avoidance system (SAS), on *rd1* mice to determine whether these mice could in fact detect light following transplantation of iPSC-derived retina. In SAS, mice can be trained to associate stimulus, such as light, with electrical shock and will move to avoid getting shocked when they sense light. Thus, if the iPSC-derived retina had formed functional synapses with host cells, the transplant recipient *rd1* mice should be able to be trained to associate light stimulus with an electrical shock and show avoidance behavior if light is detected. Their analyses revealed that some transplant recipient *rd1* mice displayed avoidance responses, while *rd1* mice that did not receive transplants moved at random regardless of light stimulus.

The team also extracted the whole retina of *rd1* mice post-transplantation to examine their ability to transmit electrophysiological signals using a microelectrode array (MEA) system. The extracted whole retina tissues were laid flat on microchips and analyzed for their responses to light signals. The team found that the retinal graft areas responded to light signals similar as seen in normal retina, and that graft-derived photoreceptors transmitted excitatory signals via bipolar cells to host retinal ganglion cells.

"Presently, we can only transplant tissue sizes equivalent to less than 5% of the whole retina with our method. If we can improve our technique to allow transplantation of larger tissues, this may lead to a marked improvement in vision," says lead author Mandai. "Our study demonstrates a proof-of-concept for considering clinical transplantation of iPSC-derived retinal tissues into patients with retinal degeneration. Aside from improving transplant techniques and testing whether human iPSC-derived retina can restore vision in blind mice, we also need to assess the safety of the transplant procedure and of the derived retinal tissue itself before we can move to human clinical studies."



Left: Structure of the retina (adapted from July 2014 issue of RIKEN News). Right: When iPSC-derived retina tissue was transplanted into *rd1* mice which has lost the photoreceptor cell layer, host bipolar cells (green) and transplanted photoreceptors (red) formed synapses.

Clinical safety study using autologous iPSC-derived retinal cell sheet for AMD

(Back) Motoki TERADA, Akishi ONISHI
Sunao SUGITA, Michiko MANDAI, Masayo TAKAHASHI, Chikako MORINAGA, Noriko SAKAI, Yumiko SHIBATA

Age-related macular degeneration (AMD) is one of the most commonly diagnosed eye disorders causing impaired vision in older populations in developed countries. There are two known subtypes of AMD—neovascular (wet-type) AMD and atrophic (dry-type) AMD. Within Japan, wet-type AMD is the more prevalent condition, and is characterized by abnormal blood vessel formation (neovascularization) in an area beneath the center of the retina (macula) at the back of the eye triggered by the age-dependent functional deterioration of the retinal pigment epithelium (PRE), which damages and impedes function of the retinal pigment epithelium. The RPE is a monolayer sheet of cells lining the outer surface of the neural retina and plays multiple roles in the eye from absorbing light entering the eye, serving as a barrier between the eye and the choroid blood vessels, to nourishing photoreceptor cells and clearing away their metabolic waste. The gradually deteriorating function of the RPE in AMD patients leads to blurriness or blind spots in the center of their vision, and while it does not often result in complete blindness, it can significantly affect the patients' quality of life.

In August 2013, a pilot clinical study was launched to examine the feasibility and safety of transplanting an RPE cell sheet generated from autologous (patient-derived) induced pluripotent stem cells (iPSCs) into AMD patients. The study was led by Project Leader Masayo Takahashi and Deputy Project Leader Michiko Mandai of the Laboratory for Retinal Regeneration, and carried out in collaboration with the Institute for Biomedical Research and Innovation (IBRI) Hospital, Kobe City Medical Center General Hospital, and Kyoto University's Center for iPS Cell Research and Application (CiRA). The first iPSC-RPE cell sheet transplantation was carried out in September 2014, and the team reported the details of their clinical study, including protocols, observations and outcomes, in a publication in *The New England Journal of Medicine*.

Currently available treatments for AMD primarily target specific symptoms, such as periodic injections of anti-vascular endothelial growth factor (VEGF) drugs to suppress further blood vessel formation, which only delays disease progression and does not cure the disease itself. Many groups have been working to establish more effective methods to restore function of damaged RPE in AMD, including transplantation of sheets or cell suspensions of RPE cells derived from human fetuses or embryonic stem cells, which has shown mixed results, from some improvement to rejection of transplanted cells as well as adverse events associated with use of immunosuppressants. Research in CDB's former Laboratory for Organogenesis and Neurogenesis led by the late Yoshiki Sasai had established methods to differentiate pluripotent stem cells into retinal cells, which was then refined by Takahashi's group to generate retinal pigment epithelial cells. This laid the foundation to launch a clinical study examining the safety of transplanting RPE cell sheets made from autologous iPSCs into the patient's eye, as well as look at the feasibility of using this approach to treat AMD.

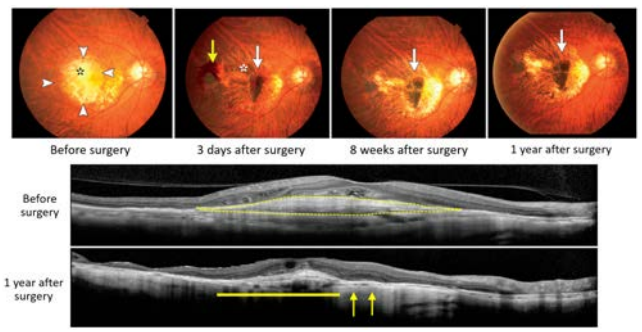
Two patients diagnosed with wet-type AMD were enrolled to participate in the clinical study, the first patient was a woman in her 70s and the second patient, a man in his 60s. The team used non-integrating episomal vectors to generate iPSCs from skin fibroblast cells taken from each patient, which were then differentiated into RPE cells. These RPE cells were purified, cultured into a cell sheet, and tested for quality and safety against the standards outlined in the protocol, which included analyses of cell viability, morphology, immunostaining, assessments of RPE cell sheet function, and tests for tumorigenicity. DNA methylation and gene expression profiles were also analyzed as reference data. Following these tests, the team obtained two RPE cell lines from the first patient and one RPE cell line from the second patient that met the protocol criteria for transplantation.

The first patient underwent transplant surgery on September 12, 2014, which involved removal of ectopically formed blood vessels and damaged RPE area, and then transplant of iPSC-derived RPE cell sheet (1.3X3.0 mm) under the retina. No serious adverse events were detected following the surgery, and changes that had resulted from abnormal blood vessel formation were resolved within a short period after the surgery. To date, the transplanted RPE cell sheet has remained intact in the originally transplanted area, and no signs of rejection or tumor formation have been observed. The patient's vision which had been deteriorating despite regular anti-VEGF injections prior to the transplant, appears to have stabilized, showing neither significant improvement or worsening after the surgery.

The RPE cells generated from the second patient also passed required quality and safety tests, including that for tumorigenicity, however, whole-genome sequencing analyses revealed some genetic changes had occurred during the derivation of iPSCs and iPSC-derived RPE cells lines. Because there were no criteria to evaluate how these changes could affect patient, as well as the fact that his symptoms had stabilized with anti-VEGF injections, the team decided not to carry out the surgery in the second patient.

"The results of this clinical study indicate that the use of autologous iPSCs as a source for cell-based therapy in AMD is safe and feasible. If ongoing studies validate safety of the approach, earlier stage interventions may stop progression or even improve visual acuity of AMD patients," explains Takahashi. "Concerns associated with autologous iPSC such as long preparation times and high costs still need to be addressed, and a shared consensus is needed among scientists and clinicians regarding acceptable quality and safety standards of iPSC-derived cells used in transplantations. We hope to clear each of these hurdles, moving closer to achieving our goal of establishing iPSC-based therapies for AMD and other retinal diseases."

RPE cells (left) and RPE cell sheet (middle) generated from iPSCs derived from first patient, and transplanted RPE sheet in eye after surgery (arrow in right panel).



Top panels: Fundus photographs of the macular region before and after surgery. Before surgery, ectopically formed blood vessels span the region around the asterisk, with arrowheads marking periphery of affected area. Three-days after surgery, normal choroid blood vessels, previously hidden by ectopically formed blood vessels, became visible. White arrow marks location of transplanted RPE sheet, which was still viable 1 year later. Bottom panels: Vertical sectional view of macular region by optical coherence tomography (OCT) before and after surgery. Ectopic neovascular tissue (yellow dotted line area) was removed, and further abnormal blood vessel formation was not observed. Transplanted RPE sheet (solid yellow line) remained intact, and retinal tissue lying next to graft (yellow arrows) also retained normal layered structure. (Adapted from figures in *The New England Journal of Medicine*.)

Graded *Wnt5* expression: Cueing left-right symmetry breaking

Katsura MINEGISHI

Vertebrates have three main body axes that are determined in turn during early stages of embryonic development: anterior-posterior (A-P) axis, dorsal-ventral (D-V) axis, and finally the left-right (L-R) axis. L-R axis determination is mediated by events in a region of the early embryo called the node, a transiently formed shallow depression at the ventral region of the embryo consisting of approximately 200 to 300 cells. Each node cell possesses a single cilium that moves in a clockwise rotation, which together creates unidirectional fluid flow (nodal flow) in a leftward direction leading to the breaking of left-right symmetry, in other words create the L-R axis, by inducing expression of a left-determining factor, *Lefty*, on the future left side of the body. There are, however, many gaps that remain in our understanding of how A-P and D-V positional information is detected by the node cells to correctly establish the L-R axis.

A study by research scientist Katsura Minegishi in the Laboratory for Organismal Patterning (Hiroshi Hamada, Team Leader) and other collaborators analyzed the molecular mechanisms involved in generating unidirectional nodal flow leading to L-R symmetry breaking using mouse embryos. Their meticulous work, published in *Developmental Cell*, revealed that a *Wnt5* expression gradient along the A-P axis of the embryo induces polarization of planar cell polarity (PCP) proteins in the node cells which causes the basal body of their cilium to shift to a more posterior position. This shift in basal body position tilts the angle of the rotational axis toward the posterior direction, consequently creating the leftward nodal flow that is critical for breaking L-R symmetry.

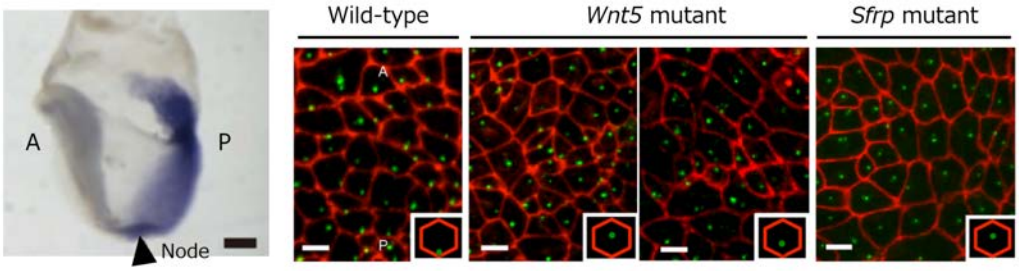
In the mouse, the node appears transiently in the embryo around embryonic day 7.5. Initially, the basal body of the cilium is found at a central region of each node cell and rotates in a clockwise direction to create small swirling fluid flows. As development progresses, the basal body position gradually shifts posteriorly, causing the rotational axis of the cilium to tilt in a posterior direction which collectively creates a strong leftward flow. The laboratory previously reported that this unidirectional nodal flow is critical for determining the region where *Lefty* expression is induced, thus breaking L-R symmetry. Furthermore, node cells in the node exhibit polarity along the A-P axis through asymmetrical localization of planar cell polarity (PCP) proteins mediated by planar cell polarity (PCP) mechanisms that control intercellular polarity across a plane of cells. This also appears to contribute to the posterior shifting of basal body needed to create leftward flow, but the signals involved in positioning of basal body remained unclear.

The group first turned their attention to Wnt5 proteins as potential candidates involved in basal body positioning, as Wnt signals are often found upstream of the PCP pathway, and *Wnt5* is known to be expressed in a gradient along the A-P axis of the embryo near the node, with lower concentrations on the apical side and higher concentrations on the posterior side. To determine whether Wnt5 plays a role in basal body positioning in node cells, they examined *Wnt5* knockout (KO) embryos and found that the basal body of many node cells

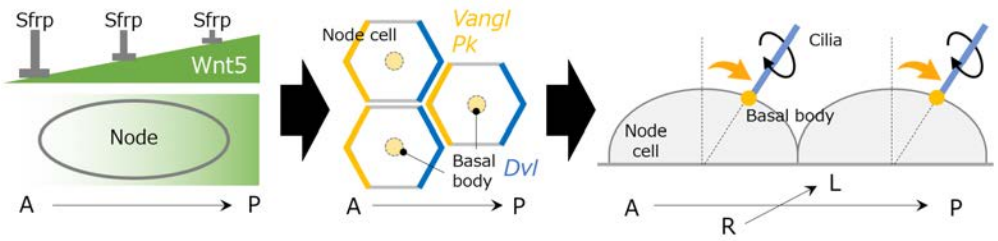
failed to shift posteriorly, consequently disrupting unidirectional leftward nodal flow. They surmised that another factor was also involved in establishing the Wnt gradient as posterior shifting of basal body was still seen in some node cells of *Wnt5* KO embryos. They examined patterns of *Sfrp*, a known Wnt antagonist, as a possible candidate and discovered that *Sfrp* KO embryos showed similar phenotypes as *Wnt5* KO embryos, if not a more pronounced effect, with respect to failure of basal body shifting. Posterior basal body shift was also not observed in a series of experiments in which *Wnt5* or *Sfrp* gradients were disrupted by ectopic or over-expression of either gene in the node, indicating that both *Wnt5* and *Sfrp* gradients are essential for shifting the basal body position posteriorly.

The group next examined how the node cells detect the *Wnt5* gradient established within the node, focusing on intracellular localization of PCP proteins within the node cells. They confirmed that anterior localizing protein Vangl, and posterior localizing protein Disheveled (Dvl) were expressed at respective poles of node cells following establishment of the *Wnt5* gradient. They also revealed that another PCP protein, Prickle (Pk), was expressed at the anterior end of node cells. *Pk* KO experiments indicated that Pk was required for anterior localization of Vangl as well as to ensure proper shift in basal body position to create leftward flow. PCP proteins were also found to interact with and influence neighboring node cells, as when Pk localization in one node cell was disrupted, abnormal basal body positioning was seen in surrounding cells. Thus, both intracellular and intercellular communication of PCP proteins appears to be involved in detecting the *Wnt5* gradient within the node and the node cells themselves.

"While we have shown in this study that Wnt signaling is the initial cue triggering the posterior shift of the basal body, it is still not clear how individual node cells can sense small differences in levels of intracellular Wnt activity at anterior and posterior ends. Nor do we understand how the basal body shifts to a more posterior position," says Hamada. "We plan to continue exploring these unanswered questions in order to fully understand the mechanisms of L-R symmetry breaking."



Left: Position of the node (black arrowhead) and pattern of *Wnt5* expression (blue) in mouse embryo at embryonic day 7.5. Right: Basal body position in node cells (red, cell membrane; green, basal body). Under normal embryos, the basal body shifts posteriorly, but when *Wnt5* or *Sfrp* is knocked out, the basal body remains near the center region.



Schematic of left-right axis determination. The Wnt gradient established in the node is detected by node cells, which activates PCP mechanisms in the node cells, eventually leading to a posterior shift of the basal body of node cell cilia. The posterior shifting of basal body tilts rotational axis resulting in nodal flow to break L-R symmetry.

Trehalose metabolism essential for adaptation to changes in dietary conditions

In insects, the disaccharide sugar trehalose is the main sugar compound and energy source circulating in the hemolymph (insect equivalent of blood), and due to its chemical stability and inertness, is also thought to play a role in protecting organisms from environmental stresses such as desiccation and starvation. Trehalose is synthesized from glucose by trehalose-6-phosphate synthase (Tps1) and is broken down by trehalose-hydrolyzing enzyme, trehalase (Treh), regulation of these processes is important for maintaining metabolic homeostasis. Whereas mammals including humans cannot synthesize trehalose, they possess the Treh enzyme to break down trehalose that is ingested into the body. Treh is a highly conserved enzyme, being found in a range of organisms from bacteria to mammals, including humans, but much of its physiological functions remain unclear.

In a study by former research scientist Tetsuo Yasugi and his colleagues in the Laboratory for Growth Control Signaling (Takashi Nishimura, Team Leader) published in *Scientific Reports*, they carried out a detailed comparison of previously reported *Drosophila* mutants carrying a mutation in the *Treh* gene, which is important in trehalose metabolism. Rearing the fly mutants under identical conditions, they found that larval lethality of *Treh* mutants are highly dependent on dietary conditions, requiring high levels of both sugar and protein. The team also revealed that under poor dietary conditions, lethality in *Treh* mutants is caused by overaccumulation of trehalose, not loss of Treh function.

The Laboratory for Growth Control Signaling previously generated several different *Drosophila* strains carrying a mutation in the *Treh* gene using the CRISPR/Cas9 system, and reported that loss of Treh function results in lethality at pupal stages and is also more susceptible to desiccation. Around the same time, two other groups—one in China in 2014 and the other in Germany in 2015—also independently generated *Treh* mutants, but reported phenotypes that appeared to differ from those observed by Nishimura's team, particularly in terms of the lethal stages. The *Treh* mutant reported in 2014, which carried a deletion or insertion within the gene, were lethal between larval and pupal stages, while the *Treh*-null mutants reported in 2015, which were generated using the TALEN system, showed lethality in early larval stages. Although in most cases, the *Treh* mutants did not develop to adult stages, it was unclear why the timing of lethal phases observed by each group differed. Yasugi and his colleagues thus decided to take a closer examination at the phenotypes of the reported mutant strains to find an answer.

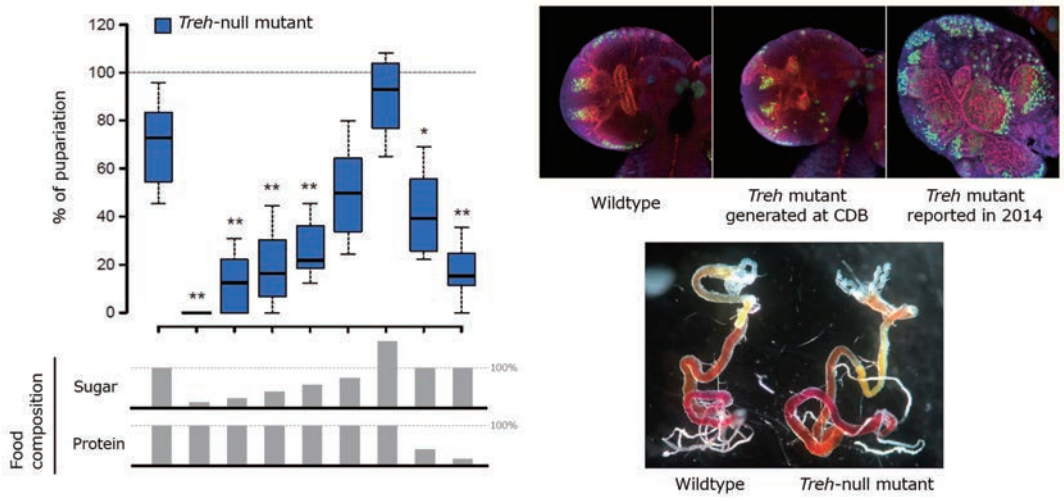
The team obtained the *Treh* mutant strains from both groups, and bred them under identical experimental conditions. In addition to larval and pupal lethality, the Chinese group had reported that their *Treh* mutants displayed abnormalities of the optic lobe (brain), which was verified by Yasugi et al. However, they did not see the same abnormal disorganization in the optic lobe of their own CRISPR/Cas9-induced *Treh* mutants. Further analyses revealed that the *Treh* mutant

reported in 2014 in fact also carried a mutation in the *lgl* gene locus, which is located at the ends of the chromosome on which the *Treh* gene is found, and backcross experiments demonstrated that it was in fact the mutation in *lgl*, not *Treh*, that caused abnormalities in the optic lobe.

The team next explored the differences in the timing of lethality among the mutant strains when bred under identical conditions. Surprisingly, the TALEN-induced *Treh*-null mutants, which reportedly displayed lethality during larval stages, were found to be capable of developing to pupal stages, similar to the CRISPR/Cas9-induced *Treh*-null strains. Furthermore, all mutant strains were discovered to have comparable trehalose and glucose levels-higher trehalose and reduced glucose levels than seen in wildtype. So why then were the TALEN-induced *Treh*-null mutants previously found to be lethal in larval stages?

The team hypothesized that diet (i.e. nutrient availability) was a possible underlying factor for differences in lethality stages. In the lab, *Drosophila* are normally reared on agar-cornmeal media, which contains a mixture of glucose (sugar), yeast (protein, etc.) and cornmeal (sugar, fat, etc.), but fly food recipes vary among groups and the quality of ingredients purchased from vendors may also show differences between lots. The lab previously demonstrated that survival rates in larval stages of *Tps1* mutants, which cannot produce trehalose, was sensitive to low sugar conditions but maintained stable survival rates under low protein conditions, supporting their idea that nutrient availability can affect lethality in mutants with problems linked to metabolism. When Yasugi et al. bred the *Treh* mutant strains under various dietary conditions, they found that low sugar or low protein conditions led to marked reduction in survival rates of *Treh* mutants in larval stages. They also generated *Tps1*, *Treh* double mutants which under low protein conditions could survive to pupal stages, with lethal effects of *Treh* mutants being mitigated by additional *Tps1* mutation. Together these results indicated the overaccumulation of trehalose due to inability to metabolize trehalose, not the loss of functional Treh, as the cause of larval lethality in *Treh* mutants. The team also confirmed that all *Treh* mutants exhibited normal feeding behavior, and relatively normal digestive and fat body (insect equivalent of the liver) functions, also ruling out possibility of these factors contributing to lethality under restricted diet conditions.

"Whereas food composition is not as critical when studying morphogenesis, we do need to be careful about food composition when looking at metabolism and growth effects, especially when using mutants with defects in metabolism-associated genes," says Nishimura. "Trehalose is a non-reducing sugar, and as such, has properties that differ from reducing sugars such as glucose, which when accumulated can trigger hyperlipidemia or arteriosclerosis. Our present study however suggests that problems in trehalose metabolism can also lead to serious defects. Our next goal will be to reveal what exactly goes on when there is an overaccumulation of trehalose in the body."



Top right: Morphological phenotypes of optic lobe (brain). No abnormalities were observed in *Treh*-null mutant generated by the lab. Left: Food composition and the survival rates of mutants to pupal stages when reared under those food conditions. *Treh* mutants are sensitive to low sugar or low protein conditions. Bottom right: Gut of wildtype and *Treh*-null mutants. No major morphological or functional differences were observed.

Large cytoplasm predisposes oocytes to meiotic errors

Hirohisa KYOGOKU

Cell division is a finely tuned process involving the partitioning of cytoplasm and the segregation of chromosomes into two daughter cells. Normally, paired chromosomes align along the spindle equator formed by microtubules, which then pulls the paired chromosomes to opposing cell poles. In somatic cells, mistakes in chromosome segregation are rarely observed thanks to a stringent checkpoint mechanism that ensures all chromosomes are correctly aligned before segregation begins. In contrast, oocytes, the female germ cells involved in reproduction that undergo meiotic cell division, display a surprisingly high rate of chromosome segregation errors (approximately 10–30%), which can lead to miscarriages or congenital diseases. The complete causal mechanisms underlying the high frequency of errors in chromosome segregation seen in oocyte meiosis remain largely unknown.

A study led by research scientist Hirohisa Kyogoku in the CDB's Laboratory for Chromosome Segregation (Tomoya Kitajima, Team Leader) examined the effects of cytoplasmic size on chromosome segregation in oocytes during the first meiotic division by manipulating the cytoplasmic volume of mouse oocytes. In their work published in *Developmental Cell*, the team demonstrated that oocytes with larger cytoplasm are more prone to display broadly dispersed spindle poles and a less rigorous spindle checkpoint before progression to anaphase, the cell-cycle phase when chromosomes begin segregating, which contributes to increased frequency of errors in chromosome segregation and aneuploidy.

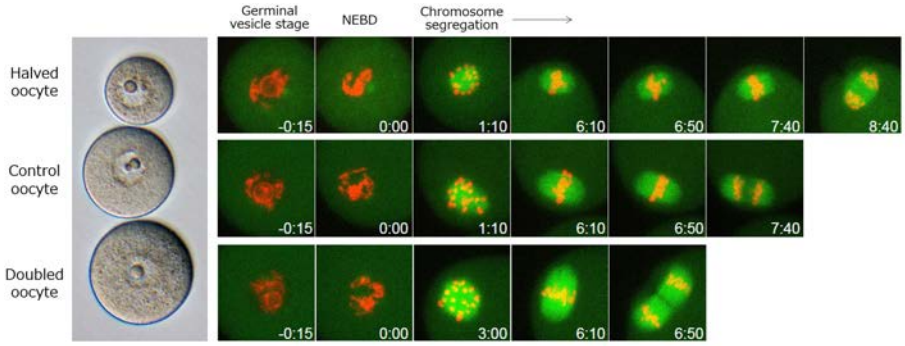
The oocyte's large cytoplasm is one feature that makes it distinct from other somatic cells, and the team hypothesized that this large cytoplasmic size was somehow linked to the higher frequency of chromosome segregation errors observed during meiosis. To test this, they made oocytes containing half the amount of cytoplasm (i.e. halved oocytes) and those containing double the amount of cytoplasm (i.e. doubled oocytes) found in normal oocytes, using micromanipulation. The halved oocytes were created by aspirating half of the cytoplasm from an oocyte using a micropipette, while the doubled oocytes were created by fusing a normal oocyte with an enucleated oocyte. They also confirmed that these artificially created oocytes could be induced to enter the first meiotic division at rates comparable to control oocytes.

Next, the team used live-cell imaging to track the movements of the chromosomes and the meiotic spindle within the oocytes undergoing meiosis, and carried out quantitative analyses of their three dimensionally reconstructed data. They first noticed a correlation between the sizes of the oocyte cytoplasm and that of the spindle—as cytoplasmic size increased, so did the relative spindle size. In addition, when they examined the dynamics of the oocyte's microtubule-organizing centers (MTOCs), which are not anchored by centrosomes as seen in somatic cells, the team observed that those with larger cytoplasm displayed less dense and more broadly dispersed MTOCs. The MTOCs in oocytes initially appear as a spherical mass of microtubules, which gradually splits

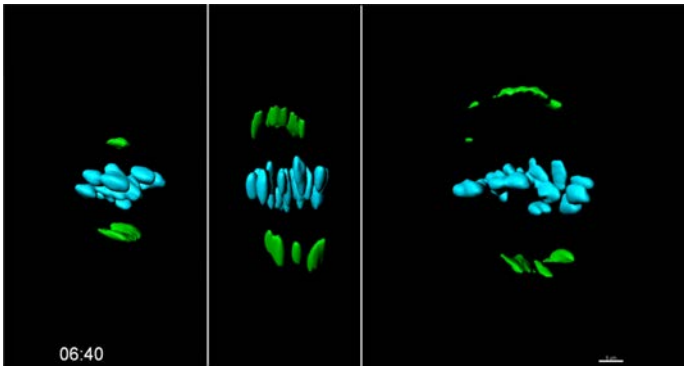
to relocate to two opposite poles creating spindle bipolarization. The doubled oocytes showed spindle formations at both poles that covered a larger area than those seen in control and halved oocytes and that were also more prone to separate into smaller units, contributing to errors in chromosome alignment along the spindle equator. Oocytes with larger cytoplasm were also unable to align chromosomes at the spindle equator as efficiently as compared to those with relatively smaller cytoplasm, requiring more time for proper alignment.

Normally, if chromosomes fail to align properly along the spindle equator, the spindle checkpoint mechanism would be triggered, blocking entry into anaphase until the errors are fixed. In oocytes, this checkpoint mechanism appears to be less rigorous, with some entering anaphase despite mistakes in chromosome alignment. Molecular factors involved in the checkpoint mechanism are produced in the nucleus and are released into the cytoplasm when the nuclear envelope breaks down at the entry of meiosis. Kyogoku and Kitajima found that these checkpoint factors become diluted within the cytoplasm as cytoplasmic size increases which results in a weakened checkpoint system that permits chromosomes to segregate prematurely before they are all properly aligned. The checkpoint mechanism was more stringent in the halved oocytes, halting anaphase entry when even one chromosome was improperly aligned, whereas anaphase entry was seen in many doubled oocytes despite errors in chromosome alignment leading to errors in chromosome segregation.

"We were able to demonstrate experimentally that the large cytoplasm is one factor contributing to the high frequency of chromosomal segregation errors seen in oocytes. As the large cytoplasm is considered to be crucial for supporting early embryonic development, our findings suggest that there is trade-off between accuracy of meiosis and post-developmental competence," explains Kitajima. "There are many other causes of chromosome segregation errors in oocytes, such as maternal aging. By understanding the different causes of chromosome segregation errors, we hope to be able to impart insights for developing strategies to prevent or rescue chromosome segregation errors."



Left: Halved, control and doubled oocytes. Right: Live imaging of the dynamics of chromosomes and spindles in the oocyte prior to and during chromosome segregation.



In oocytes with larger cytoplasm, the spindle poles (green) are found further apart and more loosely focused.

Loss of PCDH19 leads to differential behavior between sexes


Shuichi HAYASHI

The neural network in the brain is orderly yet extremely complex, and improper neural wiring during development can lead to brain disorders in humans. While many brain disorders are linked to genetic causes, in most cases, the underlying pathogenic mechanisms remain unclear. One disorder called PCDH19 Epilepsy, is known to be caused by mutations in a gene located on the X chromosome called *Protocadherin-19* (*Pcdh19*). Normally, X chromosome-linked diseases, such as color blindness and hemophilia, show severe phenotypes in males, which carry only one X chromosome. This disorder displays a unique pattern of inheritance, with symptoms appearing in females. Why phenotypes of PCDH19 mutations are female-specific, and the pathophysiological mechanisms of PCDH19 mutations remained largely unknown.

Now in a study led by former research scientist Shuichi Hayashi of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader), who is now at the University of Oxford, he and his collaborators carried out a phenotypic analysis of mice in which the X-linked gene, *Protocadherin-19* (*Pcdh19*) was knocked out. Their analyses revealed while brain tissue organization and neural morphology showed no significant abnormalities, the *Pcdh19*-knockout (KO) mice exhibited abnormal behavior in response to certain stress conditions and differential behavior between males and females. Overexpression of Pcdh19 in the cerebral cortex of wildtype mice also revealed that Pcdh19 expressing cells tended to cluster together, and this clustering might cause disease phenotypes when females have one chromosome carrying this mutation. Their findings were published in the online journal *Scientific Reports*.

Protocadherins are a group of cadherin superfamily proteins that are found in cell membranes, regulate cell-cell interactions and are expressed in central and peripheral nervous systems during development. In a previous study also led by Hayashi, they showed that Pcdh17 plays an essential role in collective axon extension of amygdala neurons during development. As PCDH17 and PCDH19 are part of the same subfamily, both are thought to be involved in brain development or its function. PCDH19, in particular, has been implicated as a causal factor in a range of brain disorders when mutated.

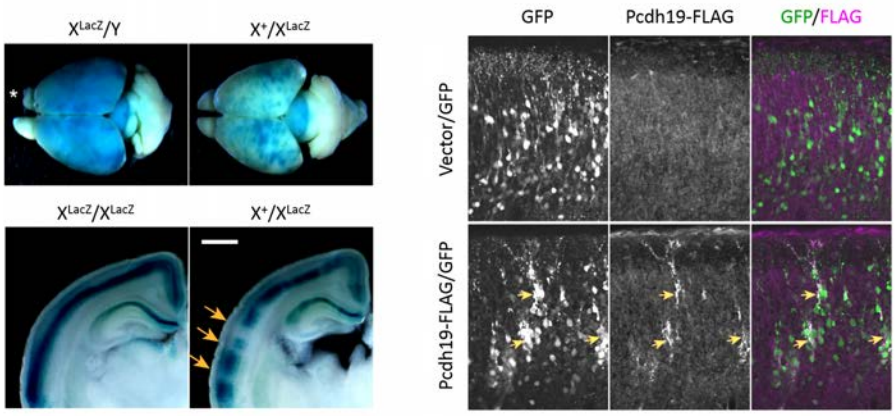
Hayashi and his collaborators first examined the role of PCDH19 in the brain by generating *Pcdh19*-KO mice. In normal mice, Pcdh19 is expressed widely throughout the brain, but is particularly strong in the cortical layer V of the cerebral cortex and the hippocampal CA1 region. It was found distributed along the dendrites of neurons, and even in some of the dendritic spines. When brains of *Pcdh19*-KO mice were examined, they found no significant abnormalities in the cortical and hippocampal structure, nor in dendritic morphology.

The team next looked at genotypic differences in the distribution of Pcdh19 in the brain. They discovered that hetero-

zygous females (X^+X^-) showed patchy distributions of Pcdh19 in the brain, with Pcdh19-positive and Pcdh19-negative cells forming respective clusters, whereas homozygous females (X^-X^-) uniformly lost Pcdh19 expression across the brain. In females, only one X chromosome is functional as the other one is inactivated through random X-inactivation. This random X-inactivation of an X chromosome lacking the *Pcdh19* gene is thought to be to reason for the patchy distribution of Pcdh-positive and Pcdh-negative neurons, and is consistent with reports from past studies. They also examined whether Pcdh19's homophilic properties also contribute to the patchy distribution by exogenously overexpressing Pcdh19 in a subset of cells in wildtype brains using *in utero* electroporation and found that clustering was enhanced in neurons strongly expressing Pcdh19. These results suggest that differences in Pcdh19 expression that arise from mosaic distribution promotes clustering of some neurons, disrupting normal neuronal patterning.

Wondering what effects loss of Pcdh19 might have on behavior, they carried out a series of behavioral analyses in a collaboration with Fujita Health University. While no significant differences were observed in social behavior, learning or memory, both male (X^-Y) and female (X^+X^-) carriers of *Pcdh19* mutations displayed increased activity during the tail suspension tests, which suggested that when placed under stress, they are predisposed to becoming hyperactive. In addition, only the female carriers (X^+X^-) showed reduced fear responses in comparison to wildtype. These findings suggest that female-specific abnormalities observed in human PCDH19 Epilepsy was in part replicated in the mouse model, and as evidenced by male carriers also displaying some behavioral abnormalities, Pcdh19 is likely important in regulating mouse behavior.

"As loss of PCDH19 does not significantly affect brain tissue or neuronal morphology, it is likely small changes in neural connections result in behavioral abnormalities. Overexpression experiments showed that *Pcdh19*-expressing cells are prone to cluster together, which is consistent with the hypothesis that the female-specific appearance of PCDH19 Epilepsy is due to mosaic distribution of Pcdh19-positive cells that triggers excessive aggregation and thereby the disease phenotype," says Takeichi. "Human brain pathophysiology is difficult to replicate in mice, but provided we continue to deepen our understanding of the disease at molecular and cellular levels, our efforts may lead to future applications in medicine."



Left: LacZ (blue) staining signals indicating the distribution of *Pcdh19*-KO cells expression in mouse brain. Male *Pcdh19*-KO mouse (X^-Y) shows relatively even *LacZ* expression throughout brain. In heterozygous females (X^+X^-) shows patchy distribution of *LacZ*-positive (*Pcdh19*-KO) and -negative (*Pcdh19*-wildtype) neurons. Right: Overexpressing Pcdh19 promotes clustering in wildtype brains. When only GFP was introduced in brain (top panels), neurons were randomly distributed in cortical plate, whereas when Pcdh19 was introduced, Pcdh19-labeled neurons formed clusters along cortical plate.

Catenins guide cell migration

Vassil VASSILEV, Anna PLATEK

Cell migration plays important roles in morphogenetic processes, and at times in pathogenetic processes such as cancer invasion. Cells will sometimes group together and collectively migrate over long distances. Neural crest (NC) cells, which give rise to several different organs and tissues, are a typical example that undergoes collective migration. During development, a group of NC cells migrate along the developing gut to establish a mesh-like enteric nervous system. Cellular level analyses have revealed that these NC cells are adhered to one another while undergoing migration. Cell adhesion and migration are both regulated by cadherins and catenins, but it remained unclear how these factors tie the two processes together.

In a new study led by CDB research scientist Vassil Vassilev and Anna Platek of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader) and other collaborators, they demonstrated that in cells undergoing collective migration, catenins play a role not only in cell-cell adhesion, but also contribute to the stabilization of front-rear polarity within an individual cell, which is essential for their directional movement. Using mouse enteric neural crest (ENC) cells extracted from embryonic guts and cultured glioblastoma U251 cells, they further showed that β-catenin/α-catenin localized along the leading-edge of the lamellipodial membrane are internalized and activate myosin at the rear regions of the cell to stabilize its front-rear polarity. Their findings were reported in the journal *Developmental Cell*.

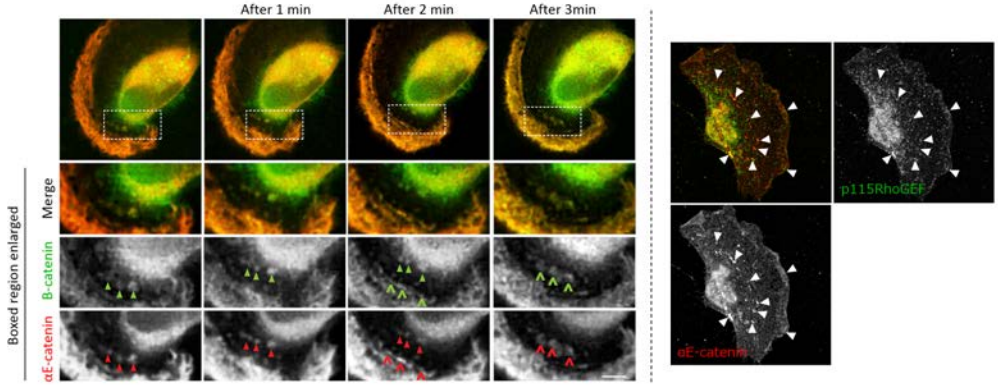
Vassilev and Platek collaborated with former CDB Team Leader Hideki Enomoto of the Laboratory for Neuronal Differentiation and Regeneration, now at Kobe University, who has long been focusing on elucidating mechanisms underlying establishment of the enteric nervous system by closely observing the dynamic collective migration of ENC cells. ENC cells express N-cadherin which forms a cadherin-catenin complex (CCC) to mediate cell-cell adhesion. The cytoplasmic region of cadherin binds β-catenin, which in turn binds α-catenin to stabilize cell-cell adhesion. The group first examined the role of N-cadherin and β-catenin/αE-catenin by conditionally knocking out (cKO) the genes for N-cadherin, β-catenin, and αE-catenin in mouse ENC cells and observed ENC cell dynamics in the gut. They found that β- and αE-catenin cKOs resulted in severe delay of ENC migration compared to wild-type gut and failed to complete migration. When single cell dynamics were tracked, they found N-cadherin cKO displayed chain migration with some disruption in the chain, whereas β- or αE-catenin cKOs lacked chain formation and instead formed clusters and disorganized movements.

The team next allowed ENC cells to migrate out from gut explants onto culture dishes to observe their movements. While wildtype and N-cadherin cKO ENC cells showed relatively unidirectional migration as noted by the persistence of lamellipodia at the front-edge, the β- and αE-catenin cKO ENC cells did not undergo unidirectional migration with the lamellipodium frequently changing positions. These observa-

tions suggested that catenins did not just function in cell adhesion, but also contributes to cell migration. Using cultured cells, they examined intracellular localization of catenins and found that in addition to being distributed at cell adhesion sites, catenins were also distributed within the cytoplasm. Time-lapse imaging revealed catenins being relocalized from the lamellipodial leading-edge toward the nucleus. Upon closer analyses, they discovered that cadherin, β-catenin and αE-catenin forming the CCC was internalized in vesicles, and further, that phosphorylation of β-catenin was important for signaling the intracellular uptake of the CCC. Cadherin also was seen to eventually segregate from the catenins during the relocation to the perinuclear region.

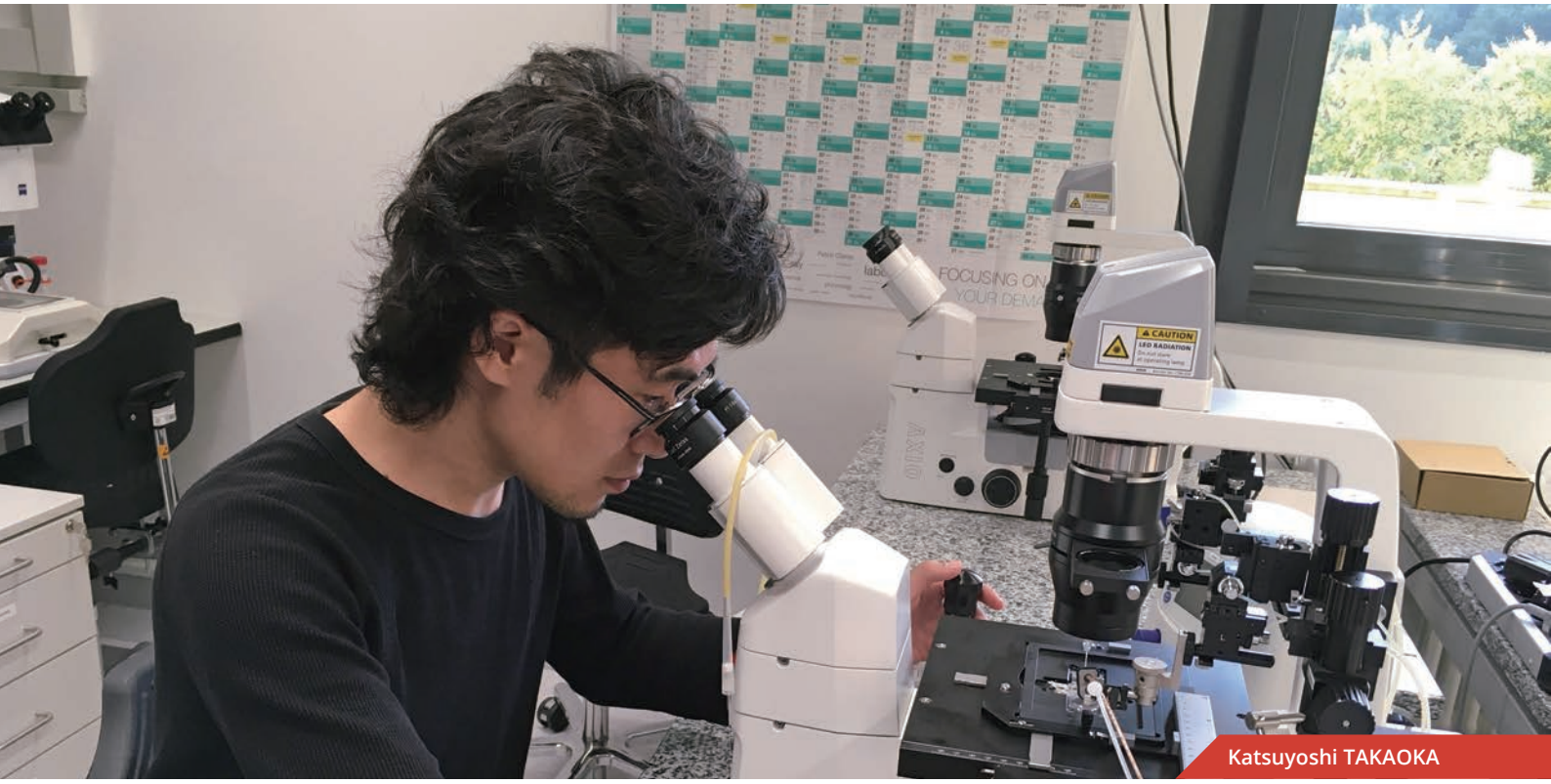
What role do internalized catenins play inside the cell? When cells move in a single direction, the leading-edge lamellipodia repeatedly extend focal adhesions in the direction of movement, while the rear-edge undergoes contraction via actomyosin. Activation of myosin to mediate contraction is regulated by activated RhoA. An examination of the relationship between catenin and RhoA revealed that p115RhoGEF, a RhoA activator, binds directly to αE-catenin to relocate and concentrate activate RhoA to the perinuclear region. When αE-catenin was depleted from the cells, active RhoA was seen dispersed throughout the cytoplasm. Their team also revealed that αE-catenin also bound transiently with myosin-IIB, indicating that internalized αE-catenin is needed to localize active RhoA and myosin-IIB at the perinuclear region, which results in a polarized activation of myosin that is necessary for stabilizing the front-rear polarity of the cell in migration.

"We showed for the first time that the CCC contributes not only to cell adhesion, but also stabilizes polarity in individual cells during migration. Together, these two functions drive collective cell migration," explains Takeichi. "We think that lamellipodia continuously expresses cadherin and catenin to prepare to form new contacts with other cells. When no new contacts are formed, the CCC is internalized and recycled, but also appear to regulate cell migration. Although cadherin-catenin mediated adhesion is specific to multicellular organisms, α-catenin was discovered in unicellular *Dictyostelium*, suggesting that catenins may have a function besides cell adhesion. It will be interesting to find out whether the mechanism we reported in this study can be found in non-adhesive cells or unicellular organisms."



Left: αE- and β-catenins accumulated in lamellipodia are relocated toward the nucleus (U251 cell line)
Right: αE-catenin and RhoGEF distributed in arc around perinuclear area. (U251 cell line)

Prospective distal visceral endoderm is randomly selected



Katsuyoshi TAKAOKA

One of the fundamental questions in developmental biology is how an initially round fertilized egg develops into an elaborately shaped embryo. In *Drosophila*, whose eggs are elliptical in shape, the anterior-posterior (A-P) axis is specified by asymmetrical distribution of maternal determinants. In mammals such as humans and mice, the egg is spherical and the three body axes—A-P, dorsal-ventral (D-V) and left-right (L-R)—are established in order, which in turn determines future positioning of organs. The A-P axis, the first axis established in mammals, is determined by a group of cells in the extra-embryonic region called, distal visceral endoderm (DVE). How prospective DVE cells are selected in the embryo, however, has remained unclear as mammals do not appear to have A-P specifying maternal determinants as found in *Drosophila*.

In a study published in *Nature Communications*, visiting scientist Katsuyoshi Takaoka of the CDB's Laboratory for Organismal Patterning (Hiroshi Hamada, Team Leader) report that, in mouse embryos, the DVE is randomly formed through a negative feedback by Lefty1 and Nodal on the cells that stochastically express the highest level of Nodal signaling during the blastocyst stage. The DVE then drives the establishment of the A-P axis by migrating to the future anterior side. Takaoka has since moved to the Max Planck Institute in Germany to continue his research.

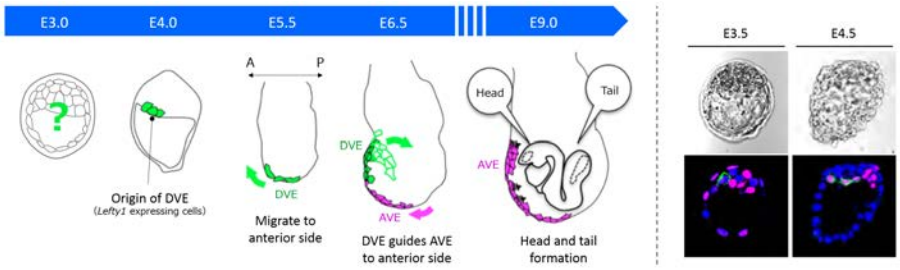
In the mouse, the DVE are selected from the primitive endoderm (PrE) at around embryonic day (E) 4, and then migrate to the future anterior end at E5.5. All visceral endoderm (VE) cells also undergo a shift at around the same time, resulting in localization of some VE cells at the embryo's distal tip to become anterior visceral endoderm (AVE). The DVE then guides the AVE migration to the future anterior side for A-P axis establishment by E6.5, and eventually induces formation of the head region. In a previous study, Takaoka showed that *Lefty1* expression is a marker for DVE progenitors, and succeeded in identifying prospective DVE cells as early as E4. But, they had not been able to pinpoint how the DVE progenitor cells, or *Lefty1* expressing cells, were selected in the embryo.

Lefty1 expression first appears in a subset of epiblast progenitor cells at around E3.5, and then in some cells of PrE at E4.5. Using mouse embryos at stages just prior to E3.5 and E4.5, the team first searched for a gene regulating *Lefty1* expression, and identified *Foxh1*, a transcription factor of Nodal signaling, as a direct regulator of *Lefty1* expression. When *Foxh1* or *Nodal*, the ligand for Nodal signaling, were inhibited in the embryos, *Lefty1* expression was not detected, whereas endogenous Lefty1 was induced in an ectopic Nodal mRNA injected cell or in the neighboring cell. They also examined *Nodal* and *Lefty1* expression in the embryo using live imaging and discovered that while *Nodal* expression expanded rapidly from one cell to the surrounding cells, *Lefty1* expression was restricted, being detected only in the first cell that expressed *Nodal* or the neighboring cells.

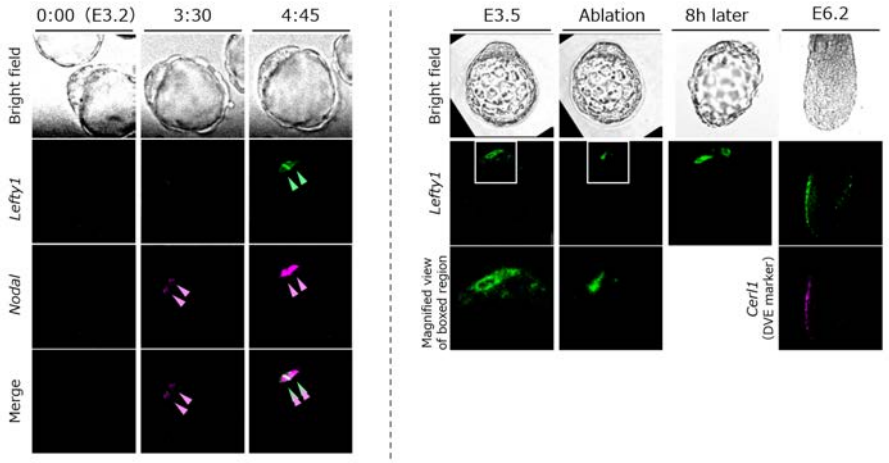
Why is *Lefty1* expression seen only in a few cells? As Lefty1 is known to be a Nodal antagonist, Takaoka and his colleagues hypothesized that *Lefty1* may serve as a negative regulator of Nodal signaling and decided to analyze the effects of inhibiting *Lefty1* in embryos. In *Lefty1* knockout embryos, they showed that Nodal signaling was enhanced and the number of prospective DVE cells increased. Thus, when the cell that initiates *Nodal* expression begins to express *Lefty1*, Lefty1 quickly diffuses to the other cells to inhibit Nodal signals, and also suppresses *Lefty1* expression in the cells. Thus, this negative feedback mechanism appears to restrict the number of prospective DVE cells. When Lefty1 expressing cells were removed from blastocysts by a laser ablation, another cell began to express *Lefty1*, and embryos subsequently continued to properly form DVE and eventually established the A-P axis. These results indicate that the cell showing high Nodal activity to begin expressing *Lefty1*.

"All PrE cells have the potential to become DVE, and the DVE is randomly formed from those cells that experience the first strong Nodal signaling," explains Takaoka. "Thus, the A-P axis is not yet determined at the blastocyst stage (E3), indicating that mammals have a different mechanism from the fly for establishing the body axes."

Hamada adds, "We created many gene knockout embryos as well as manipulated the embryo by removing prospective DVE regions. However, all embryos were able to establish a proper A-P axis regardless of small differences seen in early DVE formation stages from wild type embryos. Our results indicate that the molecular mechanism underpinning A-P axis determination is flexible yet robust. As there is still much we do not know about the early developmental mechanisms and origins of symmetry breaking in our bodies, we are keen to continue searching for the answers."

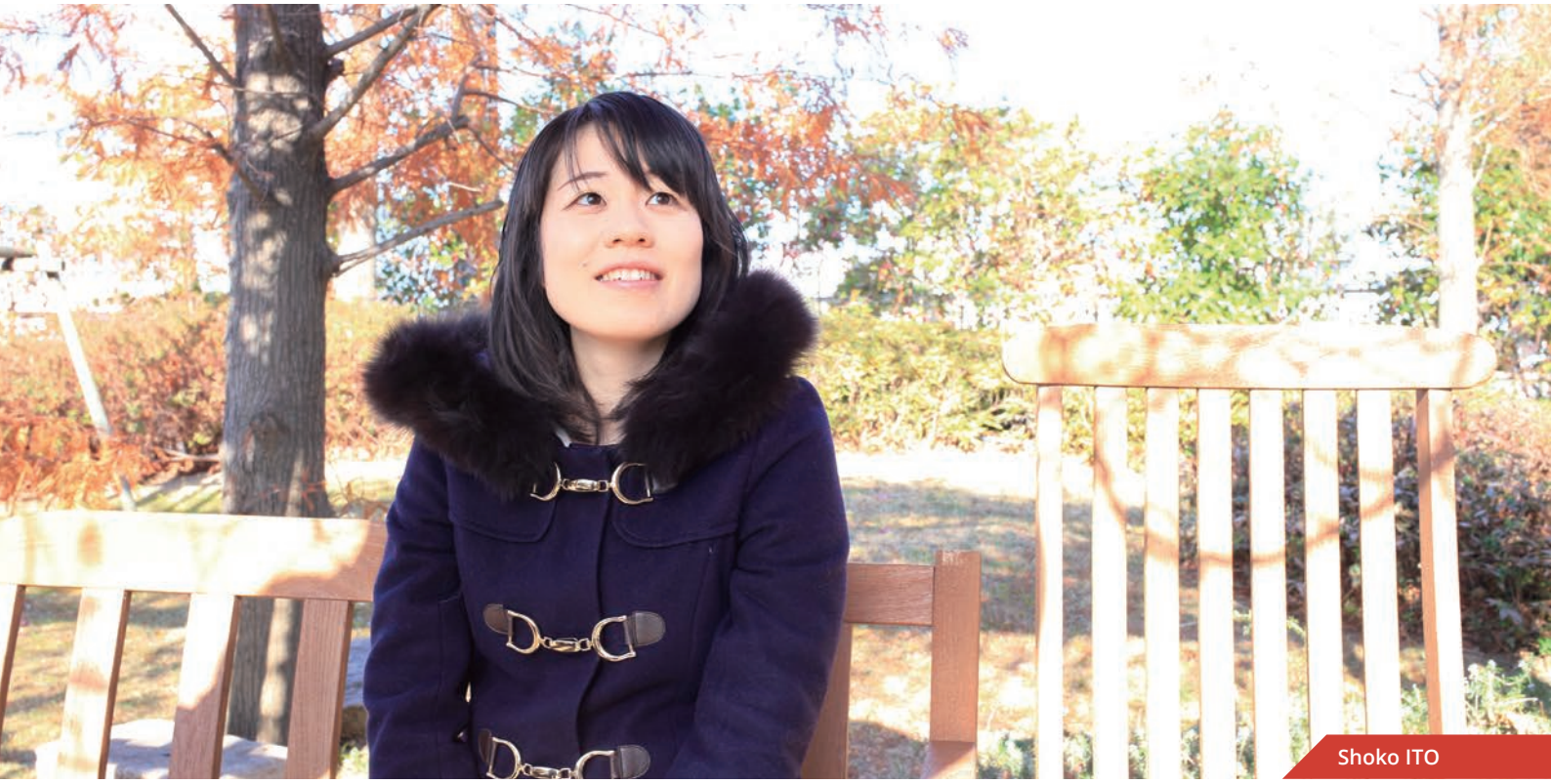


Left: Model for A-P axis determination. *Lefty1* expressing cells that appear around E4.0 form DVE, which then guides establishment of A-P axis. Right: *Lefty1* expression in E.3.5 and E4.5 embryos. Top panels are bright-field images, bottom panels are immunofluorescence images. Green, *Lefty1*; Magenta, GATA6; Blue, DAPI.



Left: Live imaging at E3.2. Cells that express *Nodal* (magenta), later begin expressing *Lefty1* (green).
Right: Even after removing *Lefty1*-expressing cells (green) in E3.5 embryos by laser ablation, cells expressing *Lefty1* begin to appear once again 8 hours later, and will continue to form a normal DVE.

Induced tension restores apical junctions in carcinoma cells



Shoko ITO

Carcinomas are the most common types of cancer, which are formed by epithelial cells that undertake major functions specific to various organs. Normal epithelial cells possess an apical-basal polarity, and are arranged in a sheet-like structure by forming a stable connection with neighboring cells via the apical junctional complex (AJC). When epithelial cells turn cancerous and undergo tumor progression, they are prone to exhibit loss of cell polarity and defects in cell-cell adhesion, which are thought to contribute to invasiveness and metastasis, at which stage effective cancer treatment options are limited. Targeting the restoration of cell-cell adhesion may be one way to limit or prevent tumor invasion or metastasis, however, to date no such therapy has been developed.

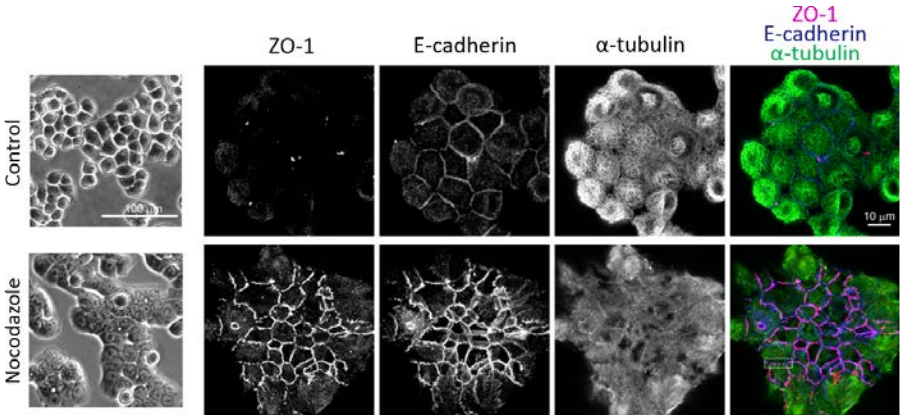
In a study by led by research scientist Shoko Ito of the CDB's Laboratory for Cell Adhesion and Tissue Patterning (Takeichi Masatoshi, Team Leader), she and her collaborators demonstrated that microtubule polymerizing inhibitors (MTIs) can restore cell-cell adhesion in adhesion-defective colon carcinoma cells. The further analyzed the mechanism at work, found that inhibiting microtubule polymerization activated RhoA to mediate the contractions of the actomyosin complex near the cell's apical cortex. The mechanical force of this contraction is transmitted to the cell boundaries, to recruit a mechanosensitive factor vinculin and other adhesion regulators to induce rebuilding of AJCs. Their findings were published in the online journal, *Nature Communications*.

Cell-cell adhesion in epithelial cells are generally stable, and are mediated by cadherins and associated cytoplasmic proteins that organize into the cadherin-catenin complex (CCC). In many types of cancer cells, however, this adhesion complex becomes defective, leading to disruption of cell-cell adhesion and thereby promoting invasion and metastasis. The laboratory and other groups have previously identified colon carcinoma cell lines that could not organize proper junctions, despite the cells expressing core components of the CCC. They also discovered that these carcinoma cell lines could at times reorganize relatively normal junctions following treatment with different chemicals, but the mechanism involved in the reorganization was not known.

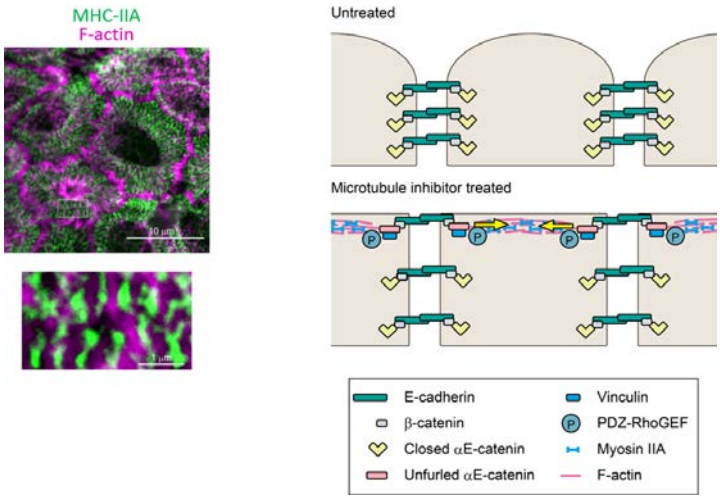
In collaboration with RIKEN's Program for Drug Discovery and Medical Technology Platforms, Ito used HT29 cells, a colon carcinoma cell line that is known to express CCC components yet exhibit adhesion defects, to carry out a comprehensive screening for chemical compounds that restore AJCs. The screening picked up 124 different types of chemical compounds out of a library of 160,960, which appeared to effectively restore junctions. Approximately 80% of these 124 compounds identified were microtubule-depolymerizing drugs, or MTIs. The team also found that following MTI treatment, the HT29 cells not only reorganized relatively normal AJCs, but also re-established apical-basal polarity.

Ito and her collaborators next examined how MTIs restored AJCs. When microtubules undergo depolymerization, the Rho guanine nucleotide exchange factor, GEF-H1, becomes activated. GEF-H1 in turn activates RhoA, and RhoA will activate cortical myosin-II via the Rho/ROCK pathway. The team analyzed time-lapse movies of HT29 cells treated with the MTI nocodazole and observed actomyosin condensing and contracting inwards. Under super-resolution microscopy, they observed that the myosin-IIA heavy chain (MHC-IIA) was organized into a striated pattern, similar to that seen in muscle sarcomeres. F-actin, which can bind to myosin-II, were also seen to reorganize into filamentous structures along the cell boundaries creating tensile forces along the cell's apical surface. The tensile force in turn helps to recruit vinculin, a mechanosensitive molecule, to the cell boundaries to further recruit other actin regulatory molecules to restore AJC.

"In this study, we showed how activation of RhoA by MTIs can restore cell adhesion in colon carcinoma cells, and further, that the MTI treatment step could be skipped as long as RhoA was activated in some manner. As other groups have reported that human cancer cells exhibit abnormal apical RhoA activity, disruption of RhoA-mediated cell adhesion may be a feature common to many cancer cells," says Takeichi. "Why cell adhesion becomes disrupted in carcinoma cells, and the mechanism underlying this phenomenon remains unknown. But, if we can find a way to regulate RhoA activity to restore functional junctions, it may lead to a new therapeutic approach for controlling cancer metastasis or invasion."



Top panels: HT29 colon carcinoma cells cannot adhere to one another. Lower panels: Cell-cell adhesion is restored in HT29 cells after treatment with MTI nocodazole. Magenta, ZO-1 (tight junctions); blue, E-cadherin (zonula adherens); green, α-tubulin (microtubules)



Left: Striated sarcomere-like organization of myosin-II at apical cortex following MTI treatment. Right: Scheme of molecular events underlying AJC restoration. Actomyosin contraction creates tensile forces along the apical surface, which in turn recruits other adhesion regulating molecules to the cell boundaries to restore AJC.

Basal polarity shift rearranges apical microtubule network to break cell size homogeneity for epithelial folding

Mustafa SAMI, Yuko FUJIYAMA, Michiko TAKEDA, Anthony ERITANO, Chun Wai KWAN, Antonio BOLEA ALBERO, Yu-Chiun WANG, Rachel STEWART

Epithelial folding plays a crucial role in morphogenesis, turning two-dimensional (2D) epithelial sheets into three-dimensional (3D) structures. To initiate the folding process, some cells within the epithelial sheet need to undergo changes in cell shape. Cell shape changes often result from contraction of actomyosin network mediated by changes in localized apical myosin activity. However, there are some instances, such as dorsal fold formation in *Drosophila* embryos, in which folding occurs despite the lack of marked changes in myosin activity, suggesting that there are alternative ways to initiate epithelial folding that are myosin-independent.

In a study carried out by technical staff Michiko Takeda and Mustafa Sami, and Team Leader Yu-Chiun Wang of CDB's Laboratory for Epithelial Morphogenesis, they use dorsal fold formation in early *Drosophila* embryo as a model to elucidate mechanisms underlying fold formation in the absence of localized myosin changes. They reported that a CAMSAP protein (microtubule minus-end binding protein), Patronin, plays a key role in reorganizing apical microtubule networks to change cell shape following a basal shift in polarity factors. Their findings were published in *Nature Cell Biology*.

Previous work led by Wang showed that prior to cell shape changes seen before dorsal fold formation, there is a change

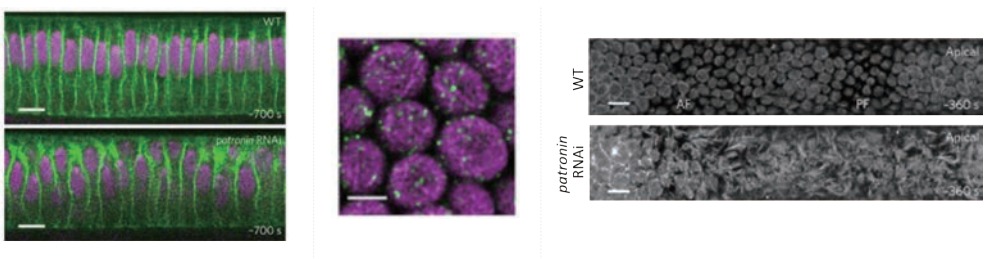
in cell polarity in the initiating cells as noted by a basal shift in proteins that regulate apical-basal polarity of the cells. In particular, the downregulation of Par-1, a MARK family kinase specifying basal-lateral membrane and involved in positioning cell-cell adhesive junctions to the subapical region of epithelial cells, resulted in the basal shift of junctions, and the shrinkage and descent of cell apices, ultimately causing a reduction in cell height. However, it remained unclear how cell polarity changes affect cell shape.

The team first turned their attention to a CAMSAP protein, Patronin, whose localization was recently reported to be regulated by Par-1, as a possible regulator of microtubule (MT) networks needed to modulate cell shape. The role of Patronin in epithelial cells was examined by generating RNAi-mediated *patronin* knockdown embryos, and loss of Patronin led to irregular apical surfaces, disparities in cell size and nuclear positions, whereas normally wildtype (WT) embryos have dome shaped apices and show uniformity in cell size and nuclear positioning. Thus, these differences confirmed that Patronin plays a role in regulating cell shape. The team then performed live imaging of the apical surface of the dorsal epithelium and saw that in WT embryos, the apices initially appear rough prior to gastrulation, but gradually become smoother, and by gastrulation onset, have formed a spherically shaped membrane called the apical dome, and subapically, just below the dome, a ring-like microtubule structure. In contrast, *patronin* RNAi embryos exhibited fibrous and protrusive apices, and showed no dome or ring-like MT structure. They next tracked Patronin localization in relation to apical cellular dynamics and found that, as the embryo prepares for gastrulation, Patronin is seen localized in apical cortex in a punctate pattern. Super-resolution microscopy revealed that lining the inside of the WT apical dome was a filamentous network of unstable, non-centrosomal MTs, anchored by Patronin puncta to form a scaffold, and differed from the more basally localized centrosomal 'inverted basket' containing a mix of stable and unstable MTs. The team also found that the smoothening of the apical dome needs the motor protein Dynein that likely crosslinks the MT filaments to produce outward pushing forces. Together these observations indicate that Patronin regulates the apical MT network to ensure the formation of the apical dome.

The team next sought to determine whether Patronin and its associated MT network is involved in dorsal fold formation. They found that Patronin was redistributed more basally in the apical dome in the initiating cells where Par-1 is downregulated, and in *par-1* RNAi embryos, Patronin displayed extensive basal distribution in all cells. To test whether the disparities in Patronin localization between initiating and neighboring cells are important for folding, the team overexpressed Patronin in the early embryo and found that saturating levels of Patronin across the tissue prevent folding. Together these results suggest that Par-1 acts to restrict Patronin localization in the apical region, and when downregulated, Patronin becomes basally redistributed to initiate folding.

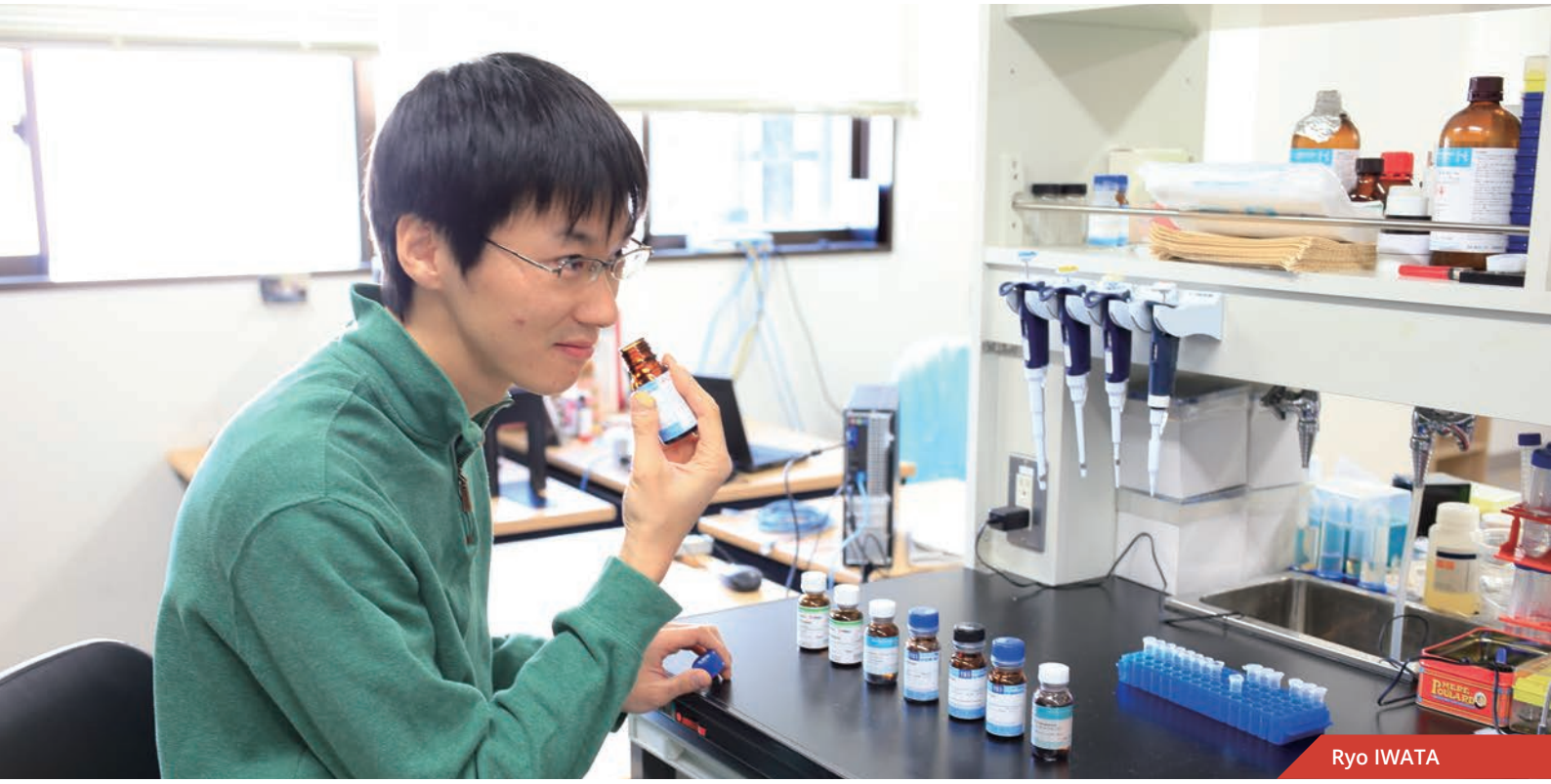
As the apical MT network, comprised primarily of unstable MTs, likely undergoes rapid reorganization in response to polarity changes, the team searched for a possible regulator of MT networks and came across the MT-severing enzyme, Katanin, which reportedly bound to Patronin. Katanin was found co-localizing partially with Patronin in the apical dome, whereas in *patronin* RNAi embryos, apical cortex localization of Katanin was reduced, suggesting that Patronin recruits Katanin to the apical cortex. Single-cell kymograph analyses of MT networks in WT, *patronin* RNAi, and Katanin depleted embryos revealed that Patronin is important for their architecture, while the concerted action of both Patronin and Katanin enhanced the remodeling. Further, both Patronin and Katanin are needed to ensure uniformity of cell size, suggesting that network remodeling is crucial to maintain cell size homogeneity.

"Our study demonstrates that CAMSAP protein Patronin has a cell shape control function in epithelial cells that display minimal myosin changes. Before dorsal fold formation, the MT network is anchored apically by Patronin, and its remodeling ensures cell size homogeneity. At gastrulation onset, the network responds to basal polarity shifts to break the local force balance so that the epithelial sheet bends inward to fold," explains Wang. "Mechanically, the apical dome of these early embryonic cells resemble the canopy of an umbrella where the runner of the stretchers (Dynein) slide along the pole to exert outward pushing forces through the ribs (MT filaments) that are anchored to the canopy (membrane) at the rib tips (Patronin). Our next step will be to look into the counteracting forces that may be present at the inner surface of the canopy—or the apical membrane—to fully understand the force (im)balance states before and during folding."



Left: Mid-sagittal section of cellularizing dorsal epithelium in WT (top) and *patronin* RNAi embryo (bottom). WT shows uniformity in shape and nuclear positioning, whereas *patronin* RNAi embryo show irregular cell apices, disparities in cell shape and nuclear positioning. (Green, cell membrane; magenta, nucleus) Middle: *En face* view of apical dome of dorsal epithelial cells in WT embryos. Patronin (green) puncta seen distributed in dome with MT (magenta) scaffolding. Right: MT arrangement in dome of WT and *patronin* RNAi embryos at initiation stage. Scale bar, left and right, 10μm; middle, 3μm.

Odors discriminated by timing of mitral cell firing



Ryo IWATA

The aroma of freshly baked cookies wafts past your nose. The source of the smell is a mixture of aromatic compounds or odorants floating in the air. Each odorant making up the aroma of freshly baked cookies will bind to and stimulate a specific neuron, called the olfactory sensory neuron (OSN), in the nasal cavity. Each OSN expresses a single type of odorant receptor; humans possess approximately 400 different types of OSNs, while mice have approximately 1,000 types. Regardless of closeness to the source of the smell or intensity of the smell, we are capable of identifying a specific smell from amongst a huge variety. The robustness in our ability to discriminate odors independent from odor concentration, has long remained unresolved.

In a study led by CDB visiting researcher Ryo Iwata of the Laboratory for Sensory Circuit Formation (Takeishi Imai, Team Leader), published in the journal *Neuron*, they examined the neuronal firing patterns in the mouse olfactory bulb, specifically the mitral cells, when stimulated by odors. Using two-photon calcium imaging system to visualize neuronal activity, they reveal that odorants are identified by differences in temporal firing of mitral cells in the glomerulus.

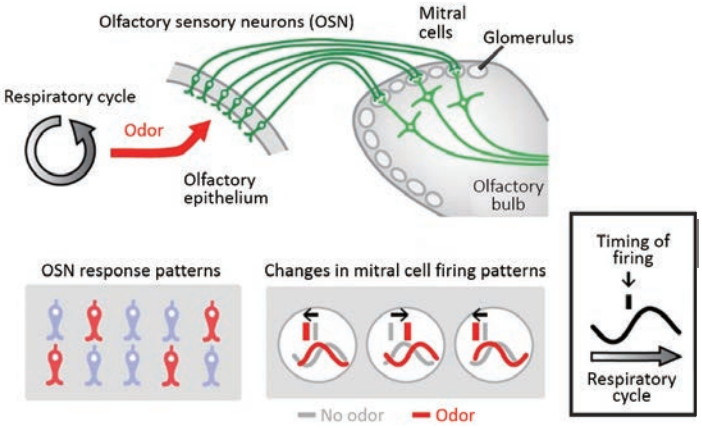
When sniffing or breathing in through the nose, odorants in the air are taken up into the nasal cavity where they bind to a specific OSNs located in the olfactory epithelium. The axons of OSNs extend to the glomeruli situated in the olfactory bulb of the brain and form synapses with dendritic spines of mitral cells. Odor stimuli transmitted from the OSNs, will excite mitral cells, which will in turn send information to the olfactory center to be interpreted as a specific odor. Past studies have demonstrated that mitral cells exhibited differences in firing rates and temporal firing patterns depending on the type of odor stimulus, and it was suggested that odor information was encoded by these two parameters. Recent studies have also shown that in addition to odor-evoked stimuli from OSNs, mitral cells also show responses to mechanical stimulation such as respiratory airflow in the nose. However, how mitral cells can differentiate between odor stimuli and mechanical stimuli remained unclear, as observing mitral cell activity *in vivo* was difficult. To try elucidating the mechanism involved in odor information processing, Iwata and his collaborators used two-photon calcium imaging, which facilitates deep tissue observations, and measured neuronal activity by looking at calcium dynamics in the olfactory bulb in response to odor stimuli.

The team first examined how OSNs in the olfactory epithelium detect mechanical signals. Their observations of the neural activity of the OSNs and the mitral cells in the glomerulus of the olfactory bulb revealed that when OSNs were stimulated by airflow, they showed robust and widespread responses, whereas downstream mitral cells showed oscillatory neuronal firing in response to the mechanical signals. Furthermore, the oscillation cycles varied between glomeruli in the olfactory bulb, and when airflow into the nasal cavity

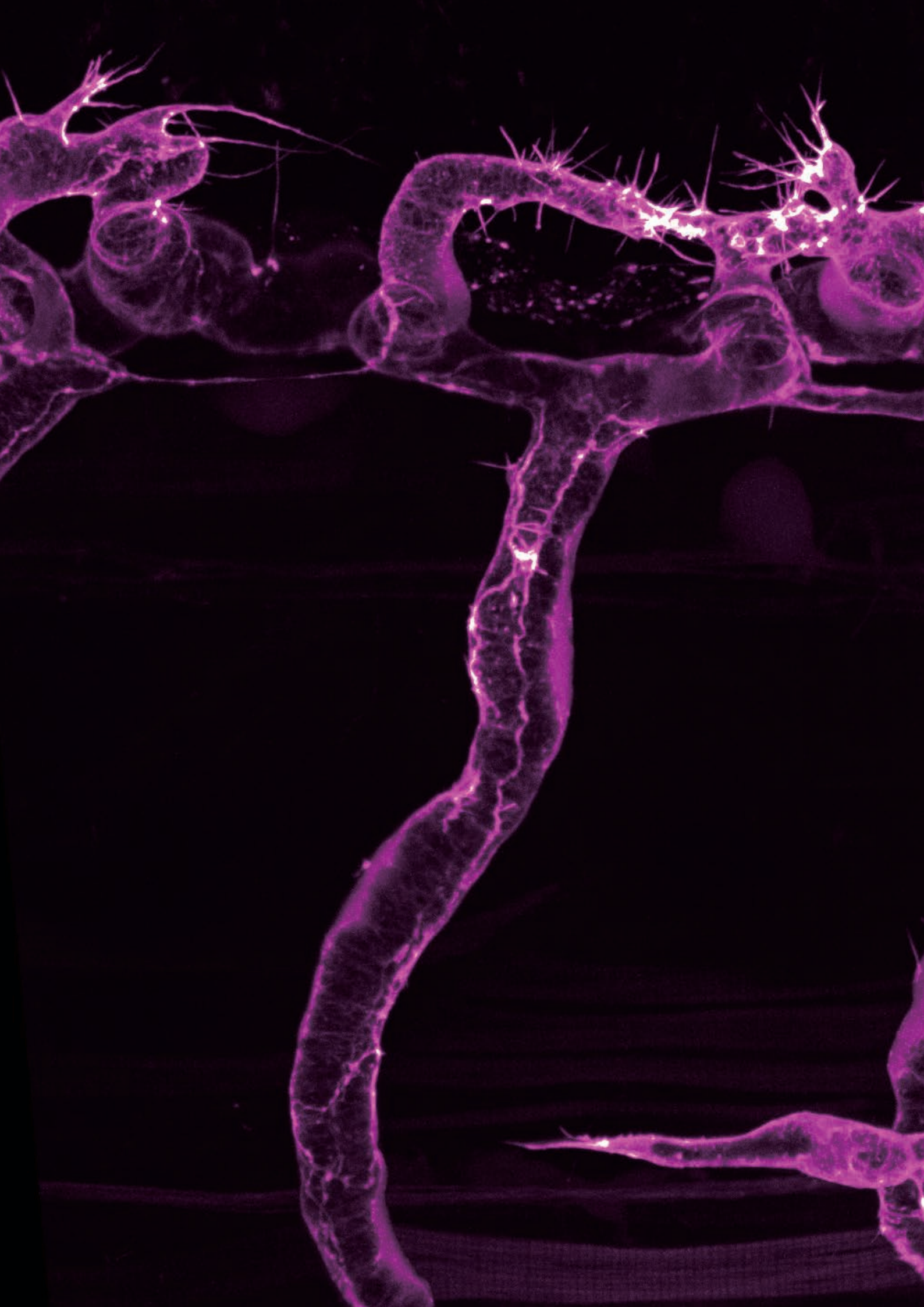
is physically blocked, no oscillatory activity was seen and instead neuronal firing was spontaneous and sporadic. These results suggested that airflow-derived mechanical signals that are transmitted by OSNs are required to produce the distinct oscillations of neural activity in mitral cells.

So what is the relationship between mitral cell firing responses triggered by mechanical signals, and those responses to odor-evoked signals? It was known mitral cells show changes in firing rates and temporal firing in response to odor stimulus, but which property was more important for discriminating odors was not well understood. The team compared and analyzed the mitral cell firing patterns when a mouse was continuously exposed to the same odor over multiple sniff cycles. They observed that temporal firing patterns of the mitral cell remained stable, while the firing rate changed dynamically. They also examined the firing patterns for different concentration of the odor, and again found that the temporal patterns are maintained regardless of odor concentration. Thus, the observations suggested that mitral cells encode odor "identity" information as temporal code. They also demonstrated that mechanosensory-driven oscillatory neural activity in mitral cells improves olfaction, acting as a pacemaker for temporal patterning, as evidenced by experiments showing reduced precision of mitral cell firing under continuous airflow.

"It has long been thought that odors are discriminated by the combination of odorants binding to OSNs. But our study demonstrated that the temporal firing pattern of mitral cells is critical for identifying odors, at the level of mitral cells. Further, mechanosensation by airflow creates oscillations of neural activity, which is important to ensure mitral cells are activated at a precise timing during a sniff cycle," explains Imai. "Our next goal is to understand the how the precise temporal neural firing patterns are generated in the olfactory bulb circuits."



Top: Odor processing in OSNs and mitral cells. Odors are detected by OSNs in the olfactory epithelium. OSNs then transmit signals to mitral cells in the glomeruli of the olfactory bulb. Bottom: Odorants are taken up each respiratory cycle into the nasal cavity, and stimulate a specific type of OSN. Odor stimuli received by mitral cells cause changes in the firing rates as well as firing timing.



Laboratories

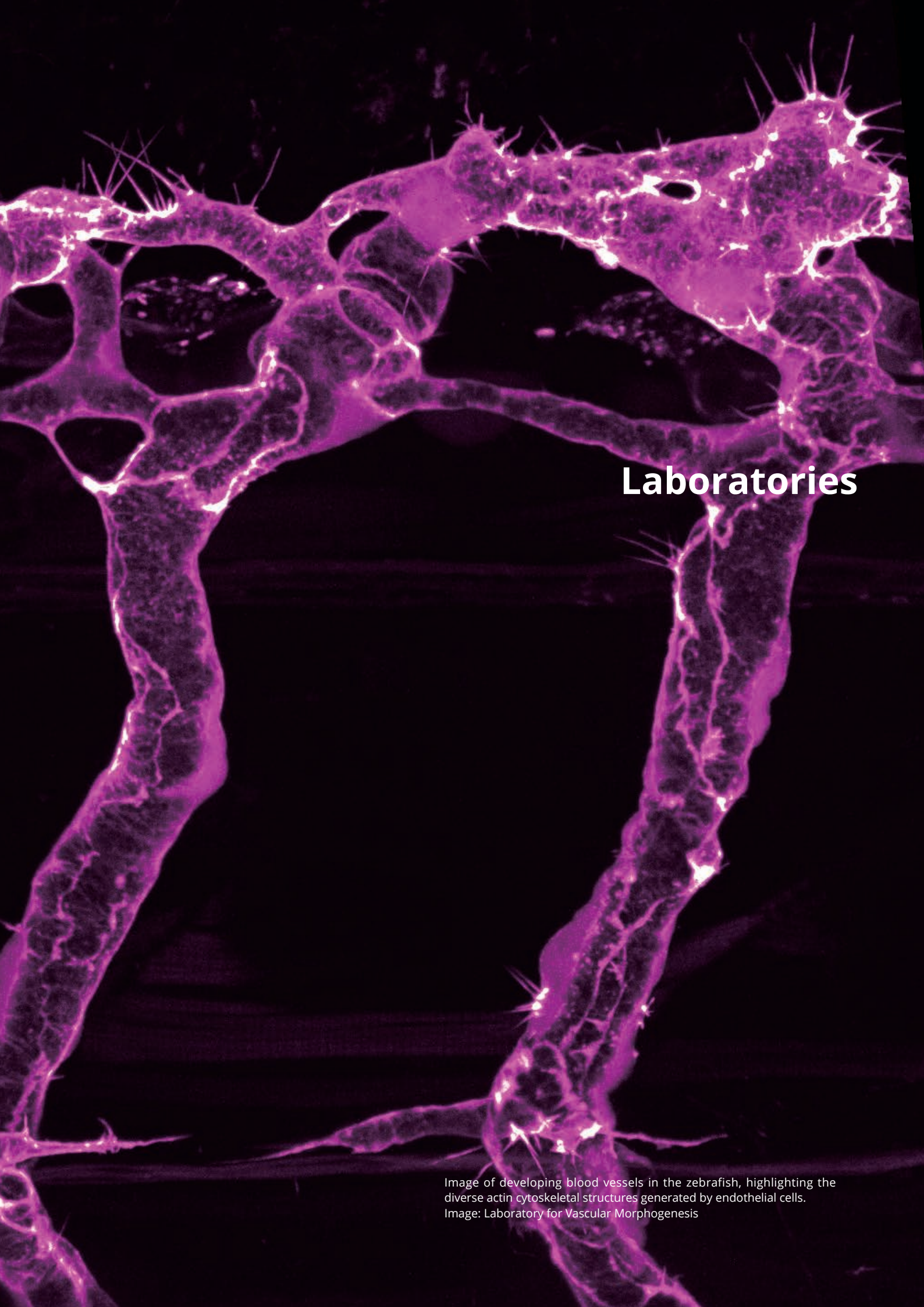
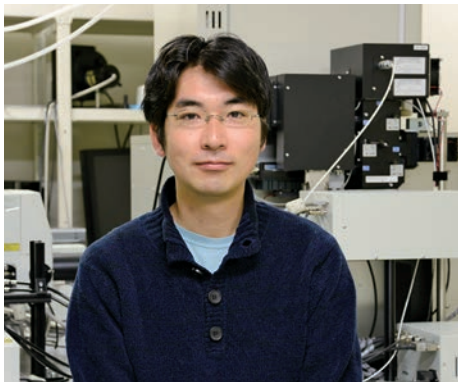


Image of developing blood vessels in the zebrafish, highlighting the diverse actin cytoskeletal structures generated by endothelial cells.
Image: Laboratory for Vascular Morphogenesis

in vitro Histogenesis



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 April 2015, he has served as Team Leader of the Laboratory for in vitro Histogenesis.

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

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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.

Recent Publications

Takata N, et al. Self-patterning of rostral-caudal neuroectoderm requires dual role of Fgf signaling for localized Wnt antagonism. *Nat Commun* 8. 1339 (2017)

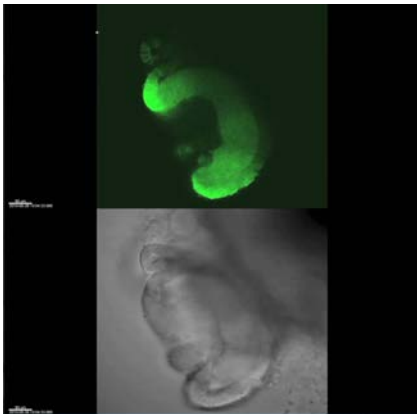
Okuda S and Eiraku M. Role of molecular turnover in dynamic deformation of a three-dimensional cellular membrane. *Biomech Model Mechanobiol* 16(5). 1805–1818 (2017)

Ohgushi M, et al. A RHO small GTPase regulator ABR secures mitotic fidelity in human embryonic stem cells. *Stem Cell Reports* 9(1). 58–66 (2017)

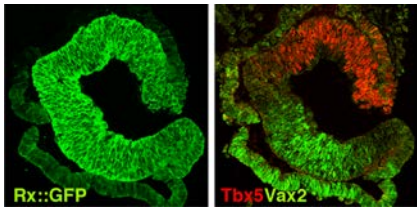
Okuda S, et al. Contractile actin belt and mesh structures provide the opposite dependence of epithelial stiffness on the spontaneous curvature of constituent cells. *Dev Growth Differ* 59(5). 455–464 (2017)

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

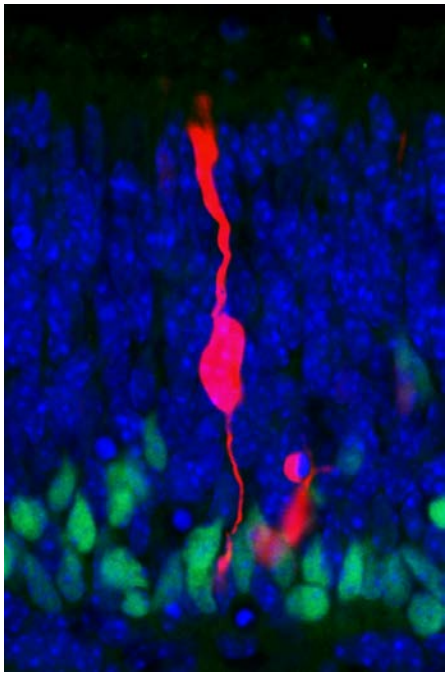
Sakaguchi H, et al. Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. *Nat Commun* 6. 8896 (2015)



Self-organized optic cup formation from ES cells



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



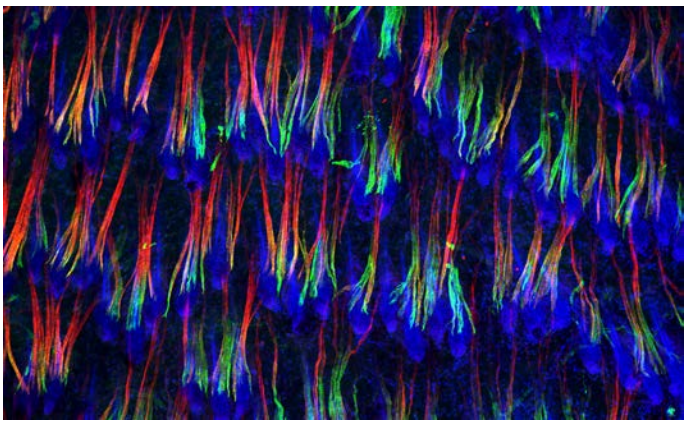
Photoreceptor cell (red) and bipolar cells (green) in ES cell-derived neural retina

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.

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

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).

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Yuichiro HIRANO

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* 69.1334–1342 (2009)

Fujiwara H, et al. Regulation of mesodermal differentiation of mouse embryonic stem cells by basement membranes. *J Biol Chem* 282. 29701–29711 (2007)

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

Organismal Patterning

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.

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

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Kei SHIOZAWA

International Program Associate
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Nor Shaheera KAMAL

Student Trainee
Lynda LAMRI

Assistant
Kaori SONE

Recent Publications

Takaoka K, et al. Both Nodal signaling and stochasticity select for prospective distal visceral endoderm in mouse embryos. *Nat Commun* 8. 1492 (2017)

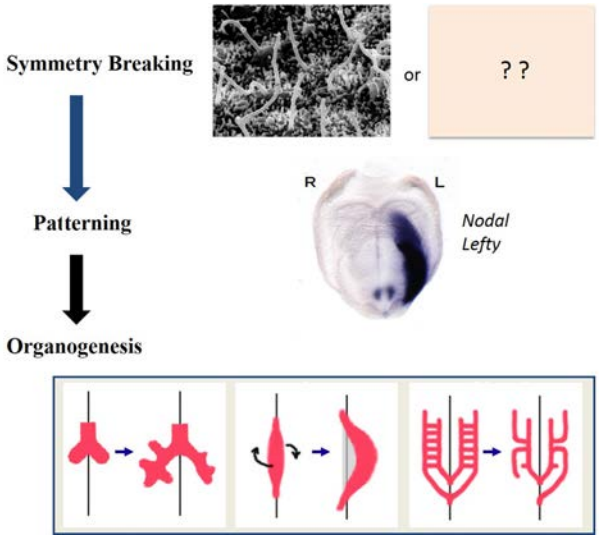
Minegishi K, et al. A Wnt5 activity asymmetry and intercellular signaling polarize node cells for breaking left-right symmetry in the mouse embryo. *Dev Cell* 40. 439–452 (2017)

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)

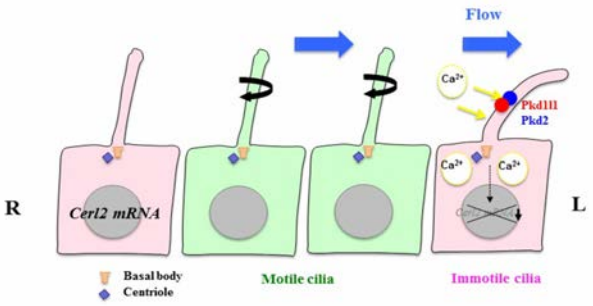
Nakamura T, et al. Fluid flow and interlinked feedback loops establish left-right asymmetric decay of *Cerl2* mRNA in the mouse embryo. *Nat Commun* 3. 1322 (2012)

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)

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 development. We address these questions by integrating live imaging, structural biology, fluid dynamics and mathematical modeling.



How left-right asymmetry is established in vertebrates

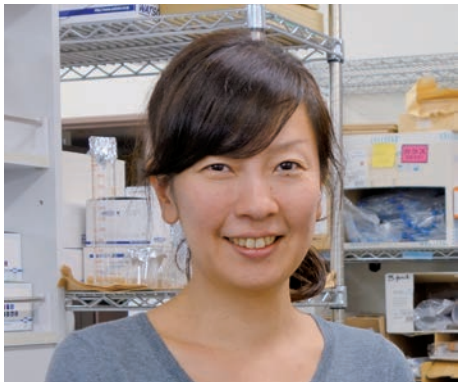


Role of motile and immotile cilia in left-right symmetry breaking

Neocortical Development

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 the 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.



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

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.

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Assistant
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Recent Publications

Hou PS, et al. A sensitive and versatile in situ hybridization protocol for gene expression analysis in developing amniote brains. *Methods Mol Biol* 1650. 319–334 (2017)

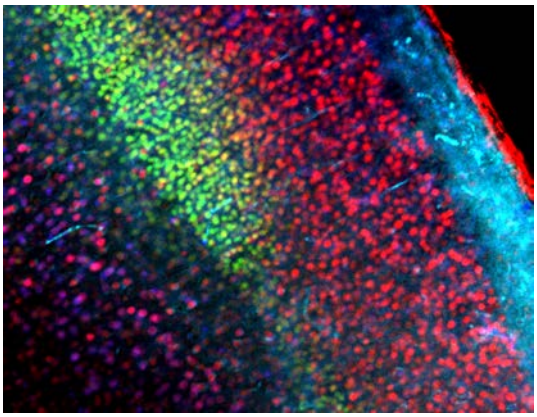
Kumamoto T and Hanashima C. Evolutionary conservation and conversion of Foxg1 function in brain development. *Dev Growth Differ* 59(4). 258–269 (2017)

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

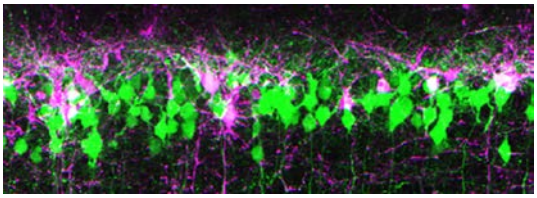
Toma K and Hanashima C. Switching modes in corticogenesis: mechanisms of neuronal subtype transitions and integration in the cerebral cortex. *Front Neurosci* 9. 274 (2015)

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* 34.13259–13276 (2014)

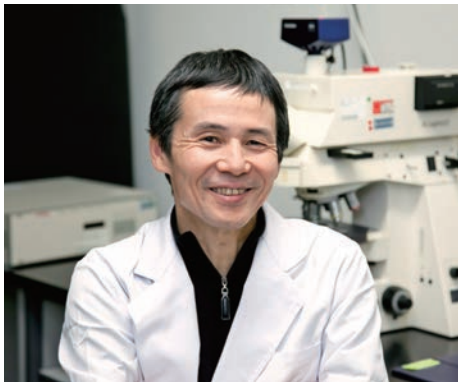


The neocortex is composed of distinct neuronal subtypes that establish six layers. Coronal section of a one-week-old mouse neocortex showing Reelin (layer I, cyan), Brn2 (layer II/III, red), RORß (layer IV, green), Ctip2 (layer V/VI, blue) expressing neurons.



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).

Morphogenetic Signaling



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*.

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

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Part-Time Staff
Ikuko FUKUZYOU
Kazumi IWASHITA
Noriko MORIMITU

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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)

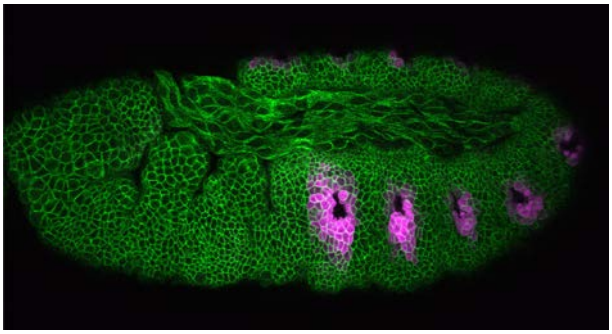
Hannezo E, et al. A cortical instability drives periodic supracellular actin pattern formation in epithelial tubes. *Proc Natl Acad Sci U S A* 112. 8620–8625 (2015)

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. 125–129 (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).

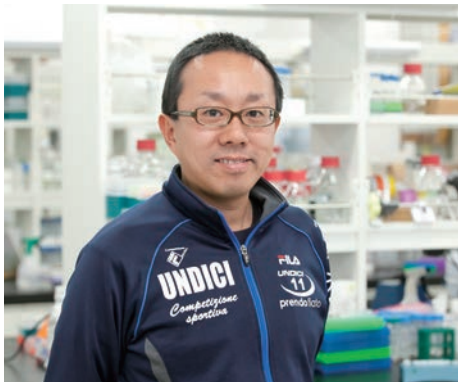


Drosophila embryo at the beginning of tracheal placed invagination (magenta). Cell boundaries are marked green.

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.

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



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.

Staff

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Akie TANIGAWA

Part-Time Staff
Yoshiko KONDO

Recent Publications

Takahashi S, et al. Epigenetic differences between naïve and primed pluripotent stem cells. *Cell Mol Life Sci* (2017) [Epub ahead of print]

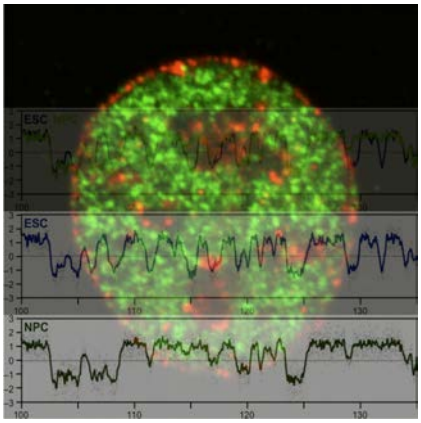
Shang WH, et al. Chromosome engineering allows the efficient isolation of vertebrate neocentromeres. *Dev Cell* 24. 635–648 (2013)

Ryba T, et al. Genome-scale analysis of replication timing: from bench to bioinformatics. *Nat Protoc* 6. 870–895 (2011)

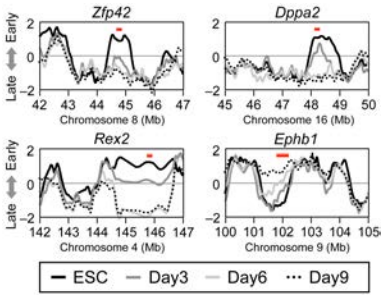
Hiratani I and Gilbert D M. Autosomal lyonization of replication domains during early mammalian development. *Adv Exp Med Biol* 695. 41–58 (2010)

Ryba T, et al. Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. *Genome Res* 20. 761–770 (2010)

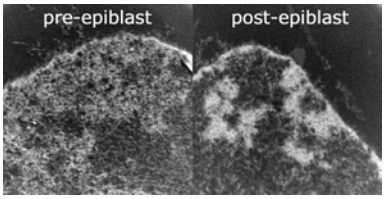
Hiratani I, et al. Genome-wide dynamics of replication timing revealed by in vitro models of mouse embryogenesis. *Genome Res* 20. 155–169 (2010)



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.

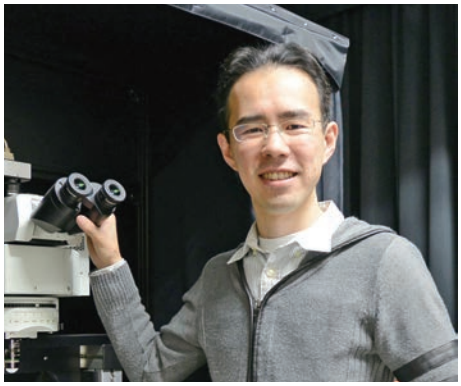


Genome-wide DNA replication profiling during ES cell differentiation can reveal domains that show large-scale changes in nuclear organization.



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

Sensory Circuit Formation



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 is also a professor at the Kyushu University Graduate School of Medical Sciences.

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

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Recent Publications

Iwata R, et al. Mechanosensory-based phase coding of odor identity in the olfactory bulb. *Neuron* 96. 1139–1152 (2017)

Murai A, et al. Distorted coarse axon targeting and reduced dendrite connectivity underlie dysosmia after olfactory axon injury. *eNeuro* 3. e0242-16 (2016)

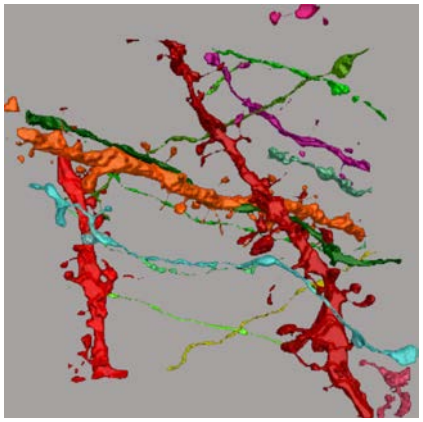
Ke M T, et al. Super-resolution mapping of neuronal circuitry with an index-optimized clearing agent. *Cell Rep* 14. 2718–2732 (2016)

Imai T. Construction of functional neuronal circuitry in the olfactory bulb. *Semin Cell Dev Biol* 35.180–188 (2014)

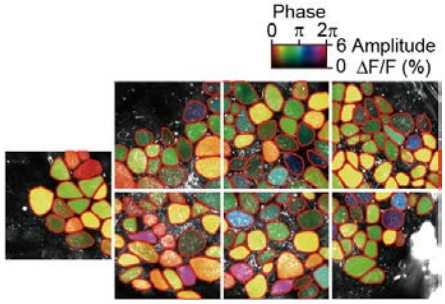
Nakashima A, et al. Agonist-independent GPCR activity regulates anterior-posterior targeting of olfactory sensory neurons. *Cell* 154.1314–1325 (2013)

Ke M T, et al. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nat Neurosci* 16.1154–1161 (2013)

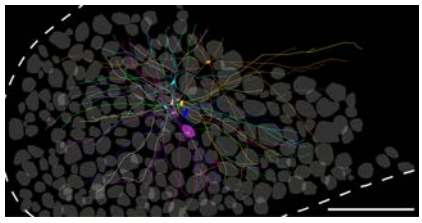
The mammalian central nervous system is composed of enormous numbers of neurons. How do these neurons establish their identity and form functional neuronal circuitries? To address this question, we are studying the mouse olfactory system as a model system. In the mouse olfactory system, odorants are detected by approximately 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.



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.



Mechanosensation in the olfactory sensory neurons produces respiration-coupled and glomerulus-specific oscillations of neuronal activity in the olfactory bulb.



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.

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 named a researcher in the Japan Science and Technology Agency (JST) PRESTO program.

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

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.

Staff

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Technical Staff
Setsuko KANAMURA
Kaori NIIMI

Internship
Anna Olomí PALLÀS

Part-Time Staff
Mako MIYAGI
Masako SUZUKI
Yoko UMEGAKI

Recent Publications

Inomata H, et al. Scaling of dorsal-ventral patterning by embryo size-dependent degradation of Spemann's organizer signals. *Cell* 153.1296–1311 (2013)

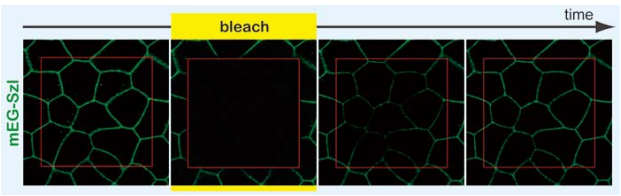
Takai A, et al. Anterior neural development requires Del1, a matrix-associated protein that attenuates canonical Wnt signaling via the Ror2 pathway. *Development* 137. 3293–3302 (2010)

Inomata H, et al. Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. *Cell* 134. 854–865 (2008)

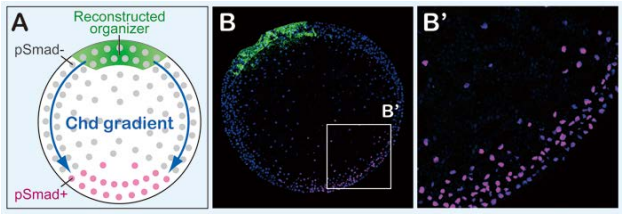
Arakawa A, et al. The secreted EGF-Discoidin factor xDel1 is essential for dorsal development of the *Xenopus* embryo. *Dev Biol* 306. 160–169 (2007)

Onai T, et al. XTsh3 is an essential enhancing factor of canonical Wnt signaling in *Xenopus* axial determination. *EMBO J* 26. 2350–2360 (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. 22946–22955 (2003)



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.

Heart Regeneration

Team Leader

Wataru KIMURA Ph.D.

Wataru Kimura completed his Ph.D. at the Tokyo Metropolitan University in 2007, and then moved to Hamamatsu University School of Medicine the same year to take a position as a post-doctoral researcher. In 2012, he obtained a position as visiting senior fellow at UT Southwestern Medical Center, U.S.A, and was later promoted to assistant instructor at the same institution. In 2015, Kimura received a joint appointment as assistant professor at the Life Science Center of TARA, the University of Tsukuba in Japan and visiting assistant professor at UT Southwestern Medical Center. He returned to Japan in 2017, to serve as Team Leader of the Laboratory for Heart Regeneration at the RIKEN CDB.

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

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Assistant
Risa IMAMURA

Recent Publications

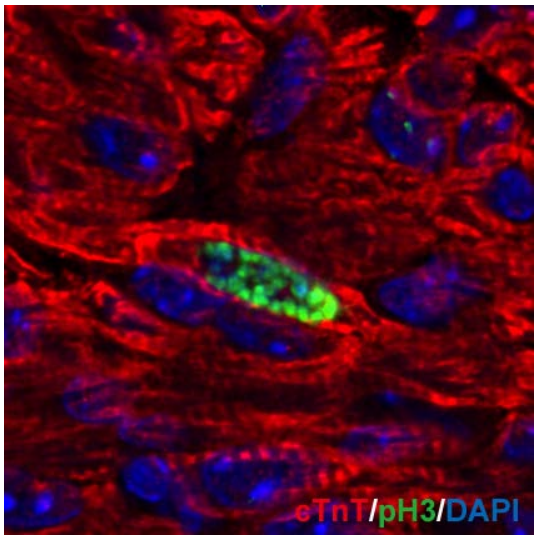
Nakada Y, et al. Hypoxia induces heart regeneration in adult mice. *Nature* 541. 222–227 (2017)

Kimura W, et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. *Nature* 523. 226–230 (2015)

Canseco D. C, et al. Human ventricular unloading induces cardiomyocyte proliferation. *J Am Coll Cardiol* 65. 892–900 (2015)

Puente B. N., et al. The oxygen rich postnatal environment induces cardiomyocyte cell cycle arrest through DNA damage response. *Cell* 157. 565–579 (2014)

Heart disease is the leading cause of death worldwide. The main reason for this is our inability to regenerate damaged myocardium in the heart. Proliferation of cardiomyocytes (heart muscle cells), is a major mediator of mammalian heart regeneration in neonates and myocardial turnover in adults. However, little is known about the mechanisms regulating the cardiomyocyte cell cycle. We have recently shown that a rapid increase in mitochondrial respiration and in oxidative stress induce cell cycle arrest in neonatal cardiomyocytes. Our research interest focuses on how the postnatal mammalian heart loses regenerative capacity following injury- and age-related myocardial damage, and whether it is possible to re-awaken endogenous regenerative capacity. We utilize molecular and cellular tools and mouse genetics to understand the role of hypoxia signaling and oxidative stress in cardiomyocyte cell cycle regulation throughout the life cycle of mammals.



An M-phase marker pH3Ser10-positive cardiomyocyte in postnatal day 3 heart

Chromosome Segregation

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 a 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.

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

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.

Staff

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Technical Staff
Kaori HAMADA

Recent Publications

Kyogoku H and Kitajima TS. Large cytoplasm is linked to the error-prone nature of oocytes. *Dev Cell* 41(3). 287–298 (2017)

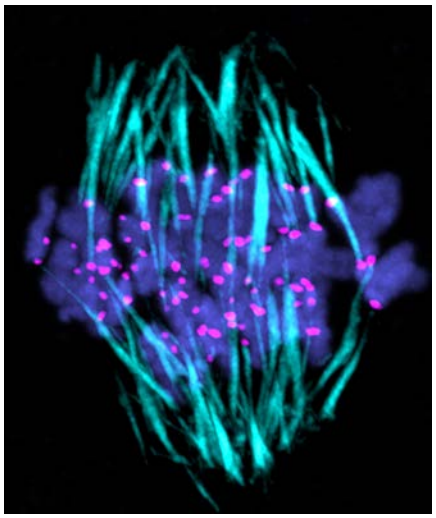
Sakakibara Y, et al. Bivalent separation into univalents precedes age-related meiosis I errors in oocytes. *Nat Commun* 6. 7550 (2015)

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

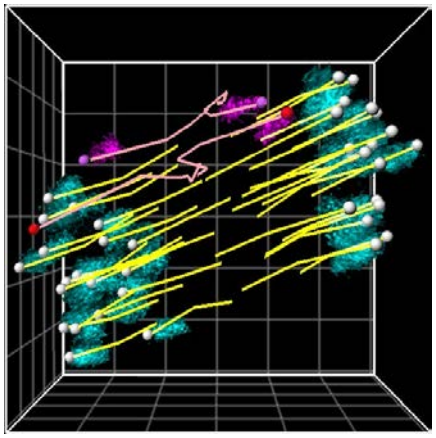
Kim J, et al. Meikin is a conserved regulator of meiosis-I-specific kinetochore function. *Nature* 517(7535). 466–471 (2015)

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

Kitajima TS, et al. Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. *Cell* 146. 568–581 (2011)



Kinetochore-microtubule attachments



Chromosome segregation error

Histogenetic Dynamics

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 an 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.

The Laboratory for Histogenetic Dynamics closed in March 2017.
Dr. Kuranaga is now at Tohoku University.

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

Staff

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Daiki UMETSU

Technical Staff
Ayako ISOMURA

Student Trainee
Yuka HAYASHI
Yuhei KAWAMOTO

Part-Time Staff
Arata KURANAGA
Yoko UMEGAKI

Recent Publications

Hiraiwa T, et al. Wave Propagation of Junctional Remodeling in Collective Cell Movement of Epithelial Tissue: Numerical Simulation Study. *Front Cell Dev Biol* 5. 66 (2017)

Umetsu D and Kuranaga E. Planar polarized contractile actomyosin networks in dynamic tissue morphogenesis. *Curr Opin Genet Dev* 45. 90–96 (2017)

Kawamoto Y, et al. Apoptosis in cellular society: Communication between apoptotic cells and their neighbors. *Int J Mol Sci* 17(12). 2144 (2016)

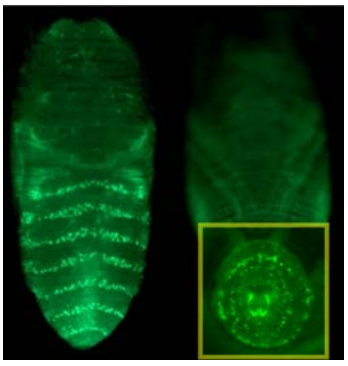
Sato K, et al. Left-right asymmetric cell intercalation drives directional collective cell movement in epithelial morphogenesis. *Nat Commun* 6. 10074 (2015)

Kuranaga E, et al. Apoptosis controls the speed of looping morphogenesis in *Drosophila* male terminalia. *Development* 138. 1493–1499 (2011)

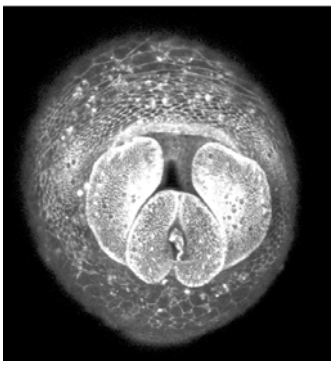
Kuranaga E, et al. *Drosophila* IKK-related kinase regulates nonapoptotic function of caspases via degradation of IAPs. *Cell* 126. 583–596 (2006)

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.

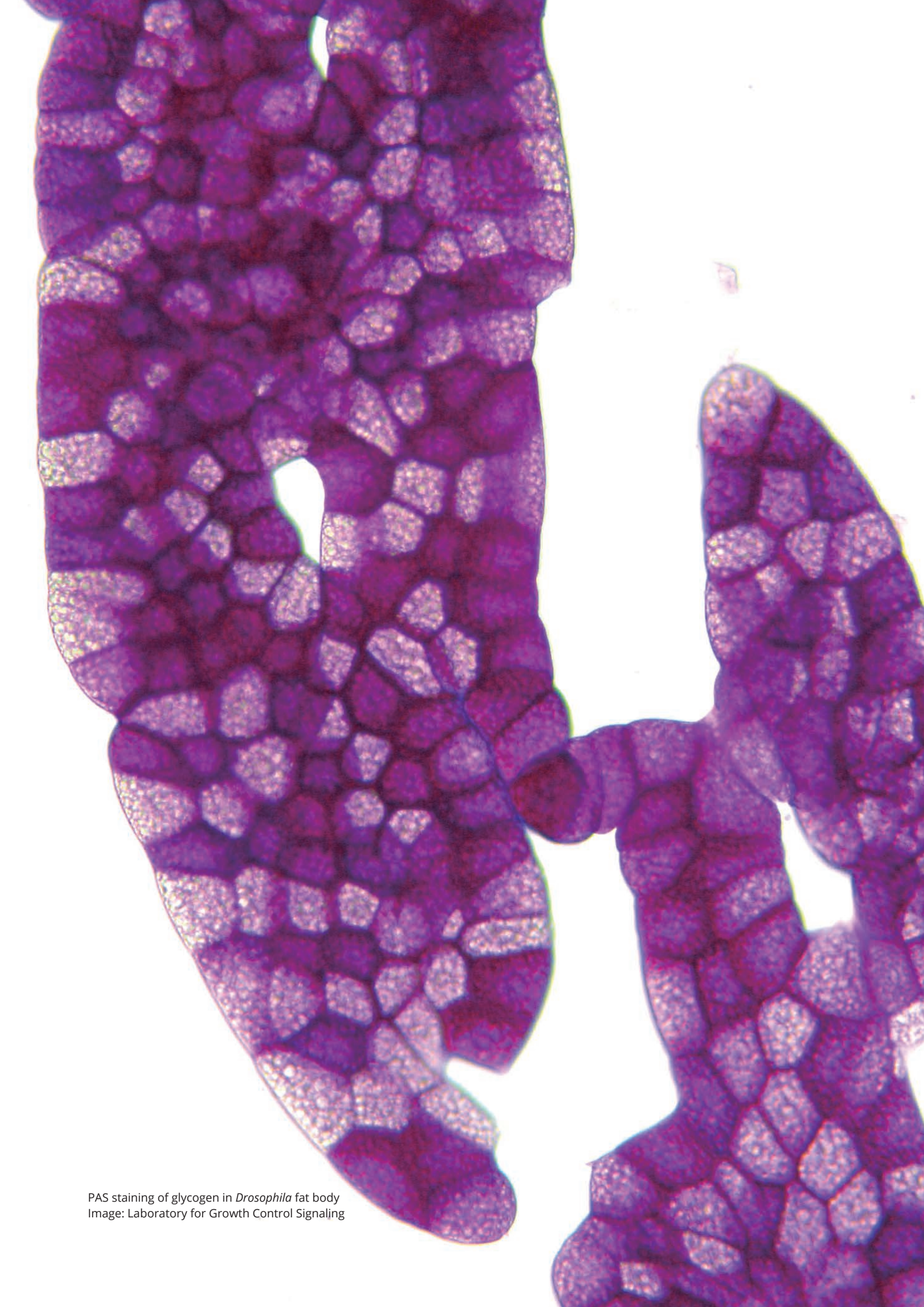
We have chosen the fruit fly *Drosophila melanogaster* as our primary research model, seeking to take advantage of its utility in developmental studies and wealth of genetic data in studying the coordination of histogenesis through live imaging and genetic screens. To elucidate the role of cell death 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.



PAS staining of glycogen in *Drosophila* fat body
Image: Laboratory for Growth Control Signaling

Cell Asymmetry

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.

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

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Noriomi EGUCHI
Mariko IKEDA
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Ayano KAWAGUCHI
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Shun MASE

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Tomomi NAKAO

International Program Associate
Merve BILGIC

Part-Time Staff
Yoko OTSUKA

Assistant
Junko ISHIGAI
Eriko MIZUTA

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. 3216–3222 (2016)

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

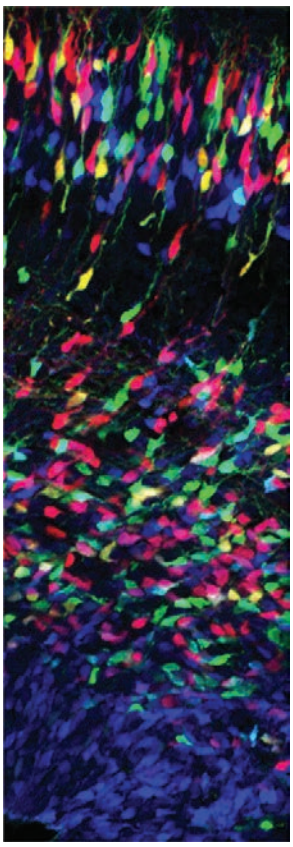
Matsuzaki F and Shitamukai A. Cell division modes and cleavage planes of neural progenitors during mammalian cortical development. *Cold Spring Harb Perspect Biol* 7. a015719 (2015)

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 and ferret) model systems, we focus our study on the 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 invertebrate. 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 (see figure). 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 as a model forming the complex brain (Tsunekawa et al., 2016).

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.

Comparative Connectomics

Team Leader
Kazunari MIYAMICHI Ph.D.

Kazunari Miyamichi received his BSc. in Biochemistry from the University of Tokyo in 2001, and completed his Ph.D. focusing on the organization and development of the peripheral olfactory neural circuit in 2006 under the supervision of Prof. Sakano. He was awarded JSPS and HFSP fellowships to perform postdoctoral research at Stanford University (mentored by Prof. Luo) where he used viral and genetic tools to map input, output, and input-output relationships of a given neural type in the mouse brain. In 2013, he formed a research group under the ERATO Touhara Chemosensory Signal Project, where he studied functions of the pheromone-processing hypothalamic neural circuit. He was appointed to his current position as Team Leader at the RIKEN CDB in September 2017.

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

The connection patterns of the billions of neurons in the mammalian brain underlie how neural circuits process information essential for perception, memory, and behavior. We have implemented viral-genetic tools that enable comprehensive mapping of input, output, and input–output relationships of specific neural types at the scale of the entire brain. Using these tools, we systematically map connection patterns of hypothalamic neurons underlying various social behaviors in mice. Specifically, we study anatomical differences in the neural circuit between male and female mice at the resolution of synaptic connection patterns, focusing on neurons that regulate sexual behaviors and reproduction. We also investigate the state-dependent circuit shift for parturition and lactation in female mice during pregnancy. These comparative connectomics approach will form a foundation upon which developmental and functional studies of neural circuits can be integrated in the future.

Currently, most genetic techniques in neuroscience are only applicable to mice, as Cre recombinase-dependent strategy is commonly used to regulate specific types of target neurons. To overcome this limitation, we combine CRISPR-mediated *in situ* gene knock-in and viral toolboxes to enable cell-type specific manipulations in non-model mammalian species without germline manipulation. We will then analyze organization and function of evolutionally orthologous neural circuits across mammalian species. This comparative connectomics will hopefully lead to an integrative platform for the study of evolution of neural circuits.

Recent Publications

Ishii KK, et al. A Labeled-line neural circuit for pheromone-mediated sexual behaviors in mice. *Neuron* 95. 123–137 (2017)

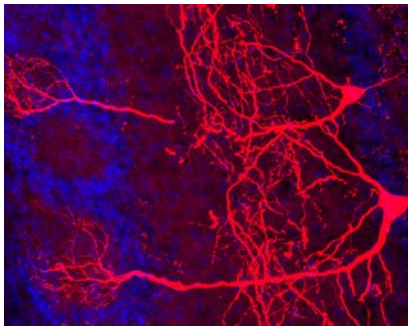
Schwarz LA, et al. Viral-genetic tracing of the input-output organization of a central noradrenaline circuit. *Nature* 524. 88–92 (2015)

Weissbourd B, et al. Presynaptic partners of dorsal raphe serotonergic and GABAergic neurons. *Neuron* 83. 645–662 (2014)

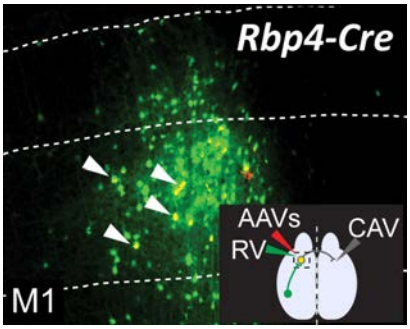
Miyamichi K, et al. Dissecting local circuits: parvalbumin interneurons underlie broad feedback control of olfactory bulb output. *Neuron* 80. 1232–1245 (2013)

Guenthner CJ, et al. Permanent genetic access to transiently active neurons via TRAP: targeted recombination in active populations. *Neuron* 78. 773–784 (2013)

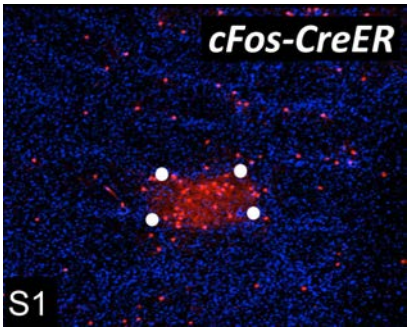
Miyamichi K, et al. Cortical representations of olfactory input by trans-synaptic tracing. *Nature* 472. 191–196 (2011)



Mitral cells in the olfactory bulb that were labeled with mCherry by rabies virus-mediated retrograde trans-synaptic tracing from the olfactory cortex.



By using cTRIO, pre-synaptic inputs were visualized in green (GFP) to layer 5 pyramidal cells (Rbp4+) in the mouse motor cortex (M1) that project their own axons to the contralateral side of the motor cortex.



Using the TRAP method, a single barrel structure was visualized with tdTomato in the primary somatosensory cortex (S1) corresponding to a single C2 whisker.

Lung Development

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.

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

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Akira YAMAOKA

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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. 8242–8247 (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. 4365–4373 (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. 213–224 (2010)

Morimoto M and Kopan R. rtTA toxicity limits the usefulness of the SP-C-rtTA transgenic mouse. *Dev Biol* 325. 171–178 (2009)

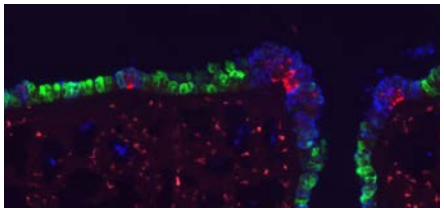
Morimoto M, et al. The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity. *Nature* 435. 354–359 (2005)

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.

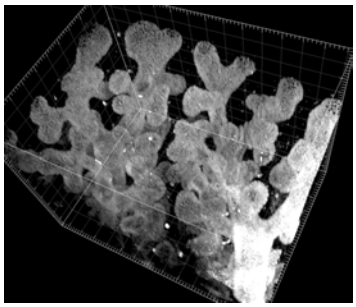
Respiratory organs in higher mammals are characterized by their efficient gas exchange, enabled by the functions of specialized cells. The development of such organs relies on the coordinated activities of both epithelial and mesenchymal tissue types, which arise from tissue-specific populations of stem 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.

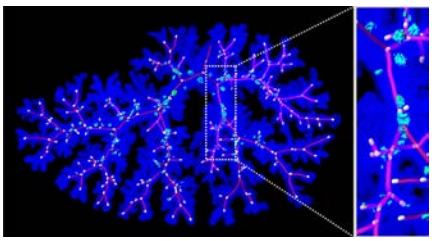
We focus on issues of how tissue morphology influences the formation of stem cell/niche tissue complexes in the development, repair and regeneration of respiratory organs, as well as mechanisms regulating cell proliferation and differentiation in developmental and regenerative processes.



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.

Single-cell Omics Research Unit

Unit Leader
Itoshi NIKAIDO Ph.D.

Itoshi Nikaido received his doctorate from the Yokohama City University in March 2004. In April of the same year he then moved to take a position as a research scientist at the Research Center for Genomic Medicine at Saitama Medical University, and then at the RIKEN Center for Developmental Biology (CDB) in 2006. In 2013, he was appointed as Unit Leader of Bioinformatics Research Unit at the RIKEN Advanced Center for Computing and Communication. He accepted a joint appointment as Unit Leader of the Single-cell Omics Research Unit at the RIKEN CDB in July 2017. Nikaido also serves as leader of several projects funded by AMED and JST's CREST program.

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

A multicellular organism is orchestrated by cell growth, death, differentiation, and communication at the single-cell level. To understand various crucial biological phenomena, we should massively perturb and measure transcriptomes and epigenomes at the single-cell level.

Our group will develop novel methods of comprehensive analysis and perturbation of transcriptome and epigenome at the single-cell level, in particular, by applying massively parallel DNA sequencing, genome editing, microfluidics, and machine learning. We focus on the development of methods for quantifying and controlling cell function, fate, and cell-cell communication at the single-cell level.

We have already reported novel single-cell RNA-sequencing methods, such as Quartz-Seq and RamDA-seq, which are highly reproducible and sensitive methods of quantifying single-cell transcriptome. Our unit will not develop new techniques but also collaborate with various life scientists within and outside of RIKEN using our new sequencing technologies.

With these techniques, our unit seeks to promote the social well-being by contributing insights into how humans can achieve health and longevity.

We are located at the RIKEN Center for Computing and Communication near Tokyo and the RIKEN Center for Developmental Biology in Kobe.

Recent Publications

Hayashi T, et al. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. *Nat Commun* (in press)

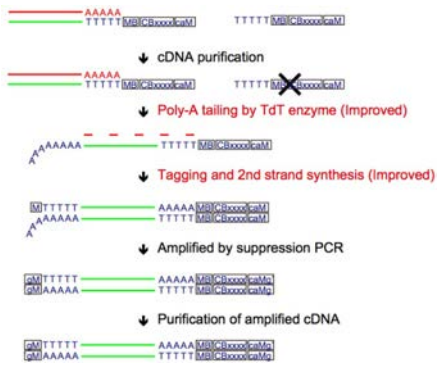
Matsumoto H, et al. SCODE: An efficient regulatory network inference algorithm from single-cell RNA-Seq during differentiation. *Bioinformatics* 33(15). 2314–2321 (2017)

Tsuyuzaki K, et al. MeSH ORA framework: R/Bioconductor packages to support MeSH over-representation analysis. *BMC Bioinformatics* 16. 45 (2015)

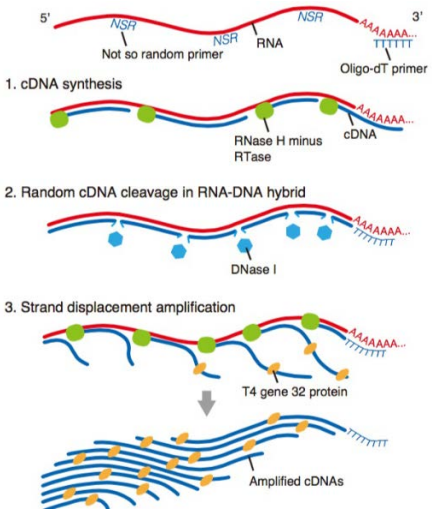
Sasagawa Y, et al. Quartz-Seq: a highly reproducible and sensitive single-cell RNA sequencing method, reveals non-genetic gene-expression heterogeneity. *Genome Biol* 14. 3097 (2013)

Adachi K, et al. Context-dependent wiring of Sox2 regulatory networks for self-renewal of embryonic and trophoblast stem cells. *Mol Cell* 52. 380–392 (2013)

Yoshimoto N, et al. An automated system for high-throughput single cell-based breeding. *Sci Rep* 3. 1191 (2013)



Quartz-Seq2: a high throughput single-cell RNA-sequencing method



Overview of RT-RamDA and single-cell RamDA-seq for detection of full-length total RNAs

Growth Control Signaling

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.

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

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Recent Publications

Yasugi T, et al. Adaptation to dietary conditions by trehalose metabolism in *Drosophila*. *Sci Rep* 7. 1619 (2017)

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. 1244–1255 (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. 2406–2411 (2012)

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)

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.

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

The establishment of a network of blood vessels is essential for the development of many tissues and organs. Tissue vascularization frequently occurs through angiogenesis, where new blood vessels arise from pre-existing ones. Angiogenesis encompasses a multitude of cellular processes including collective cell migration, cell elongation, proliferation, anastomosis and lumen formation. While many key molecules and signaling pathways have been identified to regulate 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 and behavior that lead to the final pattern of the vasculature.

My laboratory aims to unravel fundamental mechanisms that regulate endothelial cell behavior and dynamics using the zebrafish as a model system, since it is highly suited for high resolution live imaging, advanced fluorescent microscopy techniques, genetics, cell biology and chemical biology. 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-cell junctions stabilizes nascent lumens to produce a functional vascular network. In the future, we aim to investigate mechanisms regulating i) endothelial cell shape changes that are necessary for vessel morphogenesis, ii) *de novo* lumen formation and iii) blood vessel integrity. Our long-term goal is to understand how upstream angiogenic signals and hemodynamic forces regulate endothelial cell cytoskeleton to dictate cell behavior and shape and/or maintain blood vessel architecture.

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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 signaling to control vessel density in angiogenesis. *Dev Cell* 16. 70–82 (2009)

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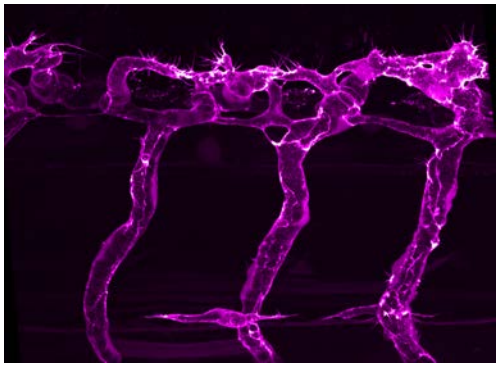
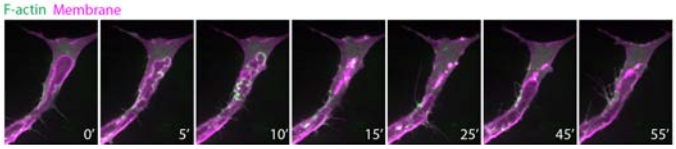
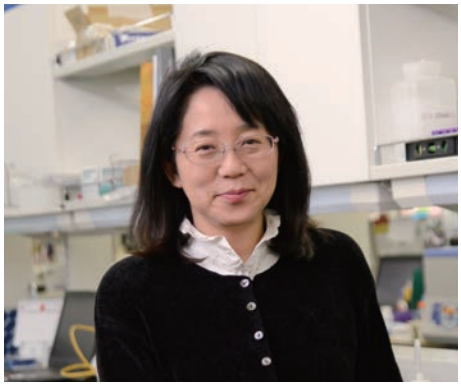


Endothelial actin cytoskeleton in zebrafish ISVs (intersegmental vessels) and developing DLAV (dorsal longitudinal anastomotic vessels).



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



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.

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

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Recent Publications

Mandai M, et al. Autologous induced stem-cell-derived retinal cells for macular degeneration. *New Engl J Med* 376.1038–1046 (2017)

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)

Shirai H, et al. Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc Natl Acad Sci U S A* 113(1). E81–90 (2016)

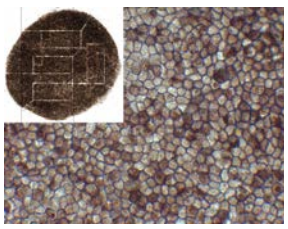
Kamao H, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Reports* 2. 205–218 (2014)

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

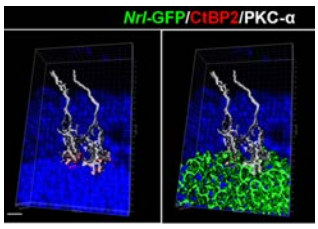
The retina has been called the “approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that in adult 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 adult retina may retain the ability to regenerate neurons and even to reconstitute the neural network. 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.

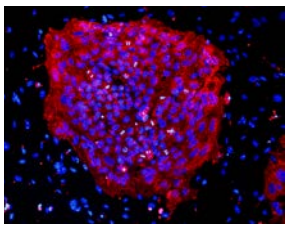
Therapeutic applications cannot be developed from basic research alone; the clinical approach—a thorough understanding of the medical condition to be treated is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily 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.



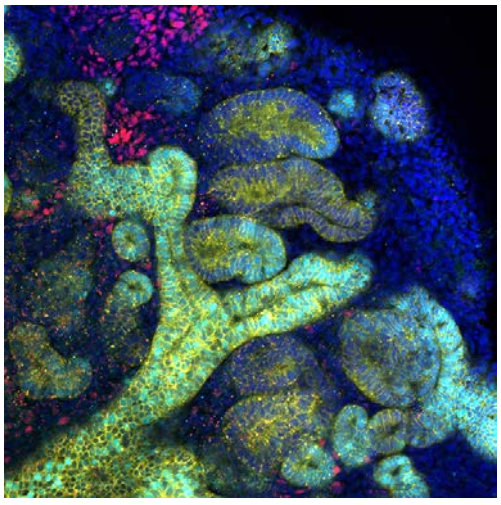
Human iPSCs (immunostained red, SSEA-4; blue, DAPI)

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 BSc. 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.

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

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).

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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)

Cell Adhesion and Tissue Patterning

Team Leader

Masatoshi TAKEICHI Ph.D.

Masatoshi Takeichi is Team Leader of the Cell Adhesion and Tissue Patterning research group. 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.

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

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Recent Publications

Ito S, et al. Induced cortical tension restores functional junctions in adhesion-defective carcinoma cells. *Nat Commun* 8. 1834 (2017)

Vassilev V, Platek A, Hiver S, Enomoto H, Takeichi M. Catenins Steer Cell Migration via Stabilization of Front-Rear Polarity. *Dev Cell* 43. 463–479 (2017)

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. 1601–1606 (2014)

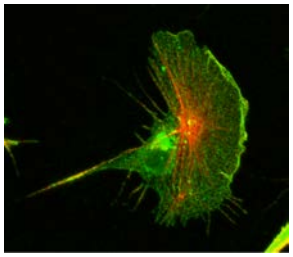
Hayashi S, et al. Protocadherin-17 mediates collective axon extension by recruiting actin regulator complexes to interaxonal contacts. *Dev Cell* 30. 673–687 (2014)

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 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:

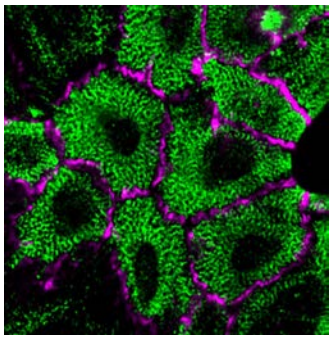
1) Cell-cell adhesion is a dynamic process, and this feature of cell adhesion is implicated in various kinds of cell behavior including tissue morphogenesis and cancer invasion. A growing body of evidence suggests that cadherins cooperate with cytoskeletal and/or motility machineries, such as actin regulators, non-muscle myosins, and Rho GTPases, via the catenins that bind the cadherin cytoplasmic domain. We are therefore studying the molecular mechanisms underlying the crosstalk between catenins and such cytoskeletal systems, and their roles in epithelial junction formation as well as in its disruption.

2) Recent studies from our laboratory have shown that the catenin-dependent regulation of cytoskeletal proteins also regulates the front-rear polarity of migrating cells, independently of cadherin or cell adhesion. We are continuing this study, and will reveal the roles of the catenin-dependent regulation of cell migration in morphogenetic or pathogenic cell behavior.

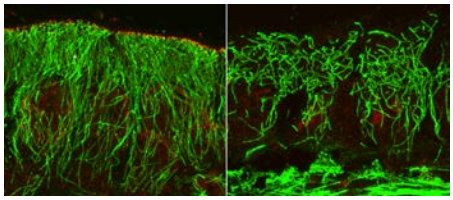
3) In addition, we have been analyzing the functions of microtubule minus end-associated proteins, Nezha/CAMSAPs. These proteins regulate microtubule assembly and dynamics, as well as intracellular architecture. We are exploring the roles of these molecules in cellular morphogenesis, such as polarized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



An isolated enteric neural crest cell, immunostained for αE-catenin (green) and F-actin (red). In migrating cells, αE-catenin accumulates in lamellipodium and then moves to perinuclear regions, to regulate actomyosin assembly.



Colon carcinoma-derived HT29 cells whose intercellular adhesion has been reestablished by nocodazole treatment. Myosin-IIA (green) organizes into a striped network, and its contraction supports E-cadherin (magenta)-mediated junction formation.



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.

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. Throughout his academic career, he has 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 reorganization of the Center in November 2014. He also holds appointments as Project Leader in Drug Discovery and Medical Technology Platforms and Deputy Team Leader in the RIKEN Innovation Center.

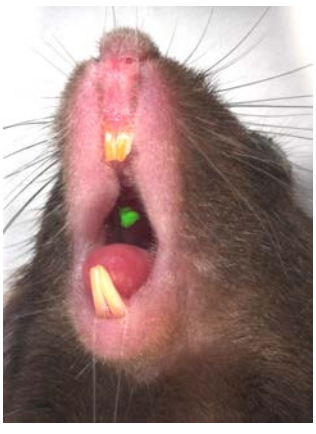
http://www.cdb.riken.jp/en/research/laboratory/tsuji.html

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. Building on these fundamental studies, we are now working to develop technologies for uses in therapeutic organ regeneration such as next-generation tooth regeneration implants and hair follicle regeneration for alopecia.

Mouse iPS cell-derived hair

Bioengineered hair follicle



Bioengineered tooth

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Sayaka NAKAMURA

Recent Publications

Bin B-H, et al. Requirement of zinc transporter ZIP10 for epidermal development: Implication of the ZIP10-p63 axis in epithelial homeostasis. *Proc Natl Acad Sci U S A* 114(46). 12243-12248 (2017)

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

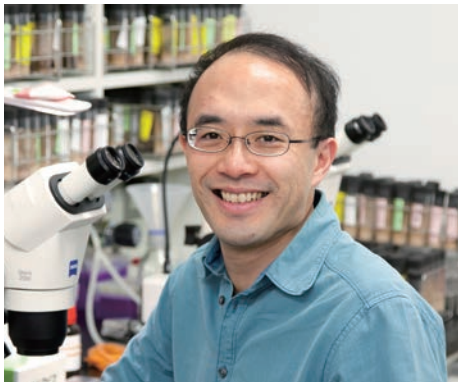
Ozone C, et al. Functional anterior pituitary generated in self-organizing culture of human embryonic stem cells. *Nat Commun* 7. 10351 (2016)

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

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. 13475–13480 (2009)

Epithelial Morphogenesis



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.

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

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Rachel STEWART

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International Program Associate
Anthony ERITANO

Assistant
Yuko FUJIYAMA

Recent Publications

Takeda M, et al. A homeostatic apical microtubule network shortens cells for epithelial folding via a basal polarity shift. *Nature Cell Biology* 20. 36–45. (2018)

Wen F-L, et al. Epithelial folding driven by apical or basal-lateral modulation: geometric features, mechanical inference, and boundary effects. *Biophys J* 112. 2683–2695 (2017)

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

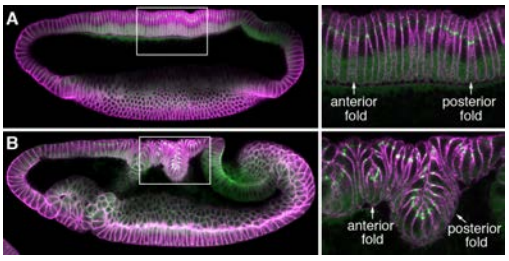
Wang Y C, et al. Differential positioning of adherens junctions is associated with initiation of epithelial folding. *Nature* 484. 390–393 (2012)

Wang Y C and Ferguson E L Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. *Nature* 434. 229–234 (2005)

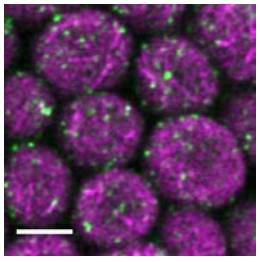
The central question in developmental biology is how cells, tissues and organs acquire their specific functions and shapes. A large body of work over the past several decades has yielded a broad understanding of how functional specialization is achieved through differential gene expression. In contrast, 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 recent work identified a novel microtubule based mechanical mechanism that is crucial for maintaining homogeneities of cell sizes and shapes prior to morphogenesis, and yet becomes coupled to the changing cell polarity, thus repurposed for cell shortening that induces folding of the epithelial tissue. The polarity-based, microtubule-dependent mechanism contrasts with the canonical myosin-dependent apical constriction. Our ongoing work promises to delineate a novel mechanical force balance/imbalance process that underlies non-myosin based epithelial folding.

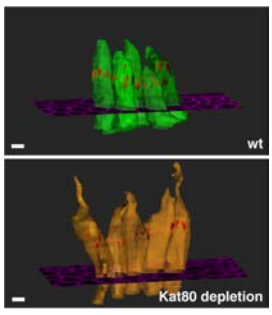
We employ an integrated approach that combines genetic manipulation, quantitative live imaging and computational mechanical modeling. We are also in the process of developing optogenetic and cell-type specific RNA-seq methodologies that could be used to manipulate and identify factors and parameters that control cell shape change and tissue deformation. Furthermore, our lab is engaged in international, multidisciplinary collaborations with scientists that specialize in evolutionary biology, computational mechanics, and theoretical physics to seek to better understand epithelial morphogenesis from a variety of different angles.



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.



The *Drosophila* CAMSAP protein, Patronin (green), decorates a non-centrosomal microtubule network at the apical surface of embryonic cells.



3D reconstruction of epithelial cells undergoing dome descent during dorsal fold initiation (top). This process is perturbed in embryos in which microtubule severing protein Katanin is depleted (bottom).

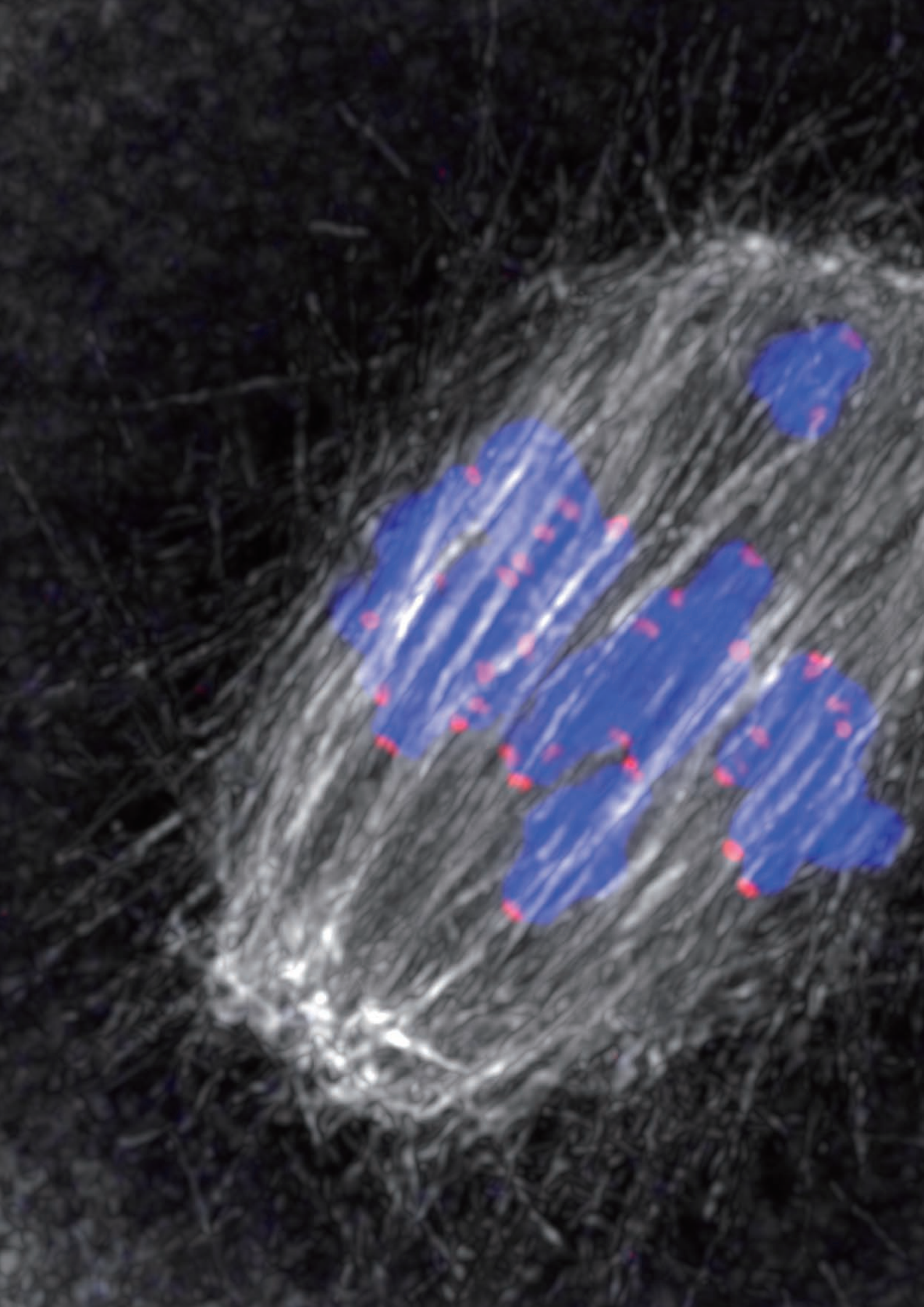
RIKEN CDB-Otsuka Pharmaceutical Collaboration Center

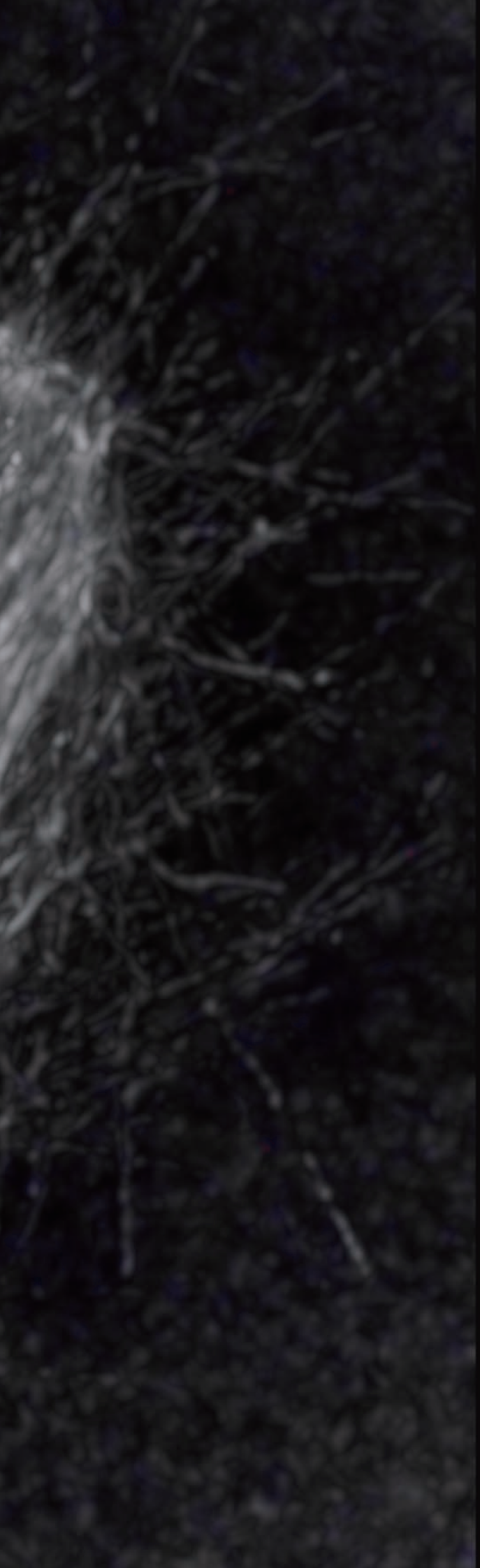
Director
Hiroshi HAMADA M.D., Ph.D.

The RIKEN CDB-Otsuka Pharmaceutical Collaboration Center (RIKEN COCC) was launched in September 2016 under one of RIKEN's Baton Zone program to promote RIKEN-industry collaboration. RIKEN COCC merges CDB's strength in creative and innovative research in elucidating mechanisms involved in development and regeneration and Otsuka Pharmaceutical's forte in research and development of medicines and health-related products, and aims to uncover innovative seeds that can be translated into practical applications, such as treatments for diseases. Presently, they are focusing on areas related to neurodegenerative diseases and kidney diseases.

The RIKEN COCC also aims to foster human resources that will lead the next generation.

RIKEN COCC Organizational Committee





Activities

Spindles, chromosomes (blue) and kinetochores (pink) in a mouse oocyte
Image: Laboratory for Chromosome Segregation

2017 CDB Symposium

Towards Understanding Human Development, Heredity, and Evolution

March 27-29, 2017

The RIKEN Center for Developmental Biology held its fifteenth annual symposium entitled, "Towards Understanding Human Development, Heredity, and Evolution," from March 27 to 29. Approximately 170 students and scientists from around the world convened at the CDB to take part in scientific exchanges on a wide range of topics related to human development, human genetics as well as evolution.

Scientists have long been trying to unveil the processes involved in human development, and the larger question of how humans evolved. While extensive research using a variety of model organisms has revealed much about universal developmental processes common to all species, there remains a gap in our understanding of processes specific to the human lineage including close primate relatives, due to experimental and ethical limitations. However, recent advances in technologies and experimental approaches, such as live-imaging, next-generation sequencing, genome editing, and stem cell culturing, have both facilitated and spurred research in human development and genetics. This year's symposium brought together leading scientists working at the forefront in these fields to try and piece together our current knowledge as well as unearth potential new avenues of research. A total of 30 talks and 56 posters were presented at the three-day meeting on a broad spectrum of topics including germline and early embryonic development, epigenetics, organogenesis from human pluripotent stem cells and other stem cells and their applications for disease modeling, and human genetics and evolution.

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 related fields, and is generally held every year in March.

Session 1 Germline Development

Azim Surani
(Wellcome Trust Cancer Center Research UK Gurdon Institute, University of Cambridge, UK)
Patrick F Chinnery (University of Cambridge, UK)
Deborah Bourc'his (Institut Curie, France)
Tomoya Kitajima (RIKEN Center for Developmental Biology, Japan)
Mitinori Saitou (Kyoto University, JST ERATO, Japan)

Session 2 Early Embryogenesis & Epigenetics

Rickard Sandberg (Karolinska Institutet, Sweden)
Austin Smith
(Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, UK)
Edith Heard (Institut Curie, France)
Jafar Sharif (RIKEN Center for Integrative Medical Sciences, Japan)
Anne Ferguson-Smith (University of Cambridge, UK)
Michael Snyder (Stanford University, USA)

Session 3 Organogenesis

Rudolf Jaenisch
(MIT, Whitehead Institute for Biomedical Research and Department of Biology, USA)
Mototsugu Eiraku (RIKEN Center for Developmental Biology, Japan)
Arnold Kriegstein (University of California, San Francisco, USA)
Ernesto Lujan (Harvard Medical School, USA)
Takashi Tsuji (RIKEN Center for Developmental Biology, Japan)






Zev J Gartner (University of California, San Francisco, USA)

Ryuichi Nishinakamura
(Institute of Molecular Embryology and Genetics, Kumamoto University, Japan)

Yoshiya Kawaguchi
(Center for iPS Cell Research and Application, Kyoto University, Japan)

Cantas Alev
(Center for iPS Cell Research and Application, Kyoto University, Japan)

Session 4 Human Genetics and Evolution

Guillaume Bourque (McGill University, Canada)

Parthiv Haldipur
(Seattle Children's Research Institute, Center for Integrative Brain Research, USA)

Agnar Helgason (deCODE Genetics, University of Iceland, Iceland)

Molly Przeworski (Columbia University, USA)

Ikuo K. Suzuki
(Institute of Interdisciplinary Research and ULB Neuroscience Institute, University of Brussels, Belgium)

Pavel Prosselkov (RIKEN Brain Science Institute, Japan)

Lluis Quintana-Murci (Institut Pasteur, France)

Session 5 Origins of Humans

Sayaka Tojima (Kyoto University, Japan)

Gen Suwa (The University Museum, The University of Tokyo, Japan)

Rasmus Nielsen (University of California, Berkeley, USA)

2018 CDB Symposium

Dynamic Homeostasis: From Development to Aging

March 26–28, 2018

The sixteenth annual symposium, "Dynamic Homeostasis: From Development to Aging," will be held on March 26–28, 2018, at the RIKEN Center for Developmental Biology.

Tissue homeostasis is a dynamically regulated process. Cells and tissues exhibit a high degree of adaptability during maintenance, maturation and renewal, and respond to a wide range of environmental changes. They also have intrinsic and extrinsic systems for damage control and repair. Stem cell systems, systemic modulation within and between tissues, and epigenetic controls may all underlie the robustness and plasticity of tissues. Defects in such homeostatic systems lead to developmental disorders, while aging is thought to involve the gradual deterioration of their function. The symposium will cover a wide range of topics on dynamic homeostasis and cell/tissue integrity from the cellular to organismal level, establish new perspectives, and look to the next frontiers of the field.

Invited Speakers

Long Cai
(California Institute of Technology, USA)

Stuart Forbes
(University of Edinburgh, UK)

Hironobu Fujiwara
(RIKEN Center for Developmental Biology, Japan)

Alex P. Gould
(The Francis Crick Institute, UK)

Ichiro Hiratani
(RIKEN Center for Developmental Biology, Japan)

Dirk Hockemeyer
(University of California, Berkeley, USA)

Tatsushi Igaki
(Kyoto University, Japan)

Shingo Kajimura
(University of California, San Francisco, USA)

Mark Krasnow
(Stanford University and Howard Hughes Medical Institute, USA)

Aaron McKenna
(University of Washington, USA)

Takashi Nagano
(Babraham Institute, UK and Osaka University, Japan)

Satchidananda Panda
(Salk Institute for Biological Studies, USA)

Emmanuelle Passegué
(Columbia University, USA)

Thomas Rando
(Stanford University, USA)

Wolf Reik
(Babraham Institute, UK)

Hans-Reimer Rodewald
(Deutsches Krebsforschungszentrum, Germany)

Toshiro Sato
(Keio University, Japan)

Akiko Satoh
(National Center for Geriatrics and Gerontology, Japan)

Ben Simons
(University of Cambridge, UK)

Shahragim Tajbakhsh
(Institut Pasteur, France)

Fiona M. Watt
(King's College London, UK)

Sa Kan Yoo
(RIKEN, Japan)

Shosei Yoshida
(National Institute for Basic Biology, Japan)

Seminars

The RIKEN CDB makes special efforts to provide a full and diverse series of invited seminars given by scientists from around the world. To date, the Center has hosted over 800 such talks, in addition to organizing 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 2017.

Date	Title	Speaker
01-30	Transcription factories: genome organization and gene regulation	Peter R. COOK
01-30	Biology of cell destruction: active roles of cell death in development and organismal homeostasis	Masayuki MIURA
01-31	Cortex-induced spindle asymmetry drives biased chromosome segregation in female meiosis	Takashi AKERA
02-10	Adaptive multicellular behaviors as mechanoresponse for radial size maintenance of epithelial tube	Tsuyoshi HIRASHIMA
02-15	Elucidating the functions of an autism-related gene in nervous system development	Igor KONDRYCHYN
02-21	Systems biology by light and electron microscopy—from protein complexes via cellular protein networks to embryonic development	Jan ELLENBERG
02-24	Coupling cell cycle to cell fate: Cell-cycle regulation of Wnt signalling by the APC/C-Nek2 axis	Yuu KIMATA
03-06	Epithelial plasticity in health and disease	Angela NIETO
03-06	Searching for the molecular basis of human-specific traits and disease	Vahan B. INDJEIAN
03-13	Drug screening on the fly: Resistance is futile	Michele MARKSTEIN
03-13	The Hippo pathway in tissue homeostasis	Toshiro MOROISHI
03-21	Oxygen metabolism and cardiac regeneration	Wataru KIMURA
03-23	Mechanosensor channel Piezos and their physiological roles—touch, proprioception and breathing	Keiko NONOMURA
03-24	Dissecting olfactory neural circuits by viral and genetic technology in mice	Kazunari MIYAMICHI
03-30	X-chromosome structure meets function during X inactivation	Edith HEARD
04-04	Do epigenetic changes cause aging in mammals?	Motoshi HAYANO
04-14	Gene ontology: biases, pitfalls, remedies	Christophe DESSIMOZ
04-18	Three-dimensional heart tissues generated from human iPS cells for regenerative therapy and disease modeling	Hidetoshi MASUMOTO
04-18	Challenges to understanding the molecular mechanisms behind hematopoietic stem cell physiology and establishing tissue stem cell-based regenerative medicine	Masanori MIYANISHI
04-20	R(e)spondin-2 WNT signaling for limb & lung development	Bruno REVERSADE
05-15	*Drosophila* genetics for human biology and medicine	Shinya YAMAMOTO
05-15	Junctional tension fluctuation and accompanying dynamics of adherens junction components during cell–cell boundary deformations in epithelia	Yusuke HARA

Date	Title	Speaker
05-26	The activation of canonical Wnt signaling in the spermatogonial stem cell pool	Hinako M TAKASE
06-05	Development of novel single-cell RNA sequencing technologies	Itoshi NIKAIDO
06-09	Why do kidneys fail to repair?	Motoko YANAGITA
06-16	Genetic mechanisms underlying fin evolution	Tetsuya NAKAMURA
07-20	Light and dark sides of aPKC	Masanori NAKAYAMA
07-28	Genetically encoded light-sensitive amino acids uncover allosteric regulation in neuronal receptors	Shixin YE-LEHMANN
08-10	Computational modeling of cellular fate and wound healing: probability landscape of stochastic networks, chromosome folding, and large-scale migration of proliferating cells	Jie LIANG
08-22	Transcriptional contol of human embryo genome activation	Juha KERE
09-08	The actin-bundling protein plastin increases cortical connectivity to promote robust polarization and timely cytokinesis in early *C. elegans* embryogenesis	Wei Yung DING
09-12	Physical bases of 3D genome organization	Masaki SASAI
09-13	Developmental role and mechanistic insight into chromatin opening by pioneer factor FoxA	Makiko IWAFUCHI-DOI
09-22	Modeling stratified epithelium: cell lineage and linearized hydrodynamics	Hsuan-Yi CHEN
10-11	Pre-oral gut, and ancient pharyngeal origins of craniofacial patterning	Robert CERNY
10-13	Comprehensive understanding of how chromosomes regulate mitosis	Hideki YOKOYAMA
10-18	Cellular control of time, size and shape during development and evolution	Richard A. SCHNEIDER
11-09	Redox signalling in development and regeneration	Sophie VRIZ
11-13	Age-related stem cell dysfunction: causes and consequences	Heinrich JASPER
11-21	Cell-based screen for altered nuclear phenotypes to reveal the nuclear events regulating cellular senescence	Mahito SADAIE
12-06	The intricate world of inter-organ communication	Norbert PERRIMON
12-07	Organ development and genetic compensation	Didier STAINIER
12-11	New mechanisms of lymphatic vascular development from zebrafish studies	Ben HOGAN
12-18	Correlative light-electron microscopy reveals how nuclear pore assembles at nano-meter resolution	Shotaro OTSUKA
12-18	TRAIL-ing the ECM for cell motility in organ morphogenesis	Dong-Yuan CHEN

2017 Educational Programs

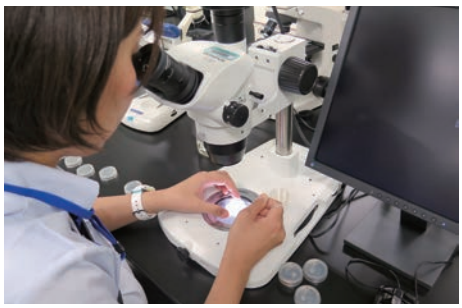
As part of its commitment to promoting awareness and understanding of development, regeneration, evolution, stem cells, regenerative medicine and related fields, and its interests in maintaining close ties with the academic and research communities, the RIKEN CDB dedicates itself to conducting training 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 established partnerships with a number of graduate schools in the Kansai region (Hyogo, Kyoto, Nara, Osaka) and organizes a two-day lecture program every year for graduate students enrolled in these partnering institutions. This year's lecture program was held August 2 to 3, and was attended by approximately 250 students. The students listened to talks by selected laboratory heads and research scientists on their research and also had the chance to visit a laboratory of interest. Most graduate students attending the lecture program can also receive academic credits towards their degree program.

High school teachers' workshop

A practical workshop for high school biology teachers was held at the CDB on August 7 to 8. 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, and for the first time, RIKEN Quantitative Biology Center (QBiC). Twenty-two biology teachers, mainly from around the Kansai region, took part in the two-day workshop. This year's program focused on genetics research using *C. elegans*, and was supervised and taught by Shuichi Onami, team leader of QBiC's Laboratory for Developmental Dynamics. The workshop program was designed to allow the participants to learn and experience several classical experiments using *C. elegans*. The workshop kicked off with a lecture by Onami, who introduced *C. elegans* and their uses in biology, including the significant discoveries made using this organism. A special lecture by Professor Atsushi Kuhara of Konan University was also included in the program on the second day, who shared his group's recent findings on thermotaxis in *C. elegans* and introduced simple taxis-related experiments that can be done in a high school classroom setting. 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 held in November.



Summer school for high school students

The eleventh annual one-day summer school program for high school students was held on August 18, 22 and 25. This program features a talk by a CDB scientist, a tour inside a working laboratory, and a hands-on scientific experiment. This year, the summer school program was organized and run jointly with the RIKEN Center for Life Science Technologies (CLST), and focused on the theme of genes and their functions. The students analyzed the DNA extracted from their own cheek epithelial cells to determine whether or not they carry a single nucleotide polymorphism in the gene encoding an enzyme involved in metabolizing alcohol. Team Leader Takashi Nishimura of the Laboratory for Growth Control Signaling contributed to the program by giving a talk on his career path as well as his current research, and also showed the students different *Drosophila* species and mutant lines species and some research equipment used in his laboratory.

Internship for undergraduate students

Thirty undergraduate students from universities around Japan were invited to spend a week, from August 21 to 25, as interns in the participating laboratories on the CDB campus. The internship is a competitive program, and the selected students are given 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 the different labs on the CDB campus. The activity-filled week concluded with group presentations by interns from each laboratory, in which they presented the findings from their projects to the other participants. Awards for best presentations were also chosen by the lab heads of participating laboratories as well as by the participants themselves.

Developmental biology tutorial for high school students

RIKEN CDB and QBiC joined hands with the Hyogo Prefectural High School Educational Committee for Biology, and the JSDB to co-organize a one-day practical tutorial program for high school students on November 19. Teachers who participated in the workshop for high school teachers that was held in August served as teaching assistants for the program, with the aim of helping them to gain confidence introducing similar experiments into the classroom. RIKEN QBiC's Shuichi Onami, team leader of the Laboratory for Developmental Dynamics, served as advisor and guest lecturer of the program which centered on developmental genetics research using the nematode, *C. elegans*. A total of 21 students from six different high schools, and 15 teachers from 12 different schools within Hyogo Prefecture took part in the tutorial.

2017 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.

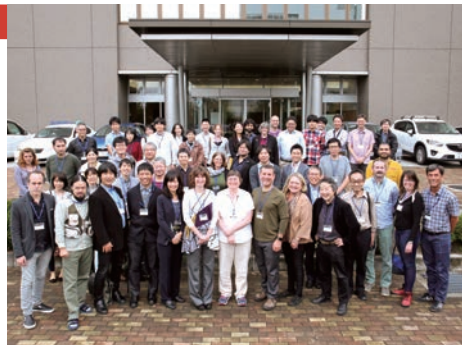
RIKEN Kansai Joint Retreat in Awaji

The CDB organizes a two-day retreat every year for the Center's research staff to encourage exchange and discussions on the latest research developments outside normal lab settings. This year's retreat took place on July 18 to 19 on Awaji Island, and for the first time, was held jointly with the RIKEN Center for Life Science Technologies (CLST) and the RIKEN Quantitative Biology Center (QBiC). A total of 381 research staff, including laboratory heads, research scientists, student trainees, and technical staff, from the three centers attended the retreat. The closed meeting facilitated discussions between research staff of the different Centers and across diverse scientific fields. The program included short introductory talks by the director of each center, a mix of talks given by PIs, younger scientists, and students from the three centers, and a poster session featuring over 200 posters.

Open House 2017

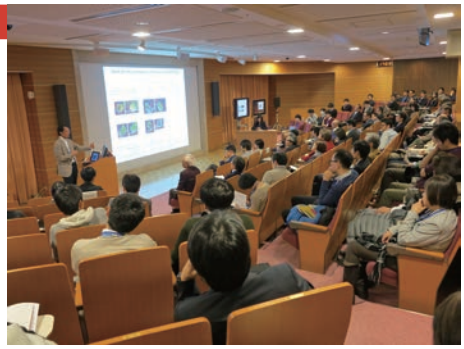
The CDB, along with other RIKEN research centers based in Kobe, held its annual Open House on October 14. Approximately 2,250 people of all ages visited the CDB campus to learn about the research being carried out at the Center. One of the most popular events was the Open Labs where visitors are permitted to enter different laboratories, see samples of cells and tissues under microscopes, and talk to scientists about their research. Other events included an exhibit introducing model organisms, a DNA experiment corner for visitors to try out techniques used by scientists for DNA analysis, and round-table talk sessions with scientists working at the CDB campus. There was also a book lounge showcasing books recommended by CDB scientists and a craft corner for kids to make button badges, straw rockets, and paper animal hats. The Open House is the largest annual event open to the public and allows the scientists to engage with the public face-to-face.

The 29th CDB Meeting "Mavericks, New Models in Developmental Biology"



The 29th CDB Meeting entitled, "Mavericks, New Models in Developmental Biology," was held on October 19 to 20. Over 80 participants from within Japan and abroad working with conventional model organisms and non-conventional model organisms, such as goldfish, dragonflies, pigeons, bats and marsupials, convened at the CDB for a lively two-day meeting. Recent advances in technology have spurred research using new models, which in turn has yielded surprising new insights into fundamental biological principles as well as evolution of different species. The attendees discussed similarities and differences across species, and also considered the impact of novel model species on the future of developmental biology.

Kobe Children's Hospital–CDB Joint Symposium



Following on the success of the inaugural joint symposium held in 2016, the second Kobe Children's Hospital–CDB Joint Symposium was held Saturday, December 16. A little over one hundred participants from both the CDB and Kobe Children's Hospital gathered in the Auditorium of the CDB for an afternoon of talks. The talks given at this Joint Symposium focused on early embryogenesis and abnormalities during these stages as well as development of the nervous system and neurodegenerative disorders. The Joint Symposium with the Kobe Children's Hospital was organized to encourage the research staff at the CDB to interact with clinicians, and for the clinicians at the Children's Hospital to learn about the latest developments in developmental biology.

2017 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
Satoru OKUDA	Special Postdoctoral Researcher	in vitro Histogenesis	Yamaguchi Medal	Asian-Pacific Association for Biomechanics
Satoru OKUDA	Special Postdoctoral Researcher	in vitro Histogenesis	JSME Young Engineers Award	The Japan Society of Mechanical Engineers
Masayo TAKAHASHI	Project Leader	Retinal Regeneration	International Member of the National Academy of Medicine (NAM)	National Academy of Medicine

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 is 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.

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 or outside the city.

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 historic 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.

Photo: Ryousuke Yano

RIKEN in Kobe

The RIKEN Center for Developmental Biology was the first research center established by RIKEN in the city of Kobe within the Kobe Biomedical Innovation Cluster, and 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 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 merger 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 in addition to the basic sciences, 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 and more recently the development of a supercomputer to succeed the K computer. AICS works 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.

Administrative support at RIKEN Kobe and CDB

Kobe Branch

- RIKEN Kobe Administrative Division
  - General Affairs
  - Human Resources
  - Accounting
- Information Networks Office - Kobe Branch
- RIKEN Kobe Safety Center
- Developmental Biology Planning Office

  The Planning Office coordinates funding and external review activities, as well as provides support for domestic and international communications and for organizing scientific meetings.
- CDB Library

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.

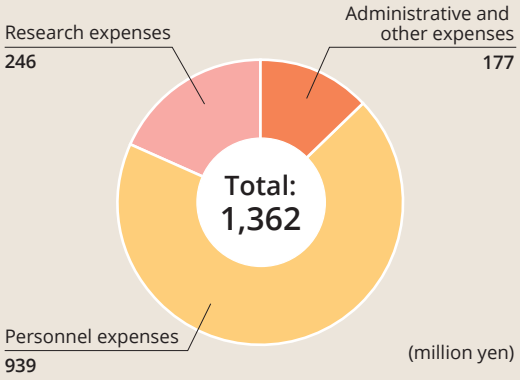
Kobe Administrative Services

Since the reform of the RIKEN organization in early 2013, the Kobe Administrative Division has been 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. There is an Information Networks Office that maintains both network access and multiple intranet services of the Kobe Branch. Within the CDB, the Developmental Biology Planning Office coordinates important activities including budget and funding management, and administrative support for the Advisory Council, laboratory performance reviews and contract renewals. The Library Office manages the CDB research literature collections and interlibrary loans.

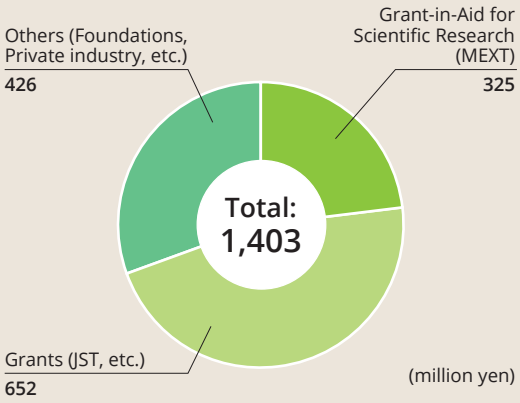
Kobe Safety Center

The Kobe Safety Center provides training and supervision for lab and workplace safety, implements and monitors disaster prevention measures, and educates staff on and ensures compliances with national and institutional regulations governing laboratory practice and technology. The office also handles all administrative affairs of the Institutional Review Board.

2017 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.



2017 CDB Staff

Laboratory heads	20
Deputy leaders	2
Research scientists	55
Research associates	3
Technical staff	63
Assistants	18
Visiting scientists	147
Student trainees	39
Part-time staff	18
Other	13
Total	378
Kobe Branch Administration	87

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 "Act on RIKEN," 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, reorganized in 2003 as an independent administrative institution under the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and then designated in 2015 as a National Research and Development Institute. RIKEN celebrated 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/en/

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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Nishina Center for Accelerator-Based Science
Advanced Center for Computing and Communication
Cluster for Industry Partnerships
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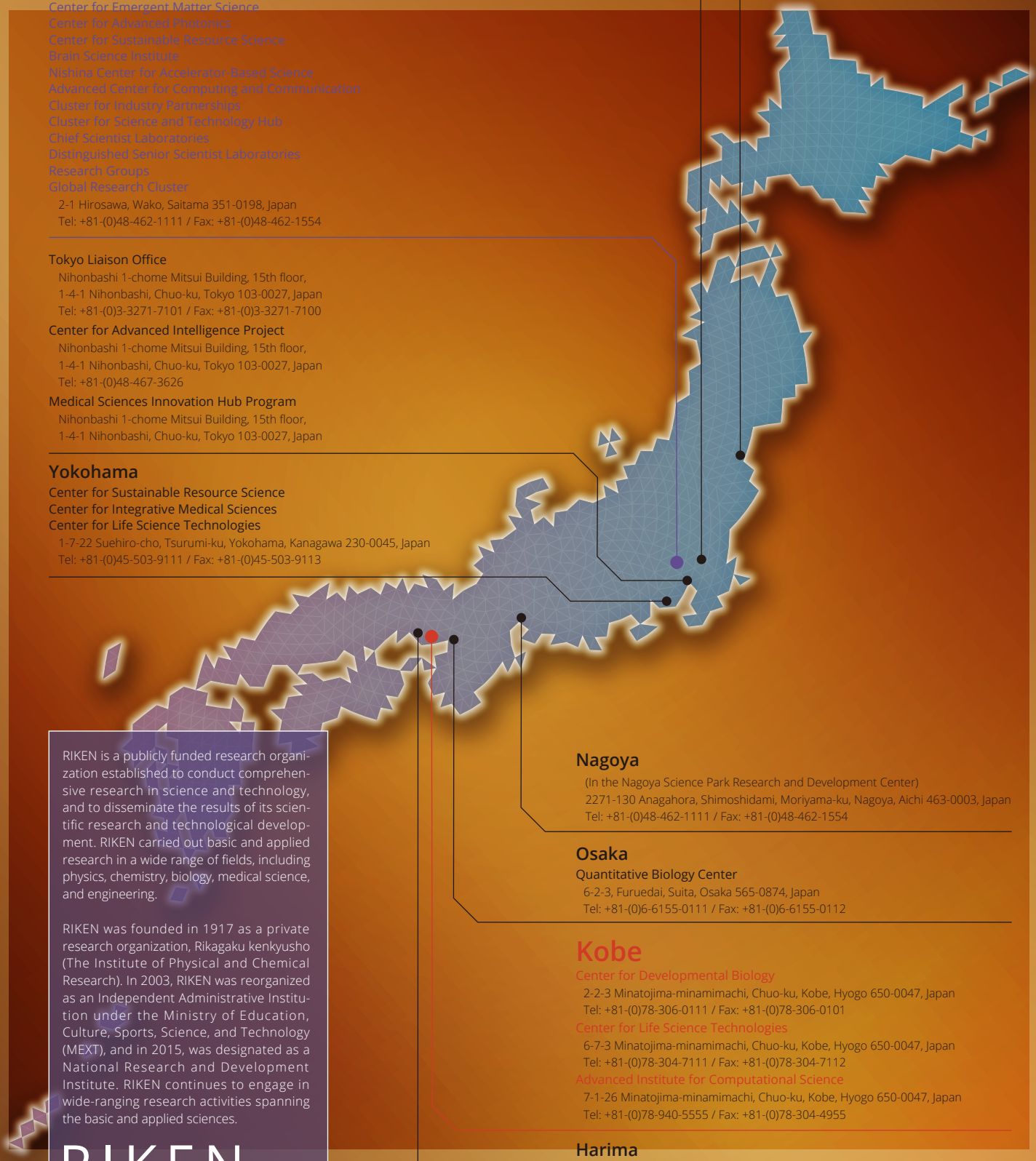

RIKEN is a publicly funded research organization established to conduct comprehensive research in science and technology, and to disseminate the results of its scientific research and technological development. RIKEN carried out basic and applied research in a wide range of fields, including physics,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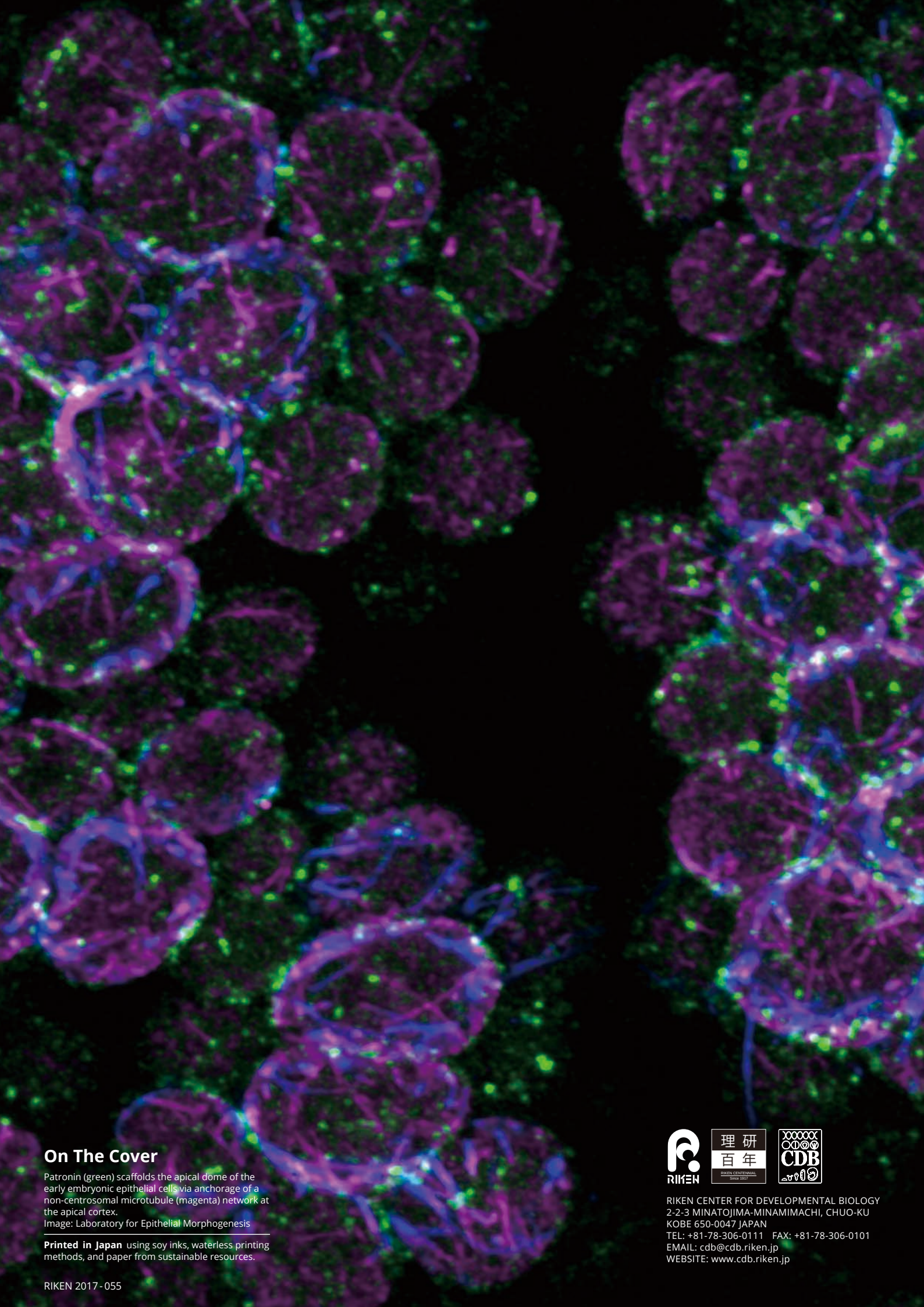

On The Cover

Patronin (green) scaffolds the apical dome of the early embryonic epithelial cells via anchorage of a non-centrosomal microtubule (magenta) network at the apical cortex.
Image: Laboratory for Epithelial Morphogenesis

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