RIKEN Center for Developmental Biology

2014 Annual Report

RIKEN
Center for
Developmental Biology

2014
Annual Report

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The RIKEN Center for Developmental Biology was launched in April 2000 under the auspices of the Millennium Project research initiative, which was established to drive research in areas of vital importance to both Japan and the world in the 21st century. The challenges of 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

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


CDB

Message from the founding director

It is now 12 years since the Center for Developmental Biology began its full-scale operations in 2002, more than a decade of exciting scientific achievements and truly enjoyable collegial interactions at the Center. In previous years, I have faced the pleasant task of reporting developments that were for the most part positive, sometimes extraordinarily so, as the CDB moved from strength to strength in its first dozen years. This year, however, my message to readers of the 2014 Annual Report is inescapably tinged with sadness and regret, even as it is tempered with hope for the future.

As is by now famous, the CDB played a central part in a research misconduct scandal that escalated into an international drama. The STAP publications, now retracted from the literature, have had an irrevocable impact on the CDB, on RIKEN, and on Japanese science. It has been a source of tremendous concern and distress that such an unforgivable breach of scientific integrity occurred during my appointment as CDB Director. I am pleased to report that the Center will appoint a new Director, Hiroshi Hamada of Osaka University, to take over this position in April 2014, and would like to extend my sincere thanks to Toshio Yanagida, the Director of the RIKEN Quantitative Biology Center (QBiC), for agreeing to serve as Acting CDB Director during the search process. I am confident that the CDB will enter a new era of success and achievement under Dr. Hamada's guidance, and I thank the international scientific community for the wonderful encouragement it has shown the CDB during an extraordinarily difficult period—my only hope that the same support is shown to the Center in its efforts moving forward.

All of us at the CDB experienced the STAP crisis on a personal level with the death of Yoshiki Sasai, a renowned scientist who had played a leadership role within the Center from the time of its establishment. It is no exaggeration to say that Dr. Sasai made fundamental contributions to the growth and success of the CDB as an internationally recognized research center, and his loss will be felt here and in the scientific community around the world for years to come.

Following an institute-wide restructuring, the CDB is now a very different organization than it has been in the past. The laboratories have been regrouped by scientific interest, and more robust systems for governance and oversight have been put in place. As part of this reorganization, many former CDB laboratories have been reassigned to other parts of RIKEN, notably the nearby Center for Life Science Technologies (CLST) and QBiC, which we embrace as an opportunity for the CDB to expand its interactions and collaborations with other RIKEN research centers.

Despite the deeply unfortunate STAP misconduct incident, I would be remiss if I did not highlight some of the many positive stories that came out of CDB labs in 2014. The Laboratory for Retinal Regeneration made headlines worldwide with its announcement that an ongoing pilot study of the use of induced pluripotent stem cell-derived cell sheets in patients with age-related macular degeneration had performed its first experimental procedure in collaboration with the Institute for Biomedical Research and Innovation. Other CDB labs continued to publish fundamental advances in such areas as tissue morphogenesis, stem cell differentiation, and new techniques for freezing and imaging biological samples.

In many ways it has been a difficult and dispiriting year, one for which we are only now beginning to achieve some clarity and closure. But I see a bright future ahead for the CDB as it enters what can only be described as a new beginning. Profound questions in the study of embryonic development remain to be answered, and with the Center's latest generation of talented scientists, ever more powerful experimental techniques, and reinvigorated sense of purpose, I look forward to what I am certain will be a time of rebirth, renewal, and revitalization for the new RIKEN Center for Developmental Biology.

Masatoshi Takeichi

RIKEN Center for Developmental Biology

RIKEN Kobe Branch

CDB Organization (Nov.2014~)

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 Kobe Research Promotion Division, which provided administrative services, and the institutional Safety Center. Following a restructuring in November 2014, the CDB has taken on a new organizational form resulting in major changes to laboratory designations and departmental affiliations. Under the new system, the great majority of laboratories are now designated as teams, and assigned to one of five programs, which are described in detail to the right. The CDB Director is assisted by a Deputy Director, and advised by the Advisory Council, while governance issues are discussed and decided by a newly formed Steering Committee.

- Center for Life Science Technologies
- Quantitative Biology Center
- HPCI Program for Computational Life Sciences
- Kobe Administrative Division
- Safety Center

Institutional Review Board

The RIKEN CDB's Institutional Review Board (IRB) includes representatives from local academic, research, medical and legal organizations, as well as CDB research leaders, and meets regularly to review and discuss investigations with potential ethical, legal, social or public health and safety implications prior to their implementation. The IRB is coordinated by the Kobe Institute Safety Center.

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.
- Growth Control Signaling
  Takashi NISHIMURA Ph.D.
- Chromosome Segregation
  Tomoya KITAJIMA Ph.D.
- Developmental Epigenetics
  Ichiro Hiratani Ph.D.

Organogenesis Research Program

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

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

Advisory Council

The CDB Advisory Council (DBAC) convenes regularly to review the Center's performance and make ecommendations on the direction of its programs and governance. The DBAC reports its findings to aid 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.

- Austin Smith
  University of Cambridge, UK
- Christopher Wylie
  Cincinnati Children's Hospital Medical Center, USA
- Margaret Buckingham
  Institut Pasteur, France
- Patrick Tam
  University of Sydney, Australia
- Stephen Cohen
  Institute of Molecular and Cell Biology - A*STAR, Singapore
- Haifan Lin
  Yale university, USA
- Toshio Suda
  Keio University, Japan
- Ryoichiro Kageyama
  Kyoto University, Japan
- Hiroshi Hamada
  Osaka University, Japan

Center Director Toshio Yanagida (Acting Director)

Senior Advisor

Office of the Director

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.

- Pluripotent Stem Cell Studies
  Hitoshi NIWA M.D., Ph.D.
- Organ Regeneration
  Takashi TSUJI Ph.D.
- Lung Development
  Mitsuru MORIMOTO Ph.D.
- Tissue Microenvironment
  Hironobu FUJIWARA Ph.D.
- Organogenesis and Neurogenesis
  Masatoshi TAKEICHI Ph.D.
- In Vitro Histogenesis
  Mototsugu EIRAKU 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.

- Histogenetic Dynamics
  Erina KURANAGA Ph.D.
- Axial Pattern Dynamics
  Hidehiko INOMATA Ph.D.

Research and Development Project

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

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

Senior Investigator Program

- Sensory Development
  Raj LADHER Ph.D.
- Early Embryogenesis
  Guojun SHENG Ph.D.

STAP misconduct incident – A look back

In 2014, a research unit in the RIKEN Center for Developmental Biology played a central role in one of the most prominent research misconduct scandals in recent history, which had far-reaching repercussions throughout Japanese society and across the international scientific community. It has been a time of unprecedented crisis and a cause for deep reflection within the Center, and this series of events ultimately resulted in sweeping changes to the CDB organization and governance, as part of RIKEN's broader efforts to ensure that such inexcusable lapses in research integrity and oversight do not take place again.

On January 29, 2014, two publications were published online in Nature by Haruko Obokata, a former Research Unit Leader at the CDB, and colleagues including former CDB Deputy Director and head of the Laboratory for Organogenesis and Neurogenesis Yoshiki Sasai; Teruhiko Wakayama, a former CDB Team Leader, was also author on both papers. The authors reported a novel phenomenon involving the conversion of adult differentiated cells to a form of pluripotency that in some ways resembled that seen in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), but with several key differences. These so-called STAP (for stimulus-triggered acquisition of pluripotency) cells purportedly showed a broader range of differentiation than either ES or iPS cells, and involved neither the destruction of human nor the use of transgenic techniques. The reports also described the generation of two secondary stem cell types from the STAP cells: STAP stem cells, which were described as functionally resembling ESCs, and FGF-induced stem cells, akin to the native trophoblast stem cells that give rise to extraembryonic lineages.

Following the publication of these apparently extraordinary results, the work was the subject of a great deal of attention by the media and the scientific community. The fanfare, however,

proved short-lived after an anonymous blogger in Japan posted concerns about apparent discrepancies and signs of manipulation in images and data in both publications. RIKEN responded by convening the first of several investing committees to look into the accusations, but the situation quickly escalated as a string of new allegations of impropriety emerged in rapid succession. Around the world, stem cell labs began reporting their inability to replicate the core STAP findings, and allegations of lax oversight and other systemic failures were leveled at both the Center and RIKEN in general.

The initial investigation found Obokata had engaged in research misconduct. RIKEN sought to determine whether the STAP findings were supportable by conducting extensive reviews of the underlying data and launching an effort to re-evaluate the STAP phenomenon, even as the authors worked to retract both papers out of concerns due, at a minimum, to the unacceptably careless and error-ridden presentation of results. Additionally, RIKEN convened an external investigation of the local and system-wide factors that enabled the increasingly dubious reports to be submitted and published in a top-tier journal, while the CDB commissioned an independent review of internal factors contributing to or enabling the misconduct.

By summer, both papers were retracted, indicating the authors' loss of confidence in their published work, and following a series of press conferences and media briefings, public interest in Japan had reached a fever pitch. A subsequent external investigative committee recommended rapid dismantling of the Center, prompting an outpouring of support for the CDB from the international developmental biology and stem cell research community. Tragically, former Deputy Director Yoshiki Sasai, a renowned stem cell scientist in his own right, took his own life on August 5.

Over the next several months, RIKEN developed a plan for the fundamental overhaul of the CDB organization as the internal effort to evaluate the central claims of the STAP publications continued. In November, a sweeping restructuring of the CDB was implemented, in which approximately half the Center's labs were reassigned to other parts of RIKEN, and the multi-tier organization of laboratories was regrouped and flattened.

In December, nearly one year from the start of a long, painful, and profoundly humbling chain of events, RIKEN made several announcements in the hopes of bringing clarity to the STAP debacle. First, it was announced that efforts to reevaluate the STAP findings led by both Hitoshi Niwa and Shinichi Aizawa and, independently, by Haruko Obokata, had failed to reproduce the reported phenomena; Obokata resigned RIKEN within days of this announcement. Second, a follow-up external investigation into the problems in the STAP publications uncovered new evidence of misconduct Dr. Obokata that was determined to be strongly suggestive of contamination of the STAP stem cell colonies with ES cells.

RIKEN remains committed to uncovering the specific acts behind this serious betrayal of the public trust and scientific integrity, and to remedying the harms caused by this deeply regrettable incident. The STAP misconduct incident has been an important and a bitter lesson to all of us at the CDB, and we can only promise to work harder than ever before to regain the trust of our peers and the public, and to renew our commitment to the highest standards of scientific conduct.

Remembering Dr. Yoshiki Sasai

On August 5, all of us at the CDB were shocked and deeply saddened to learn of the sudden, tragic death of Yoshiki Sasai. Dr. Sasai was one of the CDB's founding group directors, and an internationally regarded developmental and stem cell biologist. His laboratory was known for its innovative research into dorsoventral axis patterning in the embryo and the induced differentiation of pluripotent stem cells, particularly into neuronal lineages.

Dr. Sasai joined the RIKEN Center for Developmental Biology in 2000, leaving a professorship at Kyoto University, which he had attained at the age of only 36. In addition to his research efforts, Dr. Sasai played a central role in guiding the establishment of the CDB facilities and research infrastructure, as well as in helping to develop an international work environment.

His earliest work at the CDB focused on two complementary areas: gaining a better understanding of the molecular interactions underlying dorsoventral specification in the *Xenopus* embryo, and learning ways to steer the differentiation of mouse embryonic stem cells down specific neural lineages, such as dopaminergic or sensory neurons. This work produced a series of high-profile publications that made the Sasai laboratory a world leader in both fields.

A subsequent study on the problem of maintaining human embryonic stem cells in culture yielded an unexpected breakthrough when members of the lab discovered that inhibition of the Rho-associated protein kinase (ROCK) pathway resulted in markedly improved survival of ESCs in vitro. ROCK inhibition has gone on to become a standard technique for the culture of human ESCs around the world.

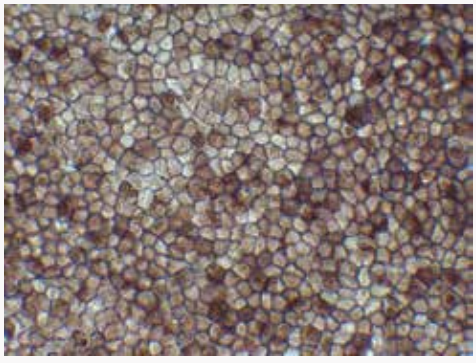
Perhaps the most noteworthy accomplishments, however, were yet to come. The following year, the Sasai lab made the first of its reports of the self-organized formation of three-dimensional neural tissue-like structures from ESCs in vitro. By minimizing external stimuli during ESC culture, the lab was able to show that mouse ESCs are able to spontaneously give rise to highly organized tissue closely resembling the embryonic cerebral cortex in vitro.

A string of related work followed soon thereafter, with demonstrations of similar activity giving rise to optic cup and pituitary (adeno-hypophysis) tissue from mouse ESCs, and recapitulations of the cortical and retinal self-organization phenomena using human ESCs as well.

Dr. Sasai's untimely death represents a loss that the international scientific community is still coming to terms with. For all of us at the CDB, he will be remembered as an enormously capable and highly driven colleague, mentor, and friend.

First patient undergoes novel iPSC-based procedure for age-related macular degeneration

In September 2014, a female patient suffering from exudative age-related macular degeneration (AMD) became the world' s first recipient of an experimental transplant of a cell sheet derived from induced pluripotent stem cell (iPSCs). The patient is part of a pilot study led by Masayo Takahashi (Project Leader, Laboratory for Retinal Regeneration) in collaboration with Yasuo Kurimoto (Head, Ophthalmology Department, Institute for Biomedical Research and Innovation Hospital), who performed the procedure. This study is designed to make a preliminary evaluation of the safety of the transplantation of iPSC-derived sheets of retinal pigment epithelium (RPE) cells. There will be a one-year intensive monitoring period to evaluate the transplant for adverse events and possible early signals of clinical benefit, after which the patient will undergo periodic follow-up for a total of three years post-transplant. The plan for the current pilot study is to enroll six volunteer participants in total. Additional information about the study is available at: http://www.riken-ibri.jp/AMD/english/index.html.

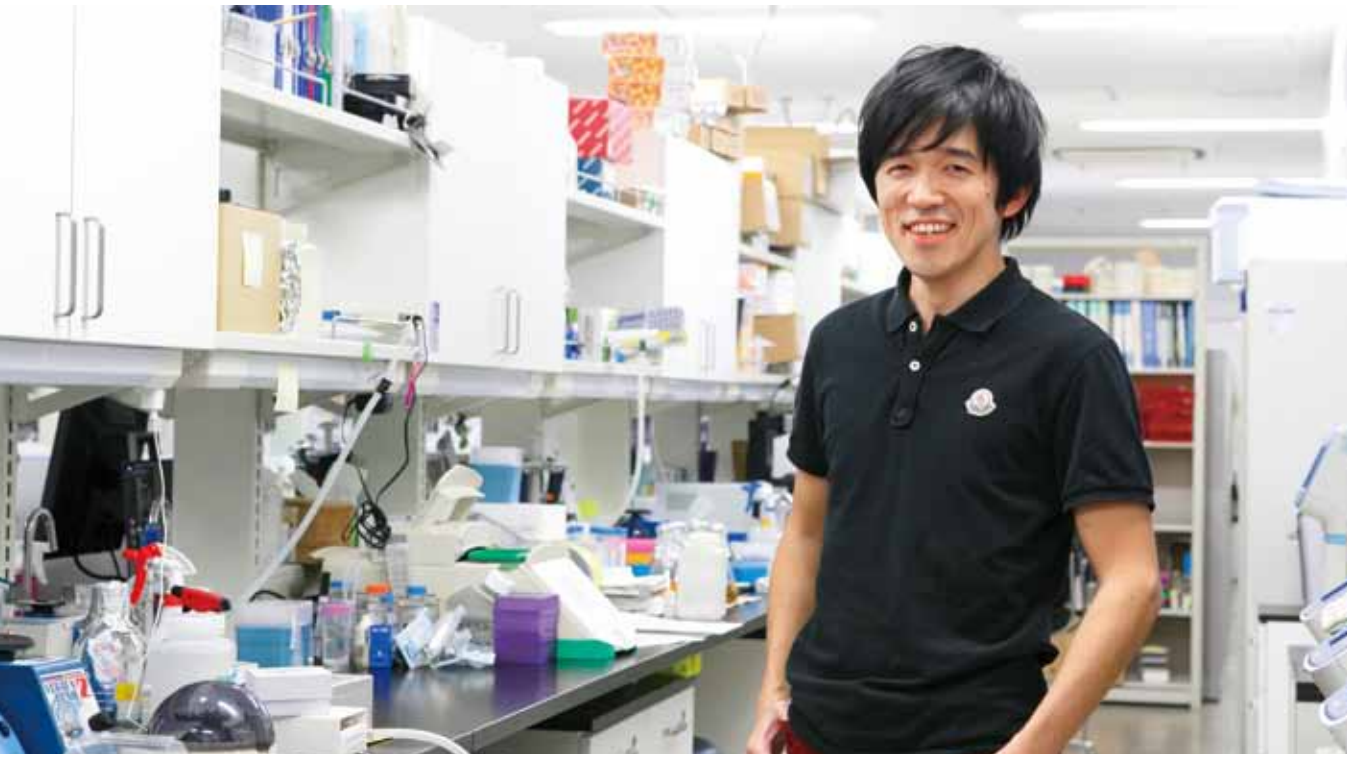


RPE cells generated from iPSCs

Restructuring of CDB in the aftermath of the STAP scandal

In response to recommendations from an external committee investing the STAP misconduct incident, RIKEN undertook a major reorganization of the Center for Developmental Biology. From November 2014, the CDB labs have been reorganized into four fundamental research programs: the Cellular Environment and Response Research Program, the Organogenesis Research Program, the Stem Cells and Organ Regeneration Research Program, the Mathematical Developmental Biology Program as well as a Research and Development Program, which will seek to develop new biomedical technologies based on CDB research results. Many laboratories were reassigned to other RIKEN Centers, including the Center for Life Science Technologies (CLST) and the Quantitative Biology Center (QBiC), which is expected to promote greater interaction between life science research centers in Kobe. The new CDB also features a flatter research organization, in which all laboratories share the same designation of Team, as distinct from the multi-tiered system employed from 2000 to 2014. A detailed overview of the post-reform organization of the CDB can be found on the CDB website, and on p.5 of this report.

Preclinical testing of iPSC-derived RPE shows efficacy and safety in animal models



Hiroyuki KAMAO

Age-related macular degeneration (AMD) is a disease of the eye that affects visual acuity in the elderly. Degeneration of the retinal pigment epithelium (RPE), a pigmented cell layer in the eye that supports and supplies nutrients to the photoreceptors, is a major pathogenic factor in AMD. Of the major forms of this disease, neovascular AMD is more prevalent in Asian populations. There is currently no cure for AMD, but several groups are exploring the possibility that pluripotent stem cells may be a useful source for generating functional RPE for use in transplantation. The efficacy and safety of such stem cell-derived tissue, however, needs to be tested extensively before studies in human can begin.

A new preclinical study by Hiroyuki Kamao and colleagues in the Laboratory for Retinal Regeneration reports that RPE cell sheets generated from induced pluripotent stem cells (iPSCs) show an excellent efficacy and safety profile in animal transplant models. Published in *Stem Cell Reports*, this work lays the groundwork for the world's first pilot clinical study on transplanting hiPSC-RPE cell sheets to AMD patients.

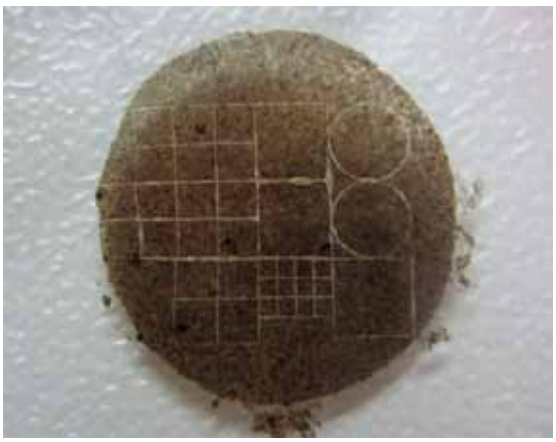
Kamao et al. produced RPE cell sheets that do not have an integrated artificial scaffold, but can nonetheless withstand the manipulations required for transplantation procedures. They were able to do so by growing the stem cell-derived RPE cells on a degradable temporary collagen scaffold. Several groups have previously reported using artificial scaffolds to generate RPE cell sheets; however, these scaffolds run the risk of causing inflammation or creating physical barriers that limit nutrient flow from choroid to photoreceptor cells. The group confirmed that, in vitro, the cell sheets expressed RPE markers and basement membrane components and had formed functional tight junctions exhibiting polarized secretion of growth factors similar to the activity of native RPE.

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 1–14 (2014)

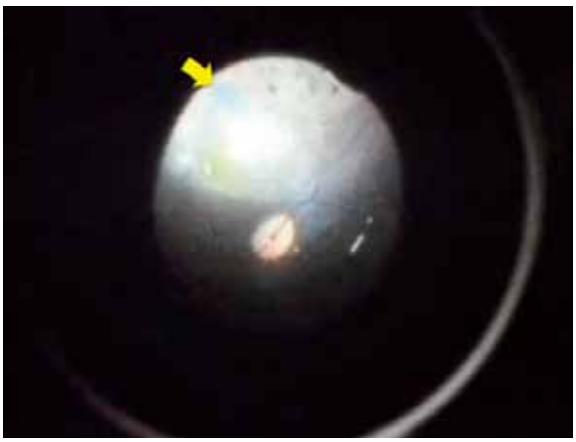
Kamao and colleagues next tested the function of these hiPSC-RPE cell sheets in vivo by transplanting them into the subretinal space in immunosuppressed three-week-old RCS rats, a model of inherited retinal degeneration. Using electroretinogram (ERG), they watched for changes in photoreceptor activity at six weeks post-transplant, and three weeks later measured the thickness of the outer nuclear layer (ONL), where the photoreceptors reside. Compared to control and sham-surgery animals, rats that received transplants of either hiPSC-RPE cell sheets or cell suspensions maintained the ONL and showed significant restoration of ERG responses, suggesting that the hiPSC-generated RPE cell sheets are functionally similar to native RPE.

To confirm the feasibility of using the iPSC-derived grafts in clinical studies, the group tested and compared the immunogenicity of autologous and allogeneic grafts in cynomolgus monkeys. To verify whether immunogenic responses are triggered by allogeneic or autologous grafts in vivo, Kamao transplanted cell sheets derived from a single donor monkey into the subretinal space of four non-immunosuppressed monkeys—the donor and three MHC-incompatible animals—and observed them for one year. The group found that tissues surrounding the allografts showed typical signs of graft rejection, while those surrounding the autograft showed no signs of rejection. The transplanted cells also did not contribute to tumor formation, which has been one of the major concerns for pluripotent stem cell-derived cell products. A final comparison between the transplantation of cell suspensions and cell sheets indicated that cell sheets offer several advantages; they can be transplanted directly at the lesion site and monitored easily, as they remain anchored in place.

"Safety is of the utmost importance, and these animal experiments show that autologous grafts are not rejected and non-tumorigenic. Tumor formation in the eye is relatively rare to begin with, and at least for AMD, transplantation does not require a large number of cells, so quality control is relatively simpler," says Takahashi. "We can also detect subtle changes in the eye, so in the event that any abnormalities develop, we can catch them at an early stage. All of these factors make AMD a good target for the world's first clinical study of iPS cells."

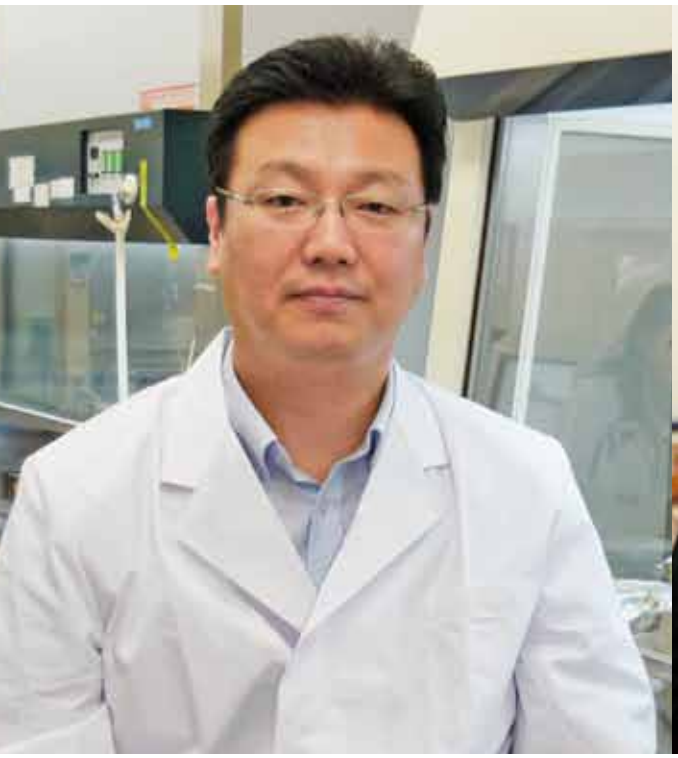


Grafts are cut from hiPSC-RPE cell sheet using a laser micro dissection system.



Monkey iPSC-RPE cell sheet (arrow) transplanted into subretinal space of a non-human primate.

New recipe for hPSC cryopreservation



Teruo AKUTA, Shin-Ichi NISHIKAWA

Human pluripotent stem cells (hPSCs) remain in the spotlight for the possibilities they hold out for uses in regenerative medicine. In the lab, hPSCs are famously difficult to handle, as they are extremely sensitive to external stresses such as those caused by experimental manipulations or changes in culture conditions, as these can lead to rapid changes in cell properties or cell death. One especially stressful technique is cryopreservation, which is characterized by low recovery rates of hPSCs after the thawing processes. Further technical developments to cryopreservation are needed to improve cell recovery rates, but any such method must also be simple, efficient, and inexpensive.

In a new study focused on hPSC preservation, Teruo Akuta, Keitaro Imaizumi, and colleagues in the Laboratory for Stem Cell Biology (Shinichi Nishikawa, Group Director; the Nishikawa lab closed in March 2013) have developed a new slow-freezing method that can be used to store large quantities of hPSCs easily and cost-effectively. Published in *PLoS One*, they took a commercially available medium used for the cryopreservation of cells from cord blood and bone marrow, and optimized it specifically for hPSCs. The group also determined the most effective dissociation solution to be used in combination with the modified medium. The study was carried out in collaboration with the laboratory of Shin Kawamata (Vice Director, Foundation for Biomedical and Innovation).

The two methods currently used for hPSC cryopreservation are vitrification and slow-freezing. Vitrification, which requires special skills, uses liquid nitrogen to quickly freeze the cells placed in a medium with high concentrations of cryoprotectants and is not suited for the cryopreservation of large quantities of cells. The slow-freezing method, where cells are suspended in cryopreservation media and then slowly allowed to freeze in a deep freezer overnight, is simpler and can be used to cryopreserve large quantities

Imaizumi K, et al. A simple and highly effective method for slow-freezing human pluripotent stem cells using dimethyl sulfoxide, hydroxyethyl starch and ethylene glycol. *PLOS ONE* 9. e88696 (2014)

of cells. The efficacy of any form of cryopreservation depends on the dissociation step carried out just prior to freezing, in which the colonies are broken up into small clusters, as the surface area of clusters bathed directly by cryoprotectant varies by the size of cell clusters following dissociation.

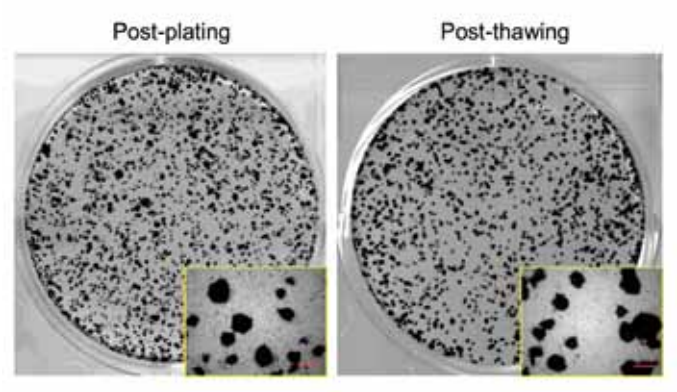
The group first examined five known dissociating solutions to determine which is most suited for the cryopreservation of hPSCs in combination with a popular commercially available freezing medium. Recovery rates of cells treated with different solutions were then examined post-cryopreservation by staining them with alkaline phosphatase (ALP). They found that treatment with Pronase/EDTA resulted in relatively small, uniform cell cluster sizes, and yielded the highest recovery frequency (44%). Trypsin/EDTA also generated small cell clusters, but the cell recovery frequency was less than half of that seen for Pronase/EDTA; other dissociation solutions tested produced large variations in cell cluster size and cell death after thawing.

Given its favorable properties, Imaizumi et.al. next looked to develop a hPSC-specific cryopreservation medium compatible with Pronase/EDTA. They prepared a panel of new cryopreservation formulas by changing the concentration of reagents in the freezing medium, and adding other known cryoprotectants. When they compared the efficacy of cell recovery after thawing, they found that the addition of ethylene glycol (EG) yielded the highest post-thaw recovery rates. They tweaked the formula further to identify the optimal concentration of reagents plus EG. The most efficient formula, CP-5E, consisted of a mix of 6% hydroxyethyl starch, a natural cryoprotectant derived from plants, 5% dimethyl sulfoxide and 5% EG.

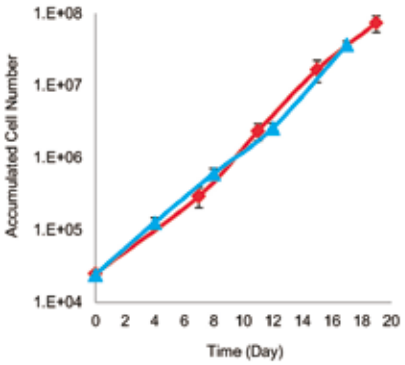
The group next tested the optimized cryopreservation medium in combination with the Pronase/EDTA solution on different hiPSC and hESC lines, and found to yield recovery frequencies of over 80%. When the group evaluated the cells before and after cryopreservation, they found no significant differences in cell properties. hPSCs subjected to the new cryopreservation method maintained their proliferative capacity and pluripotency on thawing, and showed no structural chromosomal abnormalities.

A major advantage of the hPSC cryopreservation medium developed by Imaizumi et. al. is the absence of animal and protein components. By simplifying the composition, they were able to avoid such risks as lot-to-lot variation and infections from pathogenic agents, which can make bovine serum albumin or serum solutions problematic. The coupling of this new method with a novel dissociation agent has also made cryopreservation of hPSCs more efficient and straightforward.

“The increasing demand for use of hPSCs in drug discovery and regenerative medicine has made it essential (for scientists) to establish safe, simple, efficient, and inexpensive methods for handling these cells,” says Nishikawa. “We hope that this study will lower some of the technical barriers to starting stem cell research, and help accelerate both basic and applied studies in the field.”

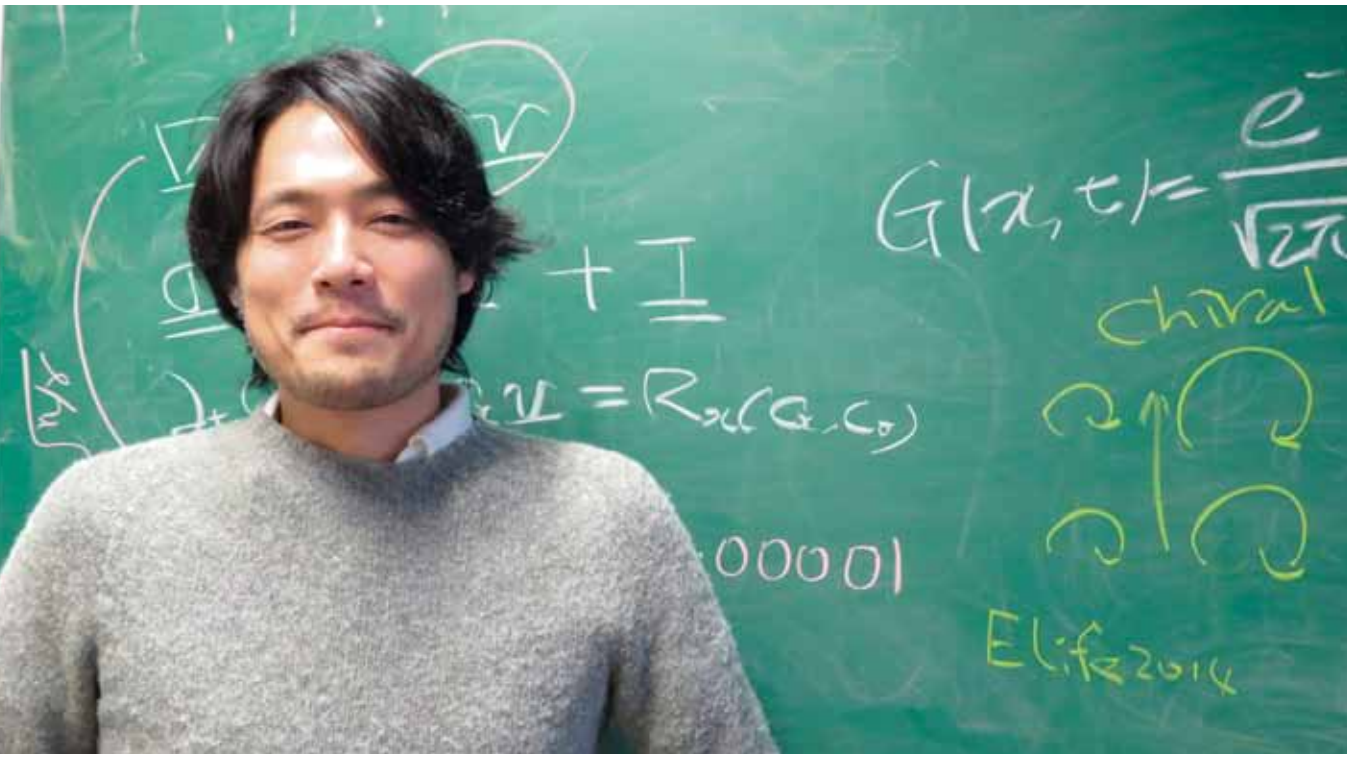


Comparison of ALP stainings of hiPSC line 201B7 before and after cryopreservation. Colony numbers are comparable.



Cell growth curve. Proliferative capacity is maintained before freezing (blue) and after thawing (red).

Mechanisms of chemotaxis and random cell movement explained



Masatoshi NISHIKAWA

For centuries, compasses have been used as navigational instruments enabling explorers to use directional information to guide them to their destinations. Likewise, during animal development, some cells follow a compass-like mechanism that guides their movements in response to external cues in a process called chemotaxis. Chemotactic cells sense chemical gradients in the surrounding environment that induce intracellular asymmetry of the signaling pathway biased in the direction of the gradient and direct the cells either toward or away from the stimulus. However, even in the absence of external gradients, intracellular asymmetry can be produced spontaneously by the cell, a process that may be linked to random cell movements. The Laboratory for Physical Biology (Tatsuo Shibata, Unit Leader) previously published a quantitative model of a gradient sensing system that reconstructs the phenomenon of spontaneous intracellular polarization and describes how this increases cell sensitivity and robustness, allowing them to respond to even shallow gradients.

Now, in a report published in *Biophysical Journal*, Masatoshi Nishikawa and colleagues in the same lab validate these models, specifically showing that excitability of the signaling pathway leads to spontaneous intracellular polarization, which can trigger both random movement in the absence of a chemoattractant gradient and biased movement in the direction of the gradient. Using the ameba, *Dictyostelium discoideum*, they show that the dynamics of spontaneous intracellular asymmetry is important for directional cell movement, and that this response is driven by an excitability-based mechanism.

The spatiotemporal dynamics of the phosphatidylinositol 3,4,5-triphosphate (PIP3) pathway plays an important role in chemotaxis in *Dictyostelium* cells, where a PIP3-enriched membrane domain is produced in response

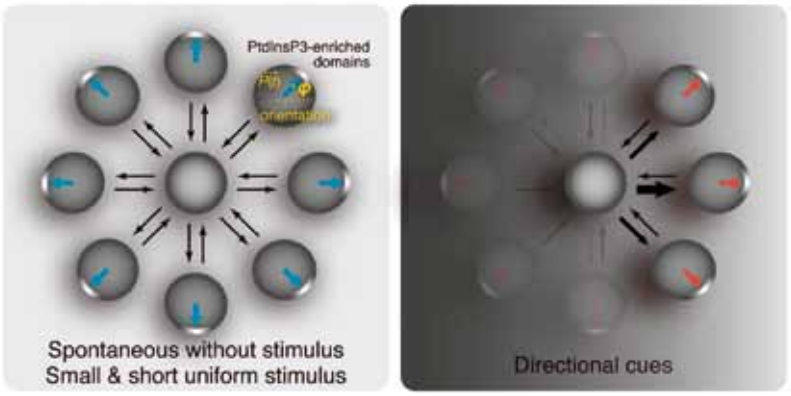
Nishikawa M, et al. Excitable signal transduction induces both spontaneous and directional cell asymmetries in the phosphatidylinositol lipid signaling system for eukaryotic chemotaxis. *Biophys J* 106.723-34 (2014)

to external stimulation (cAMP) driving changes to cytoskeleton to form pseudopods used for chemotaxis. Nishikawa et al. observed that cells exhibited random spontaneous PIP3-domain formation both in absence and presence of cAMP, with this spontaneous response appearing more frequently in presence of cAMP. They also tracked and quantified the cells' transient response levels and times when stimulated with various cAMP concentrations, and found that the average cell responses were similar, irrespective of cAMP concentration. The size of the PIP3 domain observed at peak response levels was proportional to cAMP concentration, while the overall PIP3 signal levels remained constant, suggesting that because the intensity of the responses was not influenced by the strength of the external stimulus but determined by the intracellular intrinsic activity, this is an excitable system.

If the chemotactic response is indeed driven by an excitability-based system, when the external stimulus exceeds a certain threshold to trigger a response, any responses unfolding even after the stimulus is removed can be considered to be maintained by the cell itself. When the group applied a short impulse stimulus that was shorter than the average response time, the cells showed intracellular polarization of PIP3 domains similar to that produced spontaneously, and as the length of impulses were increased, larger PIP3-domains were produced, consistent with their expectations. They also confirmed that the PIP3 signaling system has a refractory period, another indicator of an excitable system, by applying a series of successive stimuli to the cells at various time intervals.

The researchers next looked at how external gradients affected spontaneous intracellular polarization. Cells placed in conditions with a chemical gradient are known to induce localization of PIP3 in the membrane domain facing the highest cAMP concentration, driving chemotaxis in the same direction. From a theoretical perspective, spontaneous polarization of PIP3 domains, which appears in random directions in the absence of a gradient, becomes biased in the presence of an external gradient. The group found that, when presented with a gradient, the PIP3 domains appearing at random in the absence of a gradient exhibit a bias in the direction of the gradient. But, there were no significant changes in the intensity of the PIP3 response before or after the gradient was applied. These experiments demonstrate that spatial cAMP gradients orient the localization of the PIP3 domain, without changing the characteristics of the PIP3 domain.

"Our current study clearly shows that the signaling pathway for chemotaxis has excitability-based characteristics," says Shibata. "Cells do not act only in response to external stimuli. They have a set response pattern, which, when altered slightly, produces sensitive and robust responses for diverse situations. In this study, we found that the localization of the set response pattern changes according to the gradient, which may also explain how multicellular axis and polarity formation occur. The same system may function in complex multicellular morphogenesis as well."



Model of the biased-excitability system. In the absence of external stimulus (left), PIP3 domains are produced in random manner. In the presence of external stimulus (right), PIP3 domains are oriented to localize in the direction of the stimulus.

Express line to erythropoiesis

Wei WENG (with Yun-chan)

Primitive erythropoiesis during animal development requires stepwise differentiation from pluripotent epiblast cells, which undergo gastrulation to form the three germ layers: endoderm, ectoderm, and mesoderm. Newly formed mesoderm generates hemangioblast cells, the embryonic origins of the blood and vascular system, by initiating expression of early hematopoietic factors. These hemangioblasts then give rise to primitive erythrocytes. A number of studies have noted a delay between the appearance of early hematopoietic markers in mesoderm and that of erythroid lineage-specific globin genes in hemangioblasts. The purpose and the mechanisms at work during this delay, however, remain unclear.

New work by Wei Weng and colleagues in the Laboratory for Early Embryogenesis (Guojun Sheng, Team Leader) now shows that epiblast cells can be made to differentiate directly into the erythroid lineage in vivo through forced expression of five erythropoiesis-related transcription factors and inhibition of the FGF pathway. Published in *Stem Cell Reports*, these findings suggest a new mode of induced differentiation that bypasses the step-by-step routine seen during development.

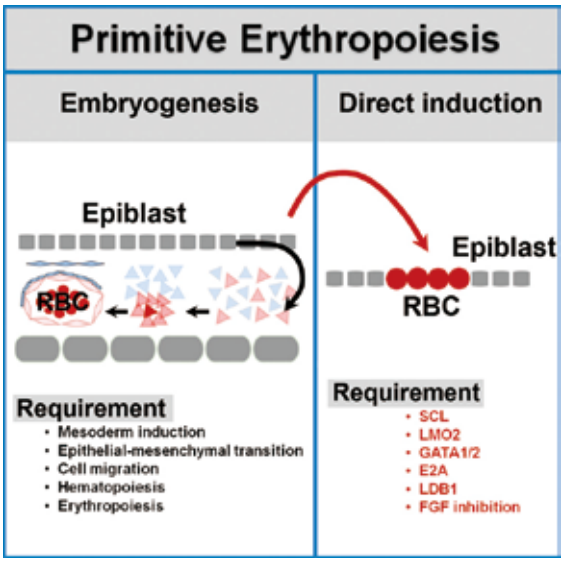
Sheng's group previously reported that FGF signaling has an inhibitory effect on primitive blood differentiation in chicken. Using an FGF inhibitor to block FGF expression levels in the embryo, this time, Weng and Sheng looked at the effect of FGF activity on early erythropoiesis and on the expression of early hematopoietic factors, *SCL* and *LMO2*. A comparison of the effects of FGF inhibition in embryos before or after endogenous expression of *SCL* and *LMO2*, suggested that FGF is needed to switch on both of these genes in mesoderm. In FGF-inhibited embryos, they found that exogenous expression of *SCL* and *LMO2* in mesoderm is sufficient to induce robust erythroid differentiation.

Steering pluripotent epiblast cells to differentiate directly into the erythroid lineage proved to be trickier than just inhibiting FGF and turning on two genes. Weng and Sheng searched transcriptomic data for candidate genes with similar expression profiles to *SCL* and *LMO2* and putative regulatory roles during the transition to erythropoiesis. They identified *LDB1* and *E2A*, both of which have been implicated in mammalian hematopoiesis, and are thought to form a complex with SCL, LMO2 and GATA2 to regulate erythropoiesis in vitro.

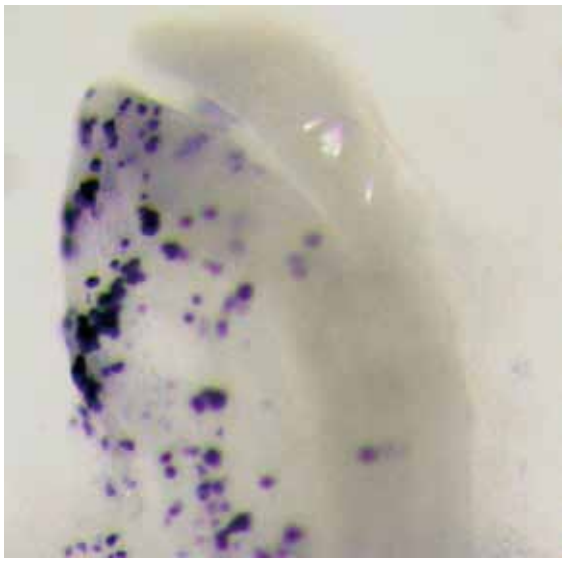
Weng W and Sheng G. Five transcription factors and FGF pathway inhibition efficiently induce erythroid differentiation in the epiblast. *Stem Cell Reports* 262-70 (2014)

They made expression constructs of these five genes (SCL, LMO2, GATA2, LDB1, and E2A) and found that when they electroporated the five constructs in combination with FGF inhibition before the endogenous expression of early hematopoietic regulatory genes, they were able to induce strong expression of *rho*, an erythroid marker, in epiblast. Interestingly, the combination of these five gene constructs and FGF inhibition also induced expression of a gene involved in heme biosynthesis in epiblast, suggesting that this directed differentiation also initiates expression of other essential factors required for primitive erythropoiesis.

"Many groups have tried using one or two key hematopoietic genes to induce erythroid differentiation from mesoderm. What we have done here is to look at inducing erythroid differentiation in mesoderm as well as other lineages," says Weng. "We have been able to get high efficiency in inducing differentiation not only of mesoderm into a specific lineage, but also in pluripotent epiblast and in ectoderm derivatives that are already less pluripotent than epiblast. But it remains to be seen whether this same efficiency can be achieved in vitro."



Primitive erythropoiesis during embryonic development (left). Direct induction of erythroid differentiation in the epiblast (right).



Globin-positive cells induced in the forebrain and head surface ectoderm by five transcription factors (*SCL*, *LMO2*, *LDB1*, *E2A*, and *GATA2*).

2014 Events

The RIKEN Center for Developmental Biology strives to engage with the public through a variety of media, including its website and printed materials, media coverage, and direct interactions such as guided tours, Open House events, and other public outreach activities. In addition to its open to the public activities, the CDB also organizes events intended to bring the Center's scientists together for discussion outside of the laboratory environment.

Start of CDB Lecture Series

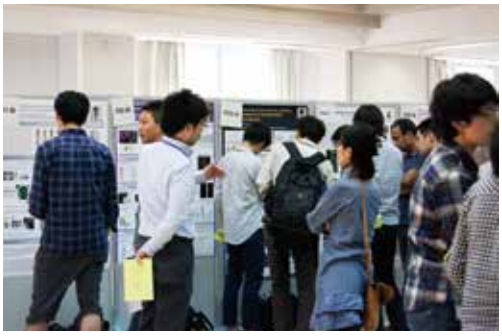
The CDB Lecture Series is the Center's new flagship forum for talks by leading international scientists, who are invited to introduce their latest research and visit individual laboratories over the course of two days for additional discussion. The annual schedule is intended to feature approximately eight CDB Lectures by eminent researchers. The program was initiated in fall of this year, and the inaugural CDB Lecture was delivered by Ben Simons of the University of Cambridge on September 8.

Open House 2014

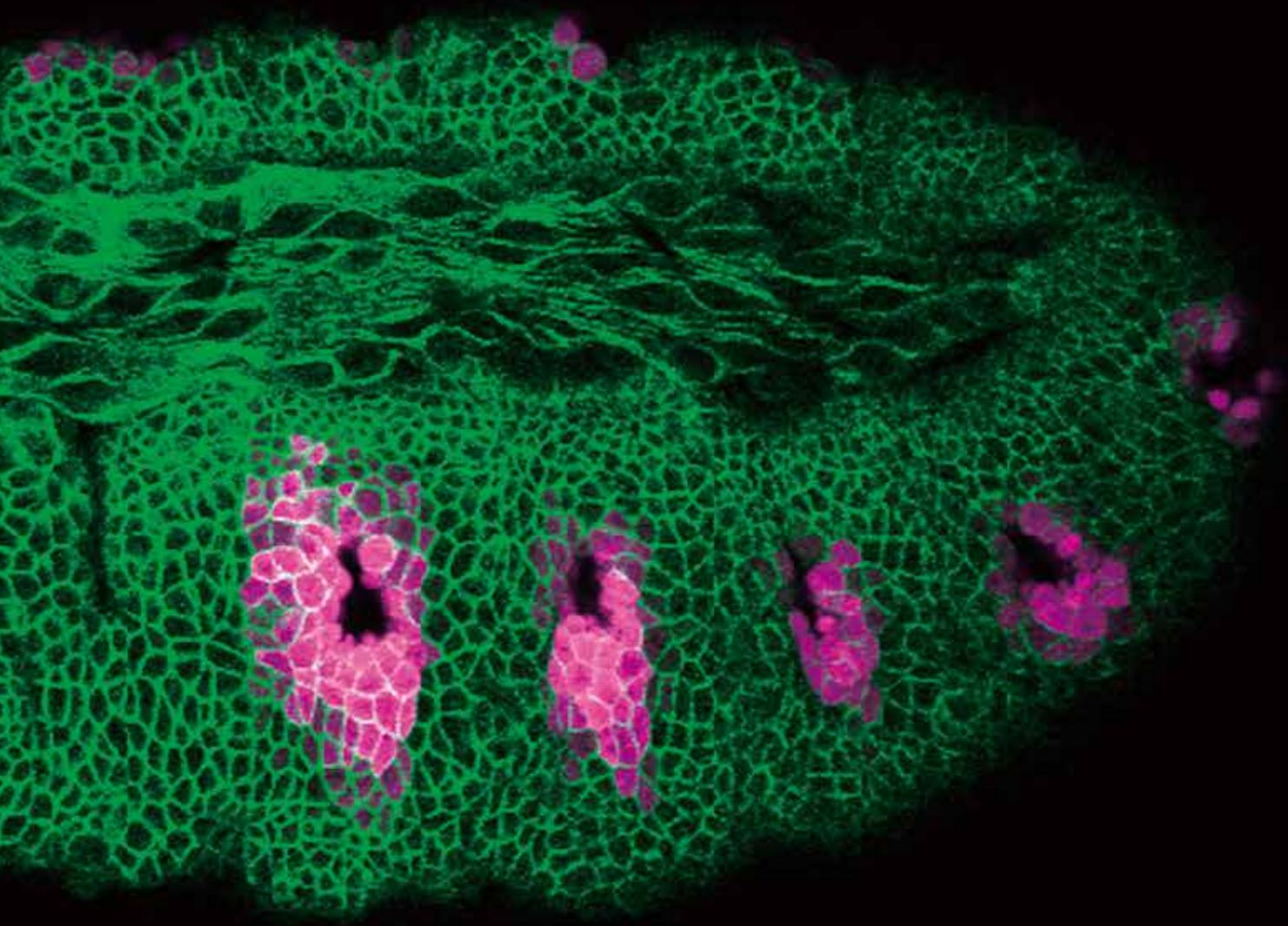
The RIKEN CDB held its annual Open House on Saturday, October 25, 2014. Around 1,500 people visited the Center to see and learn firsthand about the latest research in development and regenerative biology. This year's Open House featured public lectures by Tomoya Kitajima (Team Leader, Laboratory for Chromosomal Segregation) who talked about the causes of oocyte aging and by Takashi Tsuji (Group Director, Laboratory for Organ Regeneration) on the latest developments of research carried out in his laboratory related to teeth and hair regeneration. Twenty-four laboratories also took part in the event by organizing booths that introduced their research through posters and alluring research images. Many laboratories set up microscopes at their booths to allow the visitors to observe actual cell and tissue samples as well as some model organisms used in their research. Scientists and graduate students were on hand to explain and answer questions about their research from the visitors who dropped by their booths. In a special lecture program co-organized with Kobe City, Masayo Takahashi (Project Leader, Laboratory for Retinal Regeneration) gave a talk to a full crowd on the current state of the her clinical research using iPSC-derived retinal pigment epithelial cells at the neighboring Institute for Biomedical Research and Innovation.

CDB Retreat in Sasayama

The CDB held its annual research retreat in the mountains of Sasayama on September 29 and 30. Laboratory heads, research scientists, student trainees, technical staff and members of the administration took part in this closed meeting to share the latest developments in their work, as well as to get better acquainted with colleagues from other parts of the Center. The 2-day program featured talks by recent appointees, as well as special lectures by a number of scientists from other institutes, including CDB alumni Masahiko Hibi (Nagoya University) and Yuki Sato (Kyushu University).



Drosophila embryo at the beginning of tracheal placed invagination (magenta). Cell outline is labeled green.



Aizawa Laboratory

Shinichi AIZAWA Ph.D.

Shinichi Aizawa received his Ph.D. in biochemistry from the Tokyo Kyoiku University Department of Zoology in 1973. He spent the period from 1974 to 1979 as an investigator at the Tokyo Metropolitan Institute of Gerontology, then two years as a research fellow at the University of Washington. He returned to the Tokyo Metropolitan Institute of Gerontology in 1982, where he remained until 1986, when he moved to the RIKEN Tsukuba Life Science Center. He was appointed professor in the Kumamoto University School of Medicine in 1994, and served in that position until 2002. Since 2000 he has served as CDB deputy director and group director of the Vertebrate Body Plan lab, as well as head of the Animal Resources and Genetic Engineering Laboratory.

The Aizawa lab closed as part of the reorganization of the CDB in November 2014.
Dr. Aizawa is now affiliated with RIKEN CLST.

Pre-gastrular developmental routines have changed dramatically over the course of vertebrate evolution. The yolk, for example, has been stored in various amounts, and cleavage development has changed from the holoblastic cleavage seen in amphibian, bichir, lamprey, and mammal to the meroblastic form in teleost, reptile, and avian. Extraembryonic structures in amniotes, especially those in mammal, were acquired later in vertebrate evolution, and were formed by altering post-cleavage development. In light of these alterations, it is obscure how the mechanisms of anterior-posterior (A-P) axis formation and head development are related and have diverged in each family of vertebrates; these are among the most fundamental events in vertebrate development. Our aim is to assess A-P axis formation in an ancestral amniote and its divergence in each amniote lineage by comparative studies in extant amniote animals: mouse, rabbit, pig, *Suncus*, chick, quail, soft-shelled turtle, gecko, and gray short-tailed opossum.

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Publications

Courchet J, et al. Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. *Cell* 153.1510-25 (2013)

Kurokawa D, et al. Otx2 expression in anterior neuroectoderm and forebrain/midbrain is directed by more than six enhancers. *Dev Biol* 387.203-13 (2014)

Huilgol D, et al. Dual origins of the mammalian accessory olfactory bulb revealed by an evolutionarily conserved migratory stream. *Nat Neurosci* 16.157-65 (2013)

Abe T, et al. Visualization of cell cycle in mouse embryos with Fucci2 reporter directed by Rosa26 promoter. *Development* 140.237-46 (2013)

Inoue F, et al. Gbx2 directly restricts Otx2 expression to forebrain and midbrain, competing with class III POU factors. *Mol Cell Biol* 32.2618-27 (2012)

Suncus

gray short-tailed opossum

soft-shelled turtle

gecko

Reconstitutive Developmental Biology

Miki EBISUYA Ph.D.

Miki Ebisuya received her Ph.D. from the Graduate School of Biostudies, Kyoto University under the supervision of Prof. Eisuke Nishida in 2008. She worked briefly as a postdoctoral fellow in the same laboratory before being appointed a group leader of the Career-Path Promotion Unit at Kyoto University in 2009, where she began research in synthetic biology. In 2013, she was selected as a PRESTO researcher in the area of design and control of cellular functions, and was also appointed to her current position as unit leader of the Laboratory for Reconstitutive Developmental Biology at the CDB.

As part of the reorganization of the CDB in November 2014, the Reconstitutive Developmental Biology lab was transferred to the RIKEN Quantitative Biology Center.

Staff

Research Scientist
Mitsuhiro MATSUDA
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Publications

Matsuda M, et al. Synthetic lateral inhibition governs cell-type bifurcation with robust ratios. *Nat Commun* DOI: 10.1038/ncomms7195, in press

Imajo M, et al. Dual role of YAP and TAZ in renewal of the intestinal epithelium. *Nat Cell Biol* 17.7-19 (2015)

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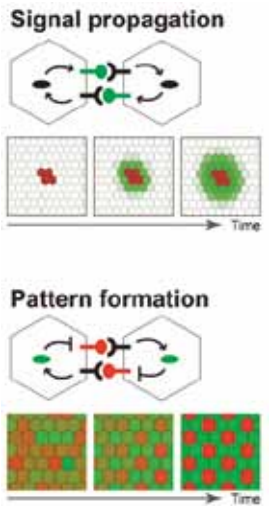
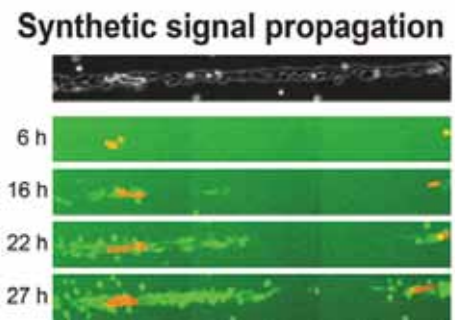
Matsuda M, et al. Synthetic signal propagation through direct cell-cell interaction. *Sci Signal* 5.ra31 (2012)

In our lab, we strive to create or reconstitute biological mechanisms. Our aim of reconstitution is to test the sufficiency of current understandings of mechanisms of interest, as well as to discover unexplained or unexpected elements through observation. We are particularly focused on reconstituting mechanisms of intercellular communications. In metazoan development, for instance, intercellular communications induce the spontaneous differentiation of groups of cells underlying the cellular patterns seen in various tissues (known as self-organized cell differentiation patterns). We use simplified, artificial networks of the Delta-Notch pathway, which is responsible for signaling between adjacent cells, in an effort to reconstitute the essence of self-organized cell differentiation patterns in mammalian cells in vitro. Our ongoing projects also include the reconstitution of asymmetric pattern formation using artificial Nodal-Lefty networks, and the reconstitution of an intercellular synchronization mechanism. Artificially reconstituted systems also have the advantage of facilitating measurements and the modification of parameters, which we hope will contribute to the quantitative understanding of intercellular communications.



Examples of gene networks for cell-cell communication.
Mutual activation between adjacent cells results in signal propagation (upper), while mutual inhibition results in cell pattern formation (lower).

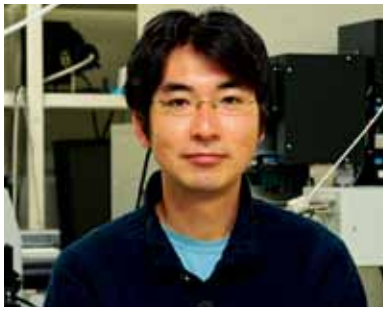


Reconstitution of signal propagation.
The gene expression signal (green) propagated to the neighboring cells along a line.

In Vitro Histogenesis

Mototsugu EIRAKU Ph.D.

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



Mototsugu Eiraku received his Ph. D. from The University of Kyoto 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 served as a research scientist until 2010. That year, he was promoted to Deputy Unit Leader of the Four-dimensional Tissue Analysis Unit within the Division for Human Stem Cell Technology, and in 2013 was appointed Unit Leader. From November 2014, he has served as leader of the In Vitro Histogenesis Unit.

Recent advances in stem cell technology have enabled the generation of various potentially medically useful cell types from ES and iPS cells, including neurons, photoreceptors, and cardiac muscle. However, the extent to which such cells mimic their in vivo function when plated on culture dishes is limited. In vitro histogenesis unit seeks to develop new approached to cell technology that will allow for more realistic in vitro recapitulation through three-dimensional tissue formation from stem cells. To this end, we establish efficient three-dimensional culture of ES cell-derived brain and retinal tissues, and also develop new cutting-edge live imaging technology and related optic devices for four-dimensional analysis of relatively large tissues. We also support and collaborate with CDB and non-CDB users of these optics technologies.

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Publications

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

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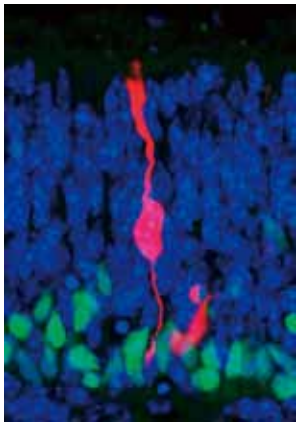
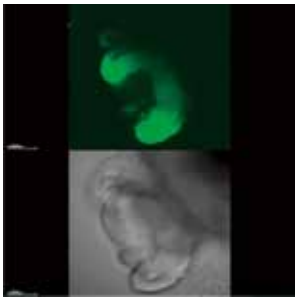
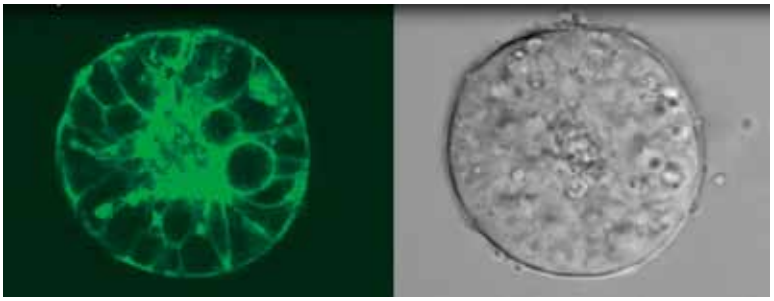


Photoreceptor cell (red) and bipolar cells (green) in ES cell-derived neural retina



Self-organized optic cup formation from ES cell



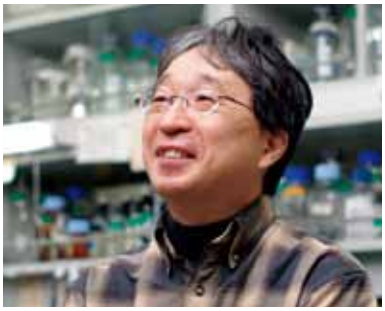
ES cell-derived neuroepithelium (lifeact-GFP)

Neuronal Differentiation and Regeneration

Hideki ENOMOTO M.D., Ph.D.

Hideki Enomoto received his M.D. from the Chiba University School of Medicine in 1988, and his Ph.D. from the same institution in 1996. After serving as the Chief of the Department of Pediatric Surgery, Matsudo Municipal Hospital in Chiba, Japan, he moved to the Washington University School of Medicine, St. Louis in 1997, to work as a research associate studying the physiological roles of the GDNF Family of Ligands in neural development and function. He returned to Japan to take a position as a team leader at the CDB in 2002.

The Neuronal Differentiation and Regeneration lab closed in April 2014.
Dr. Enomoto is now affiliated with Kobe University.



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Yuka NODA

Publications

Uesaka T et al. GDNF signaling levels control migration and neuronal differentiation of enteric ganglion precursors. *J Neurosci* 33. 16372-82 (2013)

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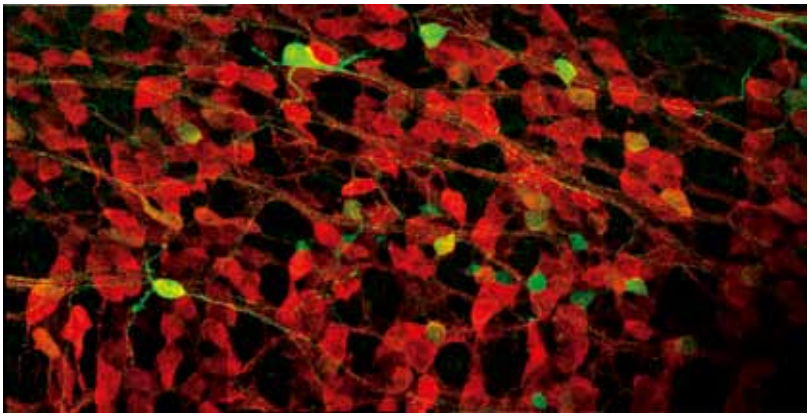
The architecture of the nervous system, which underlies much of the dynamism of animal life, is frequently described as complex, but closer examination reveals great beauty as well. The construction of this exquisite and elaborate structure involves the birth, growth and death of countless individual cells and is regulated by intercellular communication and cooperative activity.

Our laboratory is interested in questions fundamental to the development of the nervous system: determining the specific extracellular signals and cell autonomous routines that participate in the processes of neural development, and resolving the means by which diverse families of signaling molecules coordinate and interact at the molecular and cellular levels. To address these questions, we focus on a class of signaling molecules known as neurotrophic factors, most particularly the GDNF Family Ligands (the GFLs). This family of neurotrophic factors includes four known members – GDNF (Glial cell line Derived Neurotrophic Factor), Neurturin, Artemin, and Persephin. The GFLs signal via receptor complexes formed by the RET receptor tyrosine kinase and one of four co-receptors, GRFα1-4. In vitro, these four receptors show differential affinities for specific GFLs. GFL signaling has been shown to affect neuronal growth, survival and migration as well as axon guidance, and defects in this signaling system have been implicated in a number of congenital health problems.

Our efforts will focus on the analysis of the physiological roles of neurotrophic factors, which we hope will lead to an improved understanding of the molecular bases of neural development, differentiation and pathology, and ultimately to potential clinical applications in the treatment of nervous system trauma and disease.



Developing enteric nervous system (ENS) in which GDNF receptor RET was conditionally inactivated in a small population of ENS cells (mouse gut: embryonic day 14.5, Green: Ret-deficient cells, Red: enteric neurons).

Tissue Microenvironment

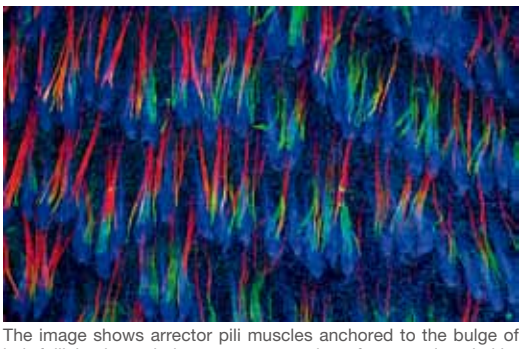
Hironobu FUJIWARA Ph.D.

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

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 served 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 Research Institute, where he completed a second postdoctoral fellowship, before returning to Japan to take a position as a Team Leader at the RIKEN CDB in 2012.

In our bodies, we have millions of different environments in which cells reside, which are known as cellular or tissue microenvironments. A series of recent studies has shown that 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 then instruct cellular behavior and communication, and the formation of organs. We are particularly interested in the role of regional specialization of the extracellular matrix (ECM) in the formation of the stem cell microenvironment, or niche. A deeper knowledge of this will provide a molecular basis to an improved understanding of the niche regulation of stem cells, and the development of tailor-made microenvironments for different lineages of stem cells.

The ECM is divided in two major groups—the fibrillar interstitial matrix, which fills the interstitial connective tissues, and the basement membrane, a thin sheet-like ECM located at the borders of tissues. Stem cells in most tissues reside at this border, adhere to the basement membrane and interact with neighboring niche cells. By virtue of its remarkable heterogeneity in composition, the basement membrane contributes to the spatial organization of niches, and modulates the local concentration of adhesive and soluble signalling molecules that are available to stem cells. A recent study by our team has shown that the molecular composition of the basement membrane in the mouse hair follicle stem cell niche, the bulge, is highly specialized. One stem cell-derived component, nephronectin, is important for the development and positioning of the bulge-residing arrector pili muscles, which, among other functions, are responsible for goosebumps. This was the first report to show that stem cells regulate the fate and positioning of surrounding niche cells through the specialization of the basement membrane. To gain further insight into fundamental aspects of the microenvironmental regulation of stem cells, we use mouse skin as a model and seek to better understand 1) the molecular landscape of basement membrane specialization in the stem cell niche, 2) mechanisms by which the basement membrane in the stem cell niche is regionally specialized, and 3) how the specialized basement membrane controls stem cell niche formation, stem cell behavior and the conversation between stem cells and their neighboring cells.



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 labelled with a nuclear counter-stain (blue).

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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)

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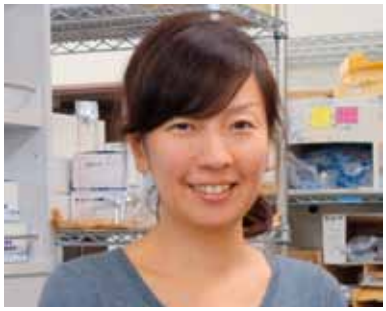
Neocortical Development

Carina HANASHIMA Ph.D.

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

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 developmental neuroscience. She moved to Skirball Institute Developmental Genetics Program in 2002 in Gord Fishell's laboratory where she extended her research to cell specification in the neocortex. She was appointed team leader at the CDB in September 2007.



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Publications

Toma K, et al. The timing of upper-layer neurogenesis is conferred by sequential derepression and negative feedback from deep-layer neurons. *J Neurosci* 34. 13259-76 (2014)

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

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

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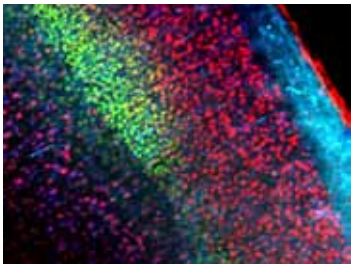
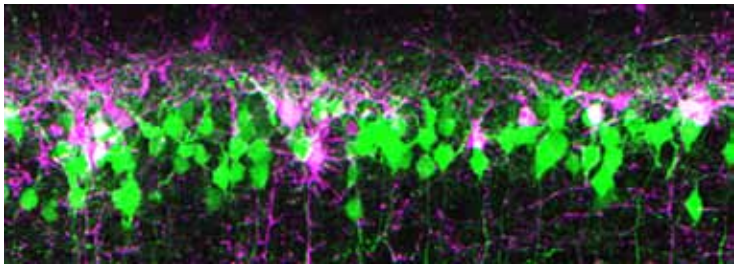
The neocortex, by far the most complex structure of all vertebrate brain systems, is organized into cytoarchitectonic groups of neurons that process unique sensory inputs, such as pressure, light and sound. This highly ordered structure nonetheless is generated from a relatively simple sheet of neuroepithelium earlier during development. Research in our laboratory aims to understand how diverse arrays of cortical neurons are specified and coordinated into high-functional territories. Despite its well-defined anatomical character and functional significance, the mechanisms underlying the precise assembly of distinct functional areas of the cerebral cortex remains largely unknown. Progress has been impeded by our present lack of understanding of the molecular mechanisms by which neuronal subtypes within cortical layers and across function domains are specified. In our laboratory, we address important questions concerning neocortical development: 1) What are the mechanisms by which diverse cell fate is determined in the neocortex? 2) How are neurons precisely arranged into distinct cortical areas and how do they establish cytoarchitectonic boundaries? 3) To what extent does the refinement of functional areas rely on environmental inputs? To investigate these questions we utilize mouse as a model to understand the assembly of functional subdivisions in the mammalian neocortex.

Previous work has shown that the fate of neocortical neurons is at least in part pre-determined within the progenitor cells. We have found that specification of deep-layer projection neurons is dependent on transcriptional repressor that function cell-autonomously to prevent neurons from acquiring an earlier neuronal phenotype. These results imply cortical intrinsic programs in which neuron fate is established by temporal changes in gene expression may be co-opted. However, in the mature cortex, cortical areas differ in their types and numbers of specific layer neurons along both the anteriorposterior (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.



The neocortex is composed of distinct neuronal subtypes that establish 6 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

Shigeo HAYASHI Ph.D.

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

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 in 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. His current research interests are dynamic aspects of cell adhesion, cell migration and cell morphogenesis in *Drosophila*.

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 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 as a respiratory organ. We are particularly interested in the mechanical control of epithelial archetechtures. 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 tracheal network is formed, tube diameter and length are enlarged to reach the final size. Tracheal size change involves increase in cell size, espacially the increase of apical cell area facing the luminal side. Key question is how indivisually controlled cellular growth is coordinated to form coherent tissue archetecture. 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 leads to destabilization of tube shape and malformation, resembling tuble shapes found in organs in pathological conditions.

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

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Publications

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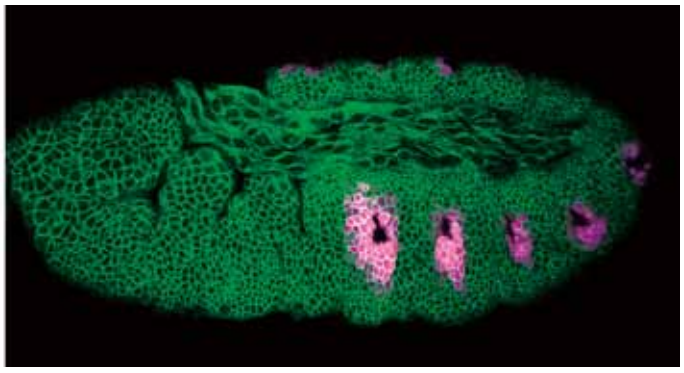

Drosophila embryo at the beginning of tracheal placed invagination (magenta). Cell outline is labeled green.

Developmental Epigenetics

Ichiro HIRATANI Ph.D.

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

Ichiro Hiratani received his B. Sc. in Biological Sciences from the University of Tokyo in 1998, and his Ph.D. from the same institute 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 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.

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Publications

Ryba T, et al. Genome-scale analysis of replication timing: from bench to bioinformatics. *Nat Protoc* 6.870-95 (2011)

Hiratani I. and Gilbert D. M. Autosomal lyonization of replication domains during early Mammalian development. *Adv Exp Med Biol* 695.41-58 (2010)

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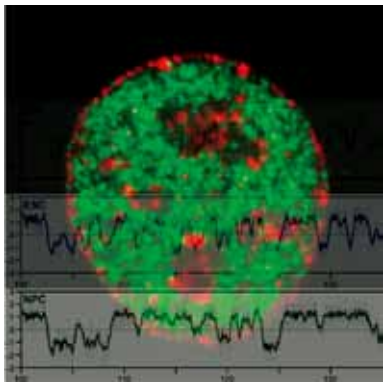
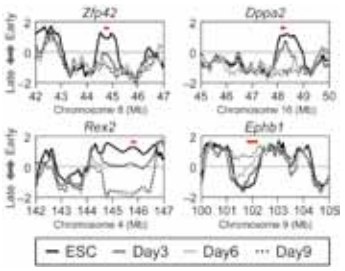
We wish to clarify the molecular mechanisms underlying global facultative heterochromatin formation during early mouse embryogenesis, with the belief that understanding the developmental regulation of higher-order chromosome organization will lead to a deeper understanding of cell differentiation.

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 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.

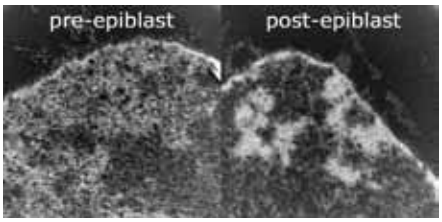
For these reasons, we combine genome-wide approaches with molecular and cell biology and imaging techniques to elucidate the molecular mechanisms underlying the facultative heterochromatin formation process. In the future, we will address the biological significance of this phenomenon and eventually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



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

Takeshi IMAI Ph.D.

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

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 in the CREST Program and the University of Tokyo in 2006 and 2007, before his appointment as Assistant Professor at the same university in 2009. He received independent funding under the JST PRESTO program that year, before joining the RIKEN CDB as team leader in 2010. He also holds an adjunct position as Associate Professor at the Kyoto University Graduate School of Biostudies.

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

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Publications

Imai T. Construction of functional neuronal circuitry in the olfactory bulb. *Semin Cell Dev Biol* 35. 180-188 (2014)

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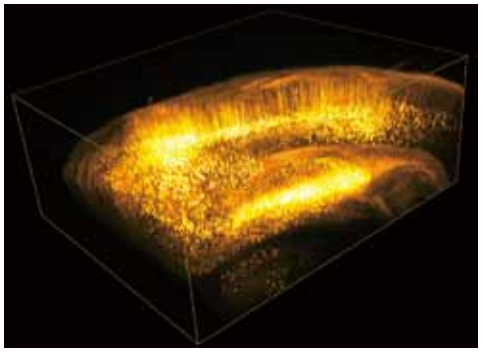
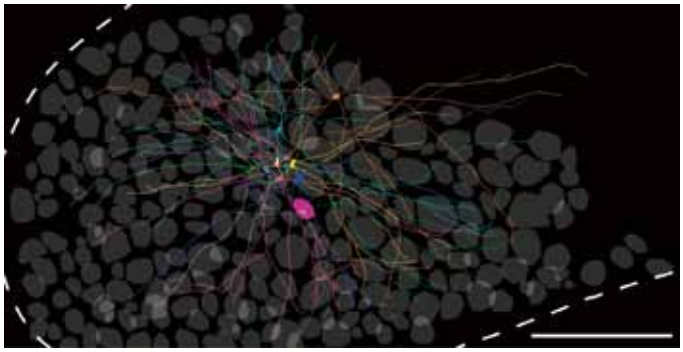


Adult Thy1-YFP-H mouse brain was cleared with an optical clearing agent SeeDB and imaged using two-photon microscopy. 3D rendering image of a volume of 4 mm x 5 mm x 2 mm, encompassing cerebral cortex and hippocampus, is shown.



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

Axial Pattern Dynamics

Hidehiko INOMATA Ph.D.

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

Hidehiko Inomata gained 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, he was appointed a Senior Scientist in the same lab. In 2011, he was also 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.

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Publications

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

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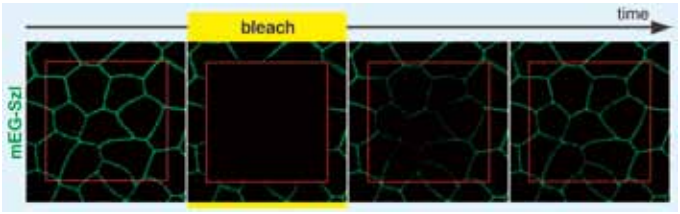
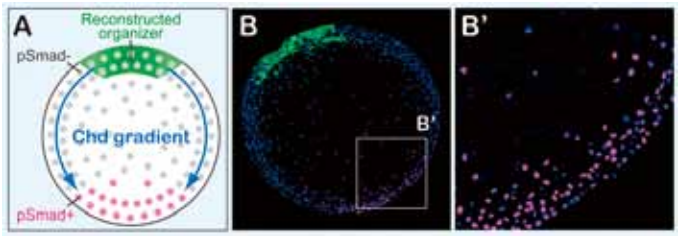
Developmental processes take place through the exchange of information by cells within the constrained spatial environment of the embryo. Such intercellular communication is essential for the formation of a well-ordered body; in its absence, our individual cells would behave in an uncoordinated fashion, and fail to follow the patterns needed for the development of a 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 mediate by morphogens need to be robust against perturbations. One example of such robustness can be seen in the response of a frog embryo when bisected: such embryos follow normal developmental patterns, despite being half the ordinary size, a phenomenon known as 'scaling.' Our team has previously shown how scaling is maintained through morphogen-mediated intercellular communication when the spatial size of the embryo is perturbed. In our lab, we address visualization of morphogen gradients and in vivo imaging along with biochemical approaches to study how developmental robustness is maintained.

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

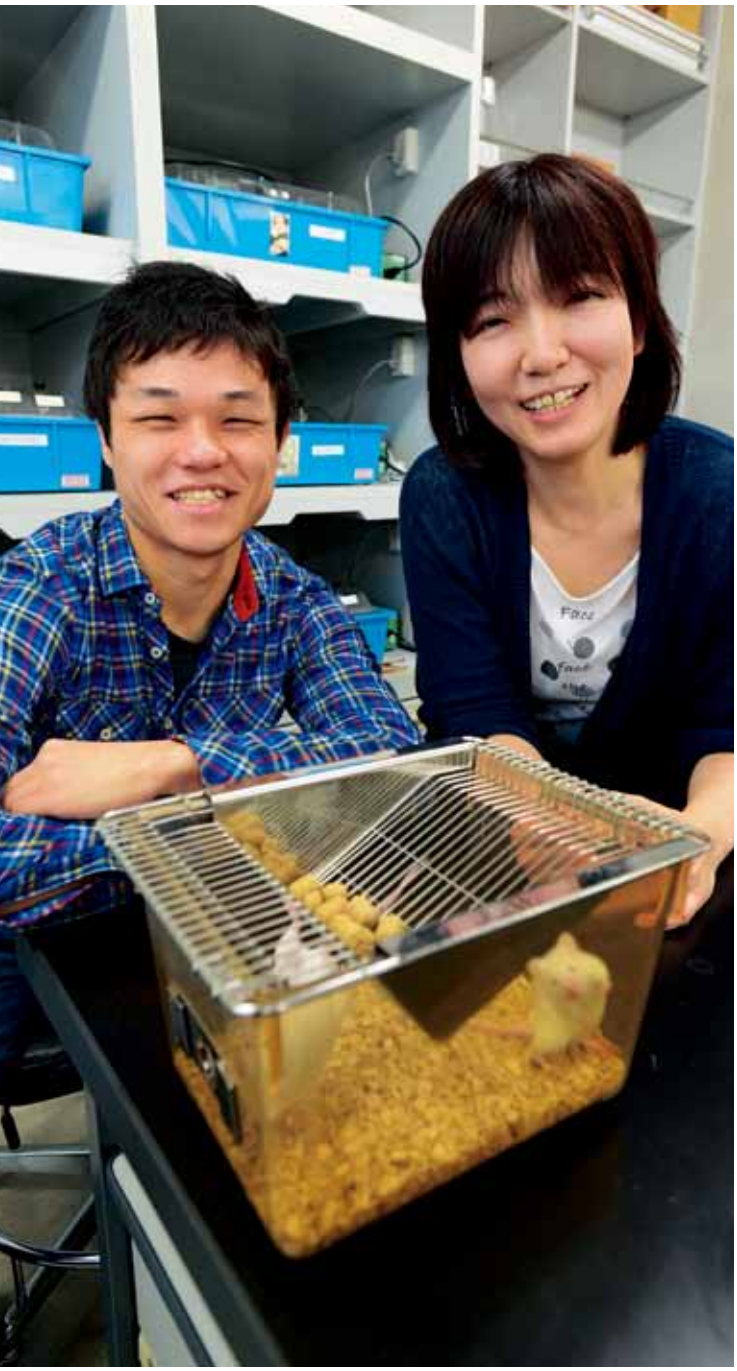


FRAP assays of mEGFP-tagged Sizzled shown by snapshots. From left to right columns; before bleaching, at the end of bleaching, 50 s after bleaching, and 230 s after bleaching.



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.

FGF makes a noise in maintaining hair cell progenitors



Kazuya ONO, Tomoko KITA

Sound is generated by vibrations of air molecules, which are detected and interpreted by the ear. These vibrations are transduced, in turn, from the ear's tympanic membrane, to the ossicular bones in the inner ear, to the perilymph in the spiral-shaped cochlea, and finally to the mechanosensory stereocilia of the hair cells (HCs). HCs then send electrical signals to the brain to be interpreted into what we perceive as sound. HCs in the cochlea are highly organized, with the mammalian cochlea comprising three rows of outer hair cells (OHCs), a single row of inner hair cells (IHCs), and populations of support cells that surround the HCs. Damage to the HCs by extrinsic or intrinsic factors can lead to hearing impairments, and once damaged, the hair cells cannot regenerate themselves. The mechanisms of HC development have long been studied as deciphering this process may lead to inroads in generating new HCs to replace damaged ones.

In a recent study, Kazuya Ono and colleagues in the Laboratory for Sensory Development (Raj K. Ladher, Laboratory head) shed new light on the role of one signaling pathway, the fibroblast growth factor (FGF) pathway, in cochlear HC development. Published in *PLOS Genetics*, they demonstrate that this pathway plays a crucial role in the maintenance of pre-hair cell progenitors and in the commitment of progenitors to differentiate into hair cells.

HCs form from an area of the inner ear epithelia which is induced to express Sox2, a transcription factor involved in maintaining pluripotency, by Notch-Jagged (Jag) 1 signaling. BMP signaling then specifies a region of cells within the Sox2-positive patch, also called sensory patch, to form the prosensory domain. These are the immediate precursors of HCs and support cells. The prosensory domain cells subsequently stop proliferating and a second wave of Notch signaling selectively induces the prosensory domain to differentiate into HCs or support cells. In addition to these pathways, studies by others have shown that the FGFR1 pathway is also important in cochlear HC development, as deleting the *Fgfr1* gene resulted in HC loss. However, the question remained: how does this signaling pathway function in HC development.

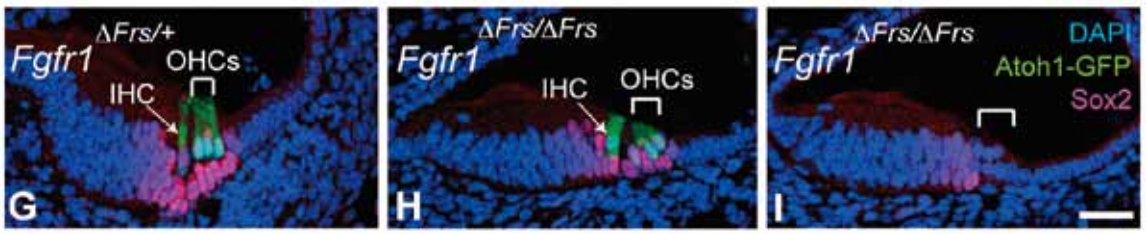
Ono and his colleagues decided to focus on addressing this question by first determining when this signal is needed by reducing *Fgfr1* levels at different stages in mice and noting its effects. They used two *Fgfr1* conditional

Ono K, et al. FGFR1-Frs2/3 signalling maintains sensory progenitors during inner ear hair cell formation. *PLoS Genet* 10.e1004118 (2014)

mutant mouse lines, which differed in the timing of *Fgfr1* deletion; in the first line, *Fgfr1* deletion occurred just prior to E10.5, before the induction of the sensory patch, and in the second line, the gene was deleted at E12.5. HC loss was observed in both lines; however, the loss was more dramatic when *Fgfr1* levels were reduced at E10.5, suggesting that FGFR1 signaling was needed before E12.5.

Having established that FGFR1 signaling is needed before E12.5, the group next turned their attention on determining which step was disrupted. The defect was traced back to the earliest step in HC development, the formation of the Sox2 positive sensory patch. They found that while Sox2 was switched on normally, in the absence of FGF signaling, Sox2 was not maintained. Thus, the FGFR1 signaling pathway appears to be important for maintaining Sox2 expression during sensory patch formation, but is not involved in initiating the exiting of the cell cycle. Further analysis of the FGFR1 signaling pathway also revealed that the MAP kinase signaling cascade, acting through Frs2/3, is turned on downstream of FGFR1 and regulates Sox2 expression.

"In this study, we answer the longstanding question of how FGF signals regulate the hair cell numbers," says Ladher. "It may be possible in the future to produce hair cells in culture, which can then be transplanted to replace the damaged inner ear hair cells, by modulating FGF signals."



Cross sections of cochleae. Left: Three outer hair cells (OHCs) and one inner hair cell (IHC) surrounded by Sox2-positive support cells (pink). Middle: Cochlea from mutant with relatively weak phenotype. Only 2 OHCs can be seen. Right: Cochlea from mutant with severe phenotype. No HCs detected.

Eye on ESC- and iPSC-derived retinal tissue transplantation

Michiko MANDAI, Juthaporn ASSAWACHANANONT

Retinitis pigmentosa (RP) is a group of genetic diseases of the eye that are characterized by the degeneration of photoreceptors, a type of neural cell which detects light. Symptoms of RP include night blindness and progressive narrowing of peripheral vision, and in some advanced cases, may lead to blindness. The pathogenesis of this disease is as varied as the large number of known causal genes or mutations, and there are currently no effective treatments available. In recent years, cell transplantation using cells derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) has emerged as a potential treatment method for RP, but progress has been hindered by technical issues to obtain sufficient numbers of photoreceptor cells at an ideal developmental stage for transplantation, which can survive and form functional synapses with neighboring neural networks within the complex retinal tissue. A groundbreaking report published in 2011 by Mototsugu Eiraku (Unit Leader, Four-dimensional Tissue Analysis Unit) and the Laboratory for Organogenesis and Neurogenesis of RIKEN CDB cleared this barrier, showing that ESCs could be induced to form photoreceptors as well as three-dimensional optic cups similar to retinal tissues in a 3D culture system, paving the way to consider tissue level transplantation.

Now, a new study carried out by International Program Associate, Juthaporn Assawachananont, Deputy Project Leader, Michiko Mandai, and others in the Laboratory for Retinal Regeneration (Project Leader, Masayo Takahashi), shows in that ESC- or iPSC-derived retina sheets transplanted in the eye can survive and integrate with the host retina over an extended period. Published in *Stem Cell Reports*, their study suggests that, at least in mouse, transplanted retina sheets derived from ESCs or iPSCs are capable of developing structures similar to normal retinal tissues and possibly neural networks, especially when grafts are prepared from younger stage cultures.

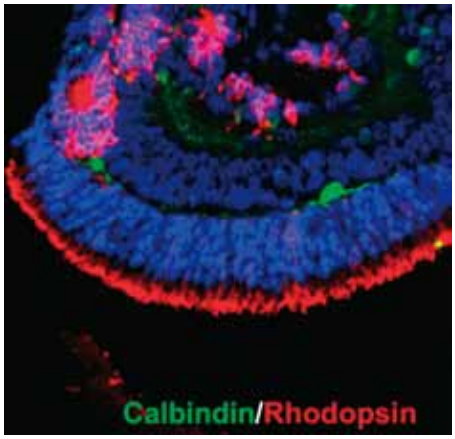
Assawachananont J, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports* 2. 662-74 (2014)

The team first modified the 3D culturing method developed by Eiraku et al. (2011) to generate larger quantities of retinal tissue from mouse ESCs and iPSCs. They tweaked the culture medium by adding a retinoic acid antagonist (RRA) and increasing the concentration of extracellular matrix, which led to a more efficient induction of mESCs and miPSCs into retinal progenitors. Using this modified medium, they examined the optic vesicle-like structures that eventually developed into retina-like structures at differentiation day (DD) 24 composed of an assembly of typical cell types found in the retina such as photoreceptors, amacrine cells, horizontal cells, and glial cells. The differentiation patterns of these cells resembled the development of the mouse retina, and cell cultures younger than DD20 were considered equivalent to embryonic retinal tissues, whereas cultures DD21 and older were similar to postnatal retinas.

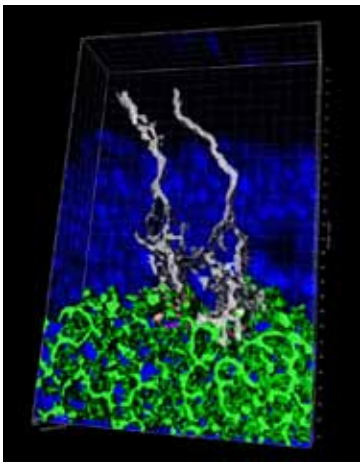
The group then examined the feasibility of transplanting grafts of retina-like sheets prepared from ESC- or iPSC-derived optic cup structures induced in 3D culture. Retina-like sheets from various stages between DD11 to 24 were prepared and then transplanted in the subretinal space of mouse models for retinal degeneration, which have lost most of their rod photoreceptors, and the post-transplantation effects were tracked over the course of six months. When retina sheets from younger cultures (DD11-17) were transplanted into host retina, close to 90% of the grafts went on to form a structured outer nuclear layer (ONL) made up of the cell body of photoreceptors. In contrast, in transplants using retina sheets prepared from cultures DD18 and older, most of the grafts (80%) failed to maintain an ONL structure.

Closer examination of the transplanted area revealed that mature photoreceptors in the grafts which formed ONL also possessed the typical outer segment and inner segment. The outer segment had stacked disc-like arrangement when they were nestled adjacent to host retinal pigment epithelial cells, while the inner segment contained mitochondria. Immunostaining experiments indicated that photoreceptors in the grafts showed assembly of their synaptic ends with dendrites of host bipolar cells, resembling normal retinal development. The grafts showed a range of patterns, but those prepared from cultures DD17 and younger showed the most potential to form synapses and maintain an ONL structure.

“Our study is the first to demonstrate that retinal tissue derived from ESCs or iPSCs can be used for transplantation, and the transplanted tissue can survive and integrate with the host retina, even in advanced stages of degeneration,” explains Mandai. “We still need to carry out more experiments to determine the functionality of the transplanted retinal tissue, but we hope to explore the possibility of translating our work into clinical studies for humans, using human derived tissues.”

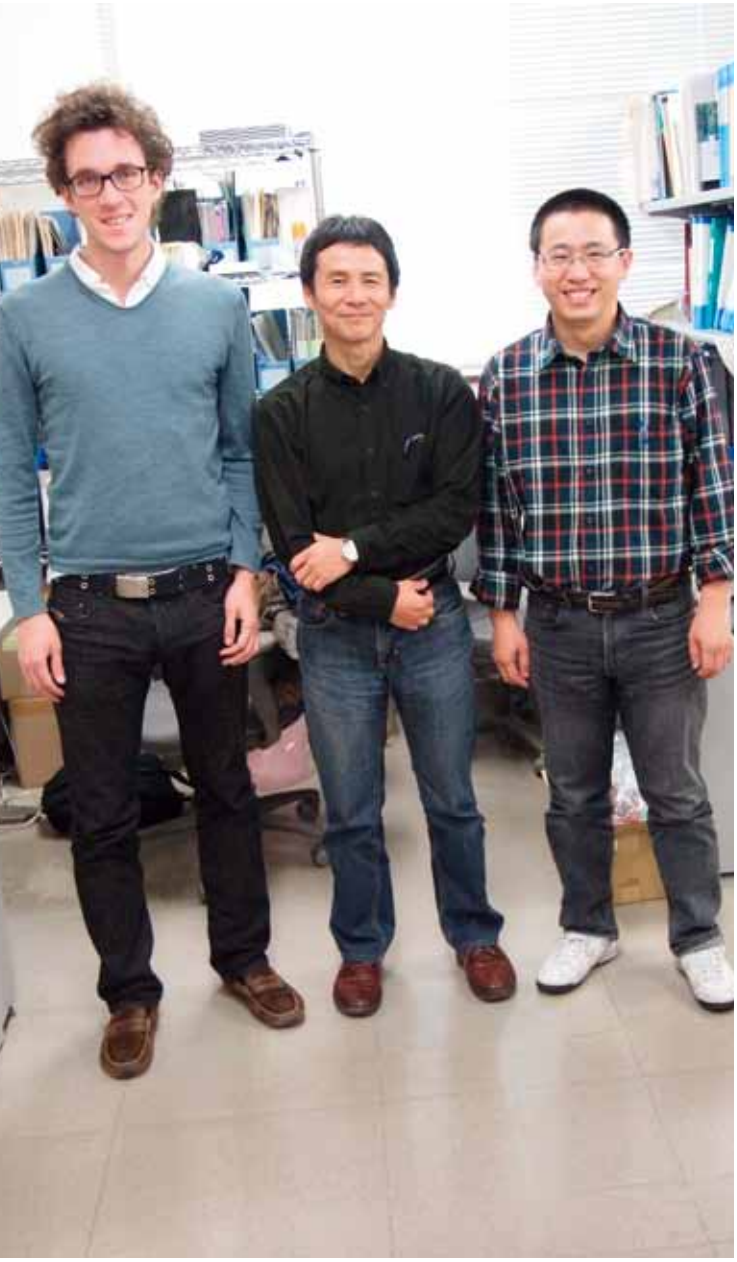


iPSC-derived retina-like sheet (DD14) transplanted to mouse forms retina-like structure, including outer nuclear layer (ONL), inner nuclear layer (INL), and inner plexiform layer.



3D reconstruction of immunostaining of transplanted area. Photoreceptors (green) from graft form synaptic connections (red) with host bipolar cells.

Balancing act by ECM regulates length of tracheal tube



Edouard HANNEZO, Shigeo HAYASHI, Bo DONG

A complex network of luminal structures plays an important role in bodily functions such as transporting gases and fluids associated with metabolism or homeostasis. The tube length and diameter of these tubular networks vary according to size of the animal, the location inside the body, and of the molecules that will circulate through the tube to ensure efficient transport. The *Drosophila* embryo, for example, prior to hatching, develops a tracheal tube that fits its tiny body. Past studies of this tracheal system indicate that apical membrane synthesis, lumen components, and cell-cell adhesion between epithelial cells have an important role in tracheal tube formation; however, the detailed mechanism has remained unclear.

In a new study published in *Cell Reports*, Foreign Postdoctoral Researcher, Bo Dong, from the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) in collaboration with Edouard Hannezo of the Institut Curie in France reveal that the extracellular matrix (ECM) plays a regulatory role in determining tube length. Using the *Drosophila* tracheal system, they demonstrate that elastic ECM and its interaction with the encircling apical membrane of the tracheal epithelium are key factors in regulating tube length.

The *Drosophila* tracheal system consists of a thick tube of epithelial cells running through the center of the body with smaller and narrower tubes branching off to the dorsal and ventral sides. During development, the tracheal lumen is filled with ECM, which is broken down prior to hatching and replaced by gases allowing it to fill its physiological role. The tracheal epithelial cells are arranged with their apical membrane facing the lumen and the basal membrane facing the outside.

Dong et al. first searched for mutants showing abnormalities in tracheal tube length, and came across a fly mutant that had an abnormally long and sinusoidal-shaped tracheal tube, but with a diameter similar to that in wild-type flies. This fly had a mutation in the *shrub* gene, which encodes part of a protein complex involved in membrane remodeling for vesicular transport. They examined the cell membrane on both apical and basal sides of trachea epithelial cells by immunostaining each side with different colored markers and found that, compared with wild-type, only the apical membrane of *shrub* mutants had elongated along the anterior-posterior (AP) axis. The basal membrane was nearly unchanged and there were no significant differences in cell number.

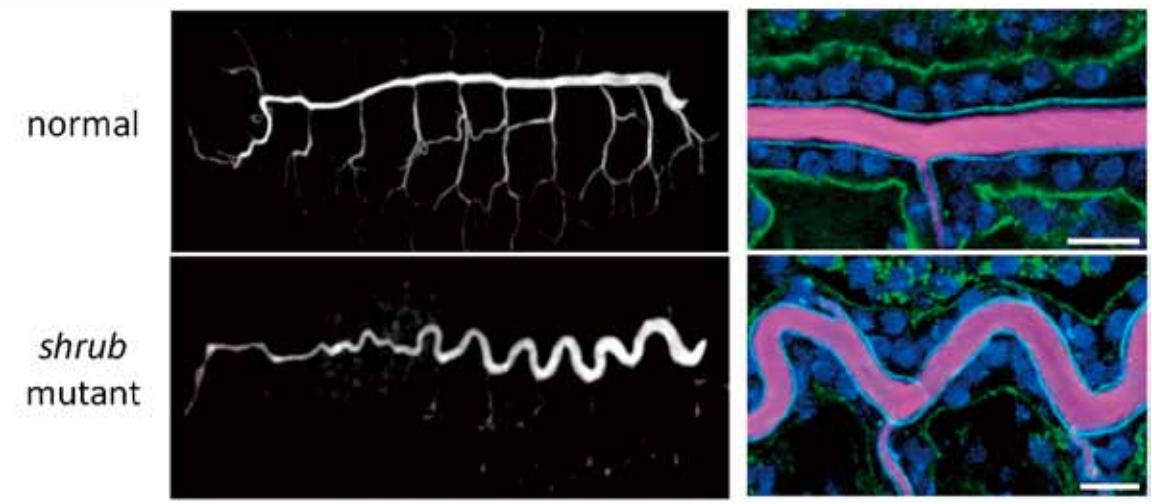
Dong B, et al. Balance between Apical Membrane Growth and Luminal Matrix Resistance Determines Epithelial Tubule Shape. *Cell Rep* 7. 941-50 (2014)

The defective transport machinery in *shrub* mutants results in many membrane proteins accumulating in the cytoplasm rather than at the membrane; the apical membrane protein Crumbs (Crb), which is involved in apical membrane biogenesis, is one such protein. Crb is normally localized to the apical membrane, but in the *shrub* mutant, Crb was found amassed in the endosome, stimulating apical membrane overgrowth.

As the orderly wave-like pattern of the trachea tube in *shrub* mutants suggests a mechanical effect, such as a force antagonistic to the elongational force of the apical membrane, the group shifted to focus on the ECM filling the tracheal lumen, which is made up of chitin, a polysaccharide that uniformly fills the lumen, and of protein components such as Dumpy (Dp) a giant protein involved in maintaining epithelial integrity. They used GFP-tagged luminal proteins and FRAP (fluorescence recovery after photobleaching) experiments to investigate the physical properties of Dp and found that while it barely moves within the lumen, the protein itself is stretched as the tube elongates. Thus, the resistance from the elastic ECM is likely the force that counteracts the elongating force exerted by the apical membrane.

Dong et al. used these findings to establish a physical model of tube length that recreates the mechanism through computer simulation. Their model assumes that the apical membrane encircles and binds to the elastic cylindrical matrix; when apical membrane growth is stimulated, it creates an elongating force along the AP axis, which will in turn stretch the ECM. The trachea will stop elongating when the apical membrane's elongating force and the ECM's elastic force are balanced. The group was able to verify the relevance of their model by confirming that it gave close predictions of the actual phenotypes observed in *shrub* and other mutants with varying lengths and shapes of elongated trachea.

"As one of the major components of the body, the ECM is often seen as having a supportive role in the body, but our current study reveals that it has a prominent role in regulating morphogenesis as well," says Hayashi. "We hope to continue closing in on the mechanisms underlying how shapes of organisms, which are closely associated with function, are determined."



Left: *Drosophila* tracheal tube. The *shrub* mutant has an abnormally elongated and wavy tracheal tube running through the embryo. Right: Staining of tracheal lumen (pink), and of apical membrane (light blue), basement membrane (green) and nucleus (dark blue) of tracheal epithelial cells shows excess apical membrane growth in *shrub* mutant, but with no major differences in cell number.

Epidermal Wnt/ß-catenin signal regulates adipocyte differentiation in underlying hypodermis

Hironobu FUJIWARA

The mammalian skin is an important multifunctional organ with roles in sensation, thermoregulation and metabolism, in addition to protecting the body from the external environment. It is organized into a complex layered structure of epidermis, dermis and hypodermis, with each layer made up of a mix of cell and tissue types. These cells and tissues use intercellular communication to guide morphogenesis, as well as maintenance (or regeneration) of the skin. The synchronicity between the growth cycle of hair follicles (HFs) and the fluctuation in the thickness of the dermal adipocyte layer (hypodermis) is one model of intercellular communication in the skin. The hypodermis increases in thickness as HFs enter the growth phase to extend deeper into the dermal layer, and becomes thinner when HFs regress and enter the quiescent phase. While the hypodermis has been reported to secrete factors that control the HF growth cycle, it remained unknown whether, conversely, the HFs regulate adipogenesis or thickness of the hypodermis.

Now, a new study by Hironobu Fujiwara (Team Leader, Laboratory for Tissue Microenvironment) and his former colleagues at the Cancer Research UK Cambridge Research Institute, in collaboration with scientists at Kings College London, reveals that activation of the canonical Wnt/ß-catenin signaling in the epidermis, which is known to promote HF growth, induces adipocyte differentiation in the hypodermis. Published in the *Proceedings of the National Academy of Sciences*, they show that epidermal Wnt/ß-catenin signal regulates morphogenesis of the hypodermis by triggering the secretion of adipogenic factors, synchronizing the HF growth cycle with adipocyte differentiation.

Because activation of the epidermal Wnt/ß-catenin signaling pathway is known to induce HFs to enter the growth phase, the research group turned their attention to this signal to determine its effects on the hypodermis. They first generated a transgenic mouse, in which the epidermal

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)

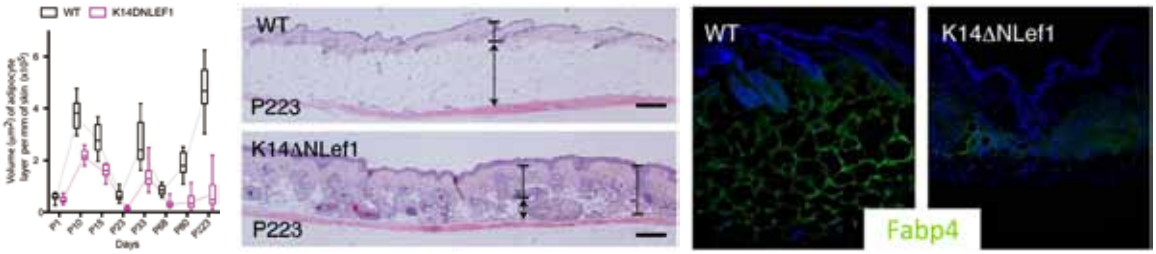
Wnt/ß-catenin signaling was blocked. During embryonic skin development, HF growth appeared normal in this mouse, but the hypodermis was thinner overall than that of wildtype. There was, however, an increase in the thickness of the reticular dermis, the lower layer of the dermis, which appeared to compensate for the thinner hypodermis. In the adult mutant mouse, adipocyte progenitor numbers were normal, but there was a significant decrease in the thickness of the differentiated adipocyte layer. Therefore, epidermal Wnt/ß-catenin signaling is important for hypodermal adipocyte differentiation, and when blocked, limits the differentiation of adipocyte progenitors to mature adipocytes.

The group next analyzed the effects of activating the epidermal Wnt/ß-catenin signal in the hair growth cycle, and found that HFs in the rest phase were induced to enter the growth phase and the hypodermis showed simultaneous expansion when Wnt/ß-catenin was activated. While there were no changes in the number of adipocyte progenitors, they did see a rise in the thickness of the differentiated adipocyte layer. And when epidermal Wnt/ß-catenin signal was activated in a transgenic mouse with impaired HF development, they observed that adipogenesis could be induced even in the absence of HFs, indicating that Wnt/ß-catenin signal acts directly on the dermal layer to induce adipogenesis, not indirectly via HF formation.

Because the epidermis and dermis are separated by a basement membrane, it was thought that the epidermal Wnt/ß-catenin signal is conveyed to the subdermal layer via a factor secreted by the epidermis. After reanalyzing gene expression profile data of previously reported transgenic mice with induced activation of epidermal Wnt/ß-catenin signal, the group discovered that genes related to extracellular regions were upregulated in the epidermis, and genes related to fat metabolism, differentiation, and proliferation were upregulated in the underlying dermis. Thus, the analyses suggest that a secreted factor is produced from the epidermis, and that responses such as adipocyte proliferation and differentiation are seen in the dermis when epidermal Wnt/ß-catenin is activated.

To confirm whether the epidermis secretes adipogenic factor(s), the group cultured adipocyte progenitors in medium conditioned with epidermis from different transgenic mice. In medium conditioned with epidermis in which Wnt/ß-catenin activation was induced, more progenitors entered adipogenesis prematurely than seen under WT conditions, and in medium conditioned with Wnt/ß-catenin–inhibited epidermis, very few adipocytes were generated. These results indicated that epidermal Wnt/ß-catenin signal triggers the secretion of an adipogenic factor from the epidermis, which in turn induces adipocyte differentiation. They tested possible candidate factors gleaned from the gene expression profile data, and identified three factors (BMP2, BMP6, and Igf2) secreted by the epidermis that triggered adipocyte differentiation.

“Our study demonstrates that epidermal Wnt signal regulates adipogenesis in the hypodermis, and synchronizes adipocyte differentiation with HF growth cycle,” says Fujiwara. “For growing HFs to extend below the skin, additional space needs to be created in the dermal layer to accommodate the downgrowth. It is possible that inducing adipocyte differentiation, which has high volume expansion rates, is an efficient way for HFs to accomplish this.”



Hypodermis in the skin is thinner in Wnt/ß-catenin blocked transgenic mouse than in WT. Left: Changes in volume of hypodermis over 223 days after birth in WT and transgenic mouse. Two center panels: Skin sections. Transgenic mouse has thinner hypodermis and thicker dermis. Right: Immunostaining of sections. Differentiated adipocytes marked by Fabp4 antibody (green).

2014 Courses

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 a degree-granting 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.

Summer School for high school students

The CDB's eighth annual one-day summer workshops for high school students were held this year on August 5 and 28. The theme of this year's workshop was "Development and Differentiation," and included a talk and laboratory tour by a CDB scientist and lab experiments. Taking *C. elegans* as a model, the students learned how molecular factors function during cell division, and how different types of cells could be produced through asymmetrical cell division by showing movies and images. In the hands-on component, students used SDS-PAGE electrophoresis to analyze proteins from brain, liver, and muscle tissues.

Undergrad internships

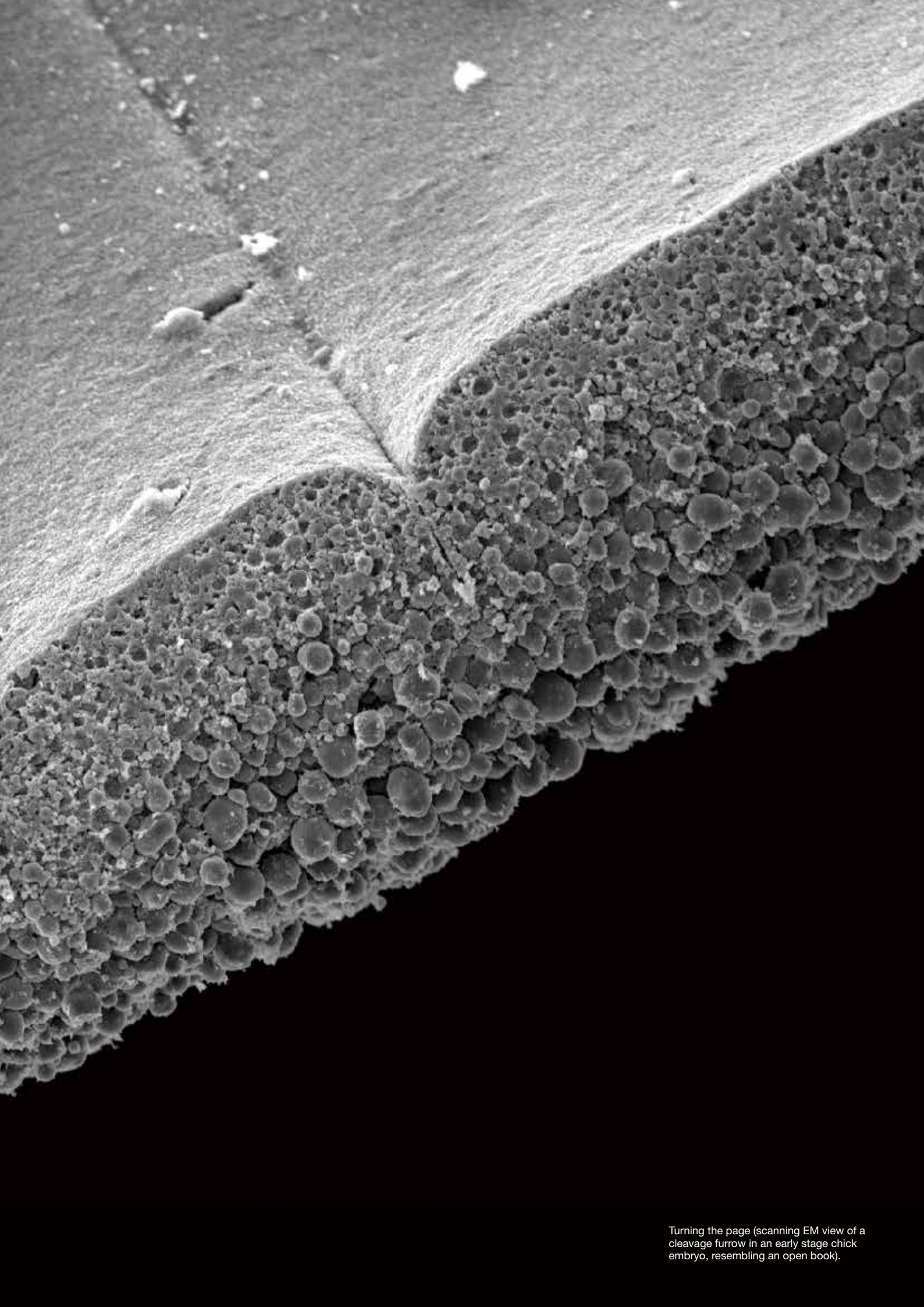
The CDB invited 34 undergraduate students from universities around Japan to spend one week in August as an intern working alongside research scientists in various labs at the CDB. In addition to carrying out small-scale projects in the host labs, the students had the chance to hear about the research being done at the CDB through lectures, and lab visits.

Graduate student lectures

The RIKEN CDB maintains close ties with a number of major graduate and medical schools in the Kansai area, and hosts a two-day lecture program every year to introduce the students to the labs at the center. Nearly 200 students convened at the CDB this year to take part in this year's program, which was held on August 27 and 28. The event also included exhibitions of model organisms and tours of the lab facilities, such as the research aquarium and electron microscopy room.

High school teacher workshop

The CDB, with support from the Japanese Society for Developmental Biologists (JSDB), organized a weekend workshop for high school teachers held on October 4 and 5. This annual workshop gives the teachers the opportunity to learn actual experimental techniques used in developmental biology, and learn how they can adapt these techniques for a classroom setting. This year's course focused on microscopic analysis of tissue morphology.



Turning the page (scanning EM view of a cleavage furrow in an early stage chick embryo, resembling an open book).

Chromosome Segregation

Tomoya KITAJIMA Ph.D.

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

Tomoya Kitajima received his Master's and doctoral degrees from the University of Tokyo, for his thesis on genetic screens for meiosis-specific proteins regulating chromosome segregation in fission yeast. After receiving his Ph.D. in 2004, he served as research associate at the Institute of Molecular and Cellular Biosciences at the same university, before moving to the European Molecular Biology Laboratory in Heidelberg, Germany as a postdoctoral researcher. He was appointed Team Leader at the CDB in 2012.

In order to maintain genetic information across generations, cells must allocate chromosomes equally to daughter cells during mitosis. Meiotic divisions of the mammalian oocyte, however, are known to exhibit a higher frequency of errors in chromosomal segregation than in other cell types. Oocytes formed from such divisions are aneuploid, meaning they have incorrect numbers of chromosomes; if these are fertilized and develop to term, the resulting individual may exhibit congenital anomalies, such as trisomy 21 (Down syndrome). Such errors in chromosomal segregation are also known to increase with the age of the mother, and this risk may be a contributing factor to the low birth rates seen in many developed nations.

Using the mouse as a model, we will seek to conduct detailed and comprehensive analyses of the dynamics of chromosomes and the molecular machinery that underlies chromosome segregation during cell division. We plan to take advantage of the latest live imaging technologies to study the chromosome dynamics of the mouse oocyte at a level detail unprecedented in other cell types. Oocyte chromosomes behave in ways distinct from those in other cells, and these unique dynamics may provide insights into novel mechanisms for chromosome allocation. By combining live imaging with genetics techniques such as RNAi and gene knockouts, we hope to study the mechanisms underlying chromosomal segregation in oocyte meiosis, and identify the causes behind age-related increases in ploidy errors.

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Publications

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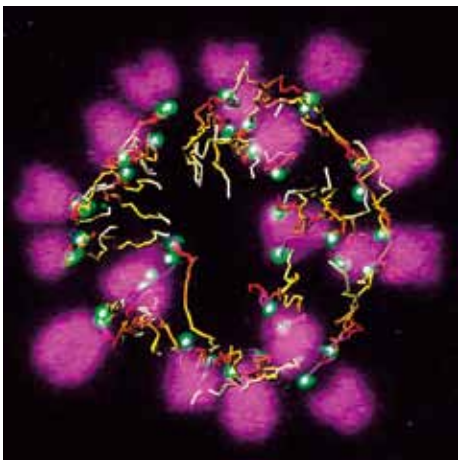
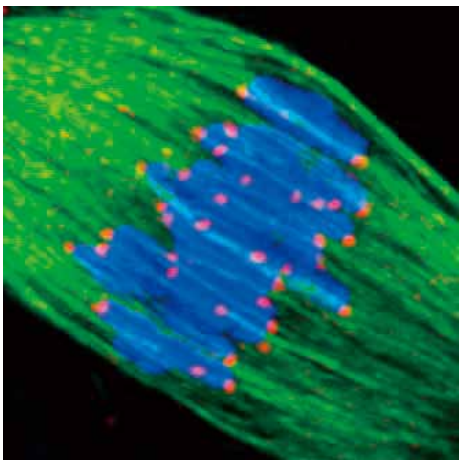


Chromosomes form the belt-like structure during prometaphase.



Kinetochore-microtubule attachment

Histogenetic Dynamics

Erina KURANAGA Ph.D.

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

Erina Kuranaga received her doctorate in medical science from the Osaka University Graduate School of Medicine in 2004, after which she moved to the University of Tokyo Graduate School of Pharmaceutical Sciences as assistant professor in the Department of Genetics. In 2006, she was promoted to associate professor in the same department, where she remained until she joined the RIKEN Center for Developmental Biology as team leader in 2011. She was awarded the Wiley-Blackwell Prize 2012 and Editor-in-Chief Prize from Development, Growth and Differentiation in 2014.

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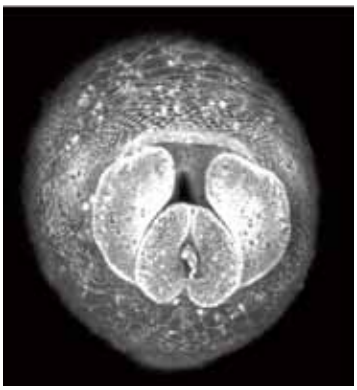
The development of multicellular organisms involves the collective effect of multiple events at the level of the individual cell, such as proliferation, differentiation, adhesion, and migration. Programmed cell death, for example, is a process by which cells are selected for death at set times in development, 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 disease. 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 a 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 caspase function and cell death control acceleration of the rotation through searching for associated genes and live imaging analysis. It has further been predicted that cell death alone cannot account for rotation that maintains 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. Location of male genitalia is pointed in yellow square.



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

Evolutionary Morphology

Shigeru KURATANI Ph.D.

Shigeru Kuratani received his masters and Ph.D. from the Kyoto University Department of Zoology. He spent the period from 1985 to 1988 studying comparative embryology in the Department of Anatomy, University of the Ryukyus, and 1988 to 1991 working in experimental embryology in the Department of Anatomy at the Medical College of Georgia before moving to the Biochemistry Department at the Baylor College of Medicine, where he was engaged in molecular embryological research. He returned to Japan in 1994 to take a position as associate professor at the Institute of Medical Embryology and Genetics in the Kumamoto University School of Medicine. He moved to Okayama University to assume a professorship in the Department of Biology in 1997, where he remained until he was appointed team leader at the CDB. He was appointed group director in 2005.

As part of the reorganization of the CDB in November 2014, the Evolutional Morphology lab was transferred to the RIKEN Chief Scientist Laboratories program.

By studying the evolutionary designs of diverse animal species, I hope to gain a deeper insight into the secrets behind the fabrication of morphological designs. Integrating the fields of evolutionary morphology and molecular genetics, our lab seeks to expand the understanding of the relationship between genome and morphology (or body plan) through investigating the evolutionary changes in developmental processes, and also the process of evolution in which phenotypic selection shapes developmental programs. Our recent studies have focused on novel traits found in the vertebrates, such as the jaw, the turtle shell, and the mammalian middle ear. By analyzing the history of developmental patterns, I seek to open new avenues toward answering as-yet unresolved questions about phenotypic evolution in vertebrates at the level of body plans.

Through the study of evolutionarily novel structures, our lab has identified changes in developmental mechanisms that have obliterated the structural homologies between organisms as evidenced in such novel structures as the jaw in gnathostomes (jawed vertebrates) and the turtle shell. Developmental studies of the cranial region of the lamprey are intended to shed light on the true origins of the vertebrate head and neck, as lampreys lack a number of important features, including jaws, true tongues, and trapezius muscles, that are possessed only by gnathostomes. We aim to resolve the question of what primary factors have changed during evolution by comparing the developmental patterns that yield such innovations, and by the experimental construction of phenocopies in one animal that mimic structures in another.

The turtle's shelled body pattern appears at the end of a graded series of changes in the fossil record. Our lab's research into turtle carapace development addresses the developmental changes that resulted in this abrupt and dramatic morphological change, and is aimed at identifying genes that function differently in turtle and other amniotes, which it is hoped will provide a key to discovering the true targets of natural selection in the acquisition of a shell.

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Publications

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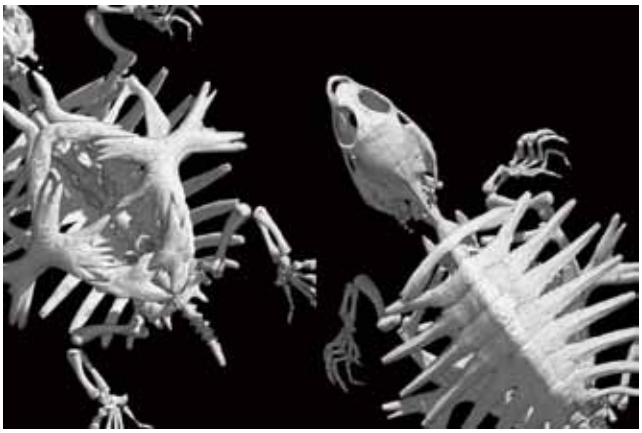

CT-scanned skeleton of *Pelodiscus sinensis* juvenile

Sensory Development

Raj LADHER Ph.D.

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

Raj Ladher received his B. Sc. in biochemistry from Imperial College, London in 1992 and his Ph. D. in developmental biology from the National Institute for Medical Research in 1996, for his thesis on the cloning of the *Xenopus* homeobox gene, *Xom*. He worked as a research associate at King's College, London from 1996 to 2000 in the lab of Pip Francis-West, and first came to Japan during that period as a visiting scientist at the Tokyo Medical and Dental University. In 2000, he moved to the University of Utah as a postdoctoral fellow in Gary Schoenwolf's laboratory. He was appointed team leader at the CDB in 2002.

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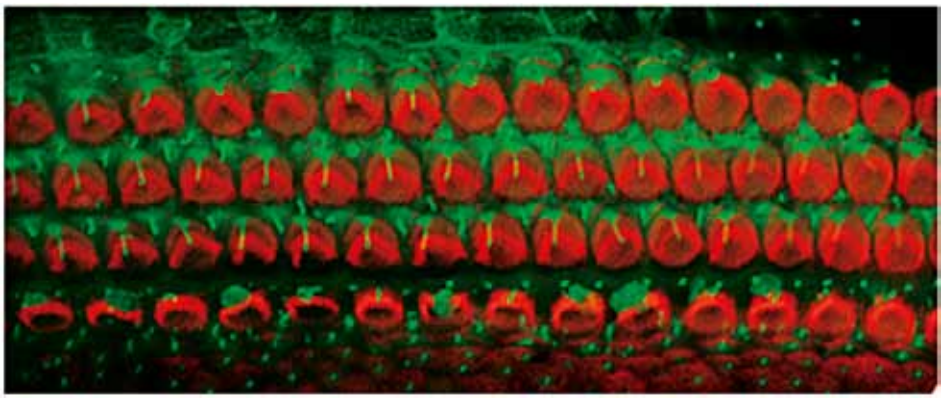
Hearing loss is the most prevalent sensory disorder not only in Japan, but also the world, with over 5% of the global population showing disabling hearing loss, rising to 50% in the aged population. Hearing occurs in the inner ear, a complex structure that houses the sensory receptors important for sound detection, as well as other non-sensory cell types that are equally important in the sensitive and precise detection of sound. This complex organ forms from a relatively simple structure known as the otic placode, found in the surface ectoderm of the early embryo. It is clear that the otic placode results from dynamic epigenetic processes that convert ectoderm into otic placode and with it establish changes in the morphology of the placode, driving the internalization of the otic placode to make the first rudiments of the inner ear.

Developmental mechanisms also subdivide the otic placode into different cellular territories that include both sensory and non-sensory progenitors. These form as a result of balancing specification and patterning with proliferation, and then subsequently the maturation of these cell-types. We want to understand the blueprint for making an inner ear, with particular emphasis on integrating extrinsic signals, the genes that they control, with the cellular and sub-cellular changes that drive phenotypic changes that adapt the cell to its function. Our ultimate aim is to use our developmental knowledge to make better cell differentiation protocols that can ultimately used to design screening protocols, diagnostic tools and cellular therapies that help in alleviating hearing loss.

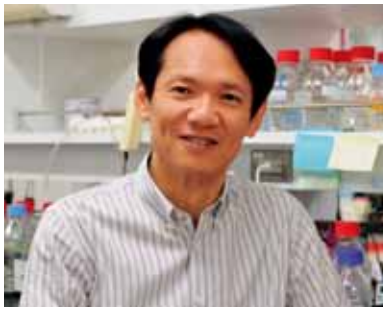


Ciliated mechanoreceptors, or inner ear hair cells, transduce sound information in the organ of corti, part of the mammalian cochlea. Shown are the four rows of inner ear hair cells that make up the organ of Corti, stained with alpha-tubulin (to show kinocilia in green) and phalloidin to show the actin-based stereocilia (in red).

Cell Asymmetry

Fumio MATSUZAKI Ph.D.

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



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.

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

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Publications

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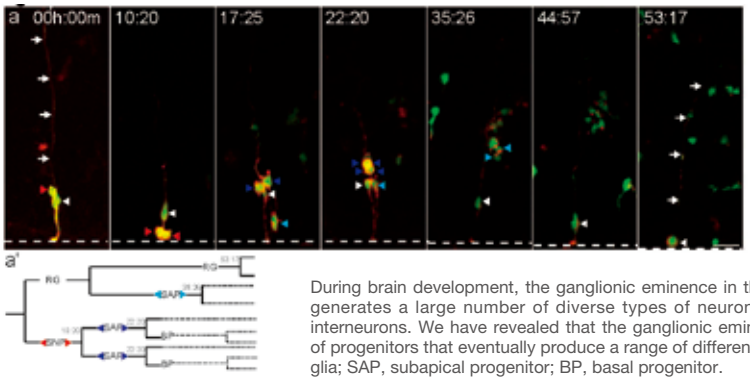


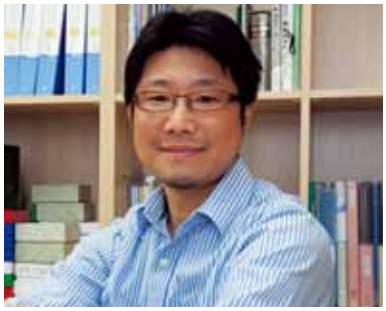
During brain development, the ganglionic eminence in the ventral telencephalon generates a large number of diverse types of neurons including GABAergic interneurons. We have revealed that the ganglionic eminence generate a variety of progenitors that eventually produce a range of different cell lineages. RG, radial glia; SAP, subapical progenitor; BP, basal progenitor.

Lung Development

Mitsuru MORIMOTO Ph.D.

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

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



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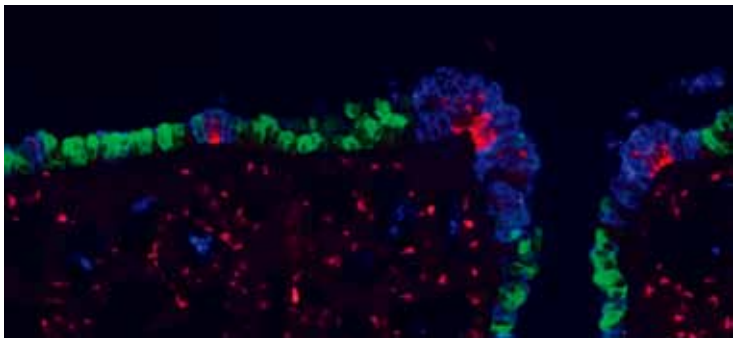
The organs of the body all play critical functional roles, which are made possible by the arrangement of differentiated cells into the structures specific to that organ. Such structures are formed throughout development, with the late embryonic and immediate postnatal periods being particularly important 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.

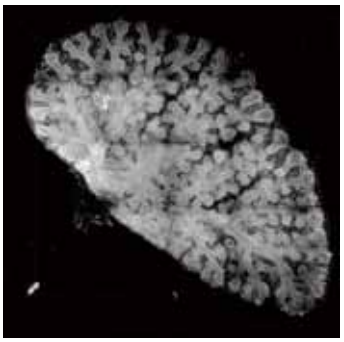
Respiratory organs in higher mammals are characterized by their efficient gas exchange, enabled by the functions of specialized cells. The development of such organs relies on the coordinated activities of both epithelial and mesenchymal tissue types, which arise from tissue-specific populations of stem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



Three dimensional image of embryonic bronchi reconstructed from multi-photon images

Developmental Morphogeometry

Yoshihiro MORISHITA Ph.D.

Yoshihiro Morishita received his M.Sc. and Ph.D. from the University of Tokyo Graduate School of Frontier Sciences. From 2005 to 2007, he served as Project Assistant Professor in the Kyushu University Department of Biology. From 2007 to 2011, he served both as assistant professor in the Theoretical Biological Laboratory at Kyushu University and as a researcher funded under several JST programs. He was named Research Unit Leader at the RIKEN CDB in 2012.

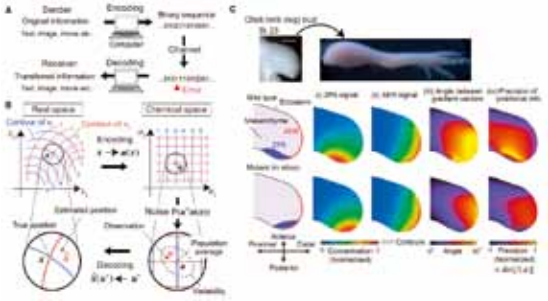
As part of the reorganization of the CDB in November 2014, the Developmental Morphogeometry lab was transferred to the RIKEN Quantitative Biology Center.

Developmental phenomena comprise a multiscalar system extending across a range of spatial scales, from molecular to cellular to histological. Such phenomena are also multiphysical, in that they involve the transmission and reception of positional information through diffusion and reactions of chemicals, and the generation of forces within tissues and concomitant geometrical deformation through the proliferation and migration of cells. The Laboratory for Developmental Morphogeometry takes theoretical and experimental approaches to the study of phenomena comprising multiple scales and properties, and the quantitative measurement of dynamic and coordinated interactions between such phenomena.

An example of this is seen in organogenesis, a process in which the various tissue regions that give rise to the organ exhibit changes in volume at different rates, or anisoptropic expansion and contraction. Such deformations can be quantified as tensor quantities (geometrical characteristics). The morphological differences between various organs, or between homologous organs in different species can thus be explained as spatio-temporal patterns in the tensor quantities of each object. We are now able to extract such patterns using organ-level quantitative imaging and statistical analysis. By combining and comparing such data with the accumulated body of molecular and cellular evidence, we hope to develop clearer insights into the relationships between macro-scale organ morphogenesis and micro-scale phenomena.

The ability of individual cells to recognize and respond to (for example, through proliferation and differentiation) their positions within a tissue is also essential to tissue growth and patterning. This necessitates accurate “spatial recognition” on the part of cells, which receive environmental cues (such as gradients of growth factors or interactions with neighboring cells), but this is complicated by uncertainty arising from perturbations within the organism (such as inter-individual variations in morphogen expression levels). Questions of how to maximize the accuracy of the transmission and reading of information against a background of uncertainty is formalized as problems in information coding. Analysis of such problems has revealed optimal sites of the expression of information sources (morphogens) and optimal designs for the form and parameter values of response functions implemented by intracellular biochemical reactions. By comparing the results of these theoretical analyses with experimental observations, we can begin to assess the extent to which actual developmental systems are designed to optimal criteria.

Our lab will seek to use 1) analysis of measured data and 2) study system designs through theoretical formulation and computer simulations with an eye to developing a better understanding of these phenomena.



Information coding process in computer science (A) and in developmental biology (B). In former, information is coded through binary electronic signals, while in latter, the information, spatial coordinate, is coded through chemicals. (C) Optimal arrangement of morphogen source (Shh-source) to maximize the precision of positional information in vertebrate limb bud.

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Growth Control Signaling

Takashi NISHIMURA Ph.D.

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

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Matsuda H., et al. Flies without Trehalose. *J Biol Chem* 290.1244-55 (2015)

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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 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 of peptides have conserved roles in the regulation of growth and metabolism in a wide variety of metazoans. We are now analyzing the molecular mechanism underlying the nutrient-dependent expression of Dilp genes. 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. The identification of SDR protein in *Drosophila* provides a new concept for the regulation of insulin/IGF signaling.

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

Strategy of bending by giant cadherins

Yoshikazu TSUKASAKI

The cadherin superfamily of transmembrane proteins play an important role in mediating cell-cell adhesion, which is fundamental for maintaining organization of multicellular organisms. They require calcium ions (Ca^{2+}) to function, and also regulate many developmental processes such as tissue and neural circuit formation through their intercellular interactions. Cell-cell adhesion is achieved through homophilic or heterophilic binding between cadherins of adjacent cells through their filamentous extracellular domains (ectodomains). The so-called "classical" cadherins have ectodomains consisting of five repetitive units called, extracellular cadherin (EC) domains, which are joined together with a segment containing a Ca^{2+}-binding motif (CBM), an amino acid sequence that can bind Ca^{2+}. When Ca^{2+} binds to the CBM, it activates the cadherin by causing the ectodomain to elongate into a filamentous morphology and interacts with its partner molecule in cell adhesion. But when Ca^{2+} is depleted, the ectodomain loses its elongated morphology, and consequently, cell-cell adhesion is also lost. The ectodomains of "non-classical" cadherins such as protocadherins, Fat, and Dachsous have varying numbers of EC domains, ranging from five up to 34. However, these larger cadherins have also been found to occupy intracellular spaces with spans smaller than the linear length of its ectodomain. Thus, this raises the question of how larger cadherin molecules are able to fit into relatively narrow intercellular spaces.

Now, a new study by research scientist Yoshikazu Tsukasaki of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader) and other colleagues demonstrates a strategy of bending adopted by the giant cadherins, Fat and Dachsous, which allows them to squeeze into narrow intercellular spaces despite their large size. Published in the *Proceedings of the National Academy of Sciences (PNAS)*, their findings indicate that some segments of the large ectodomain of Fat and Dachsous have been modified to prevent Ca^{2+}-binding, and facilitate the folding of these domains to assume a more compact form. Tsukasaki is now at the University of Texas Health Science Center.

The mammalian Fat4 and Dachsous1 form a heterophilic binding that regulates planar cell polarity and proliferation. Fat4 is the largest molecule within the cadherin superfamily with 34 EC domains, and Dachsous1 is also large with 27 EC domains. Tsukasaki et al. first examined purified ectodomain structures of both cadherins under transmission electron microscopy (TEM), and found that while the N-terminal end of ectodomains, the region furthest from the cell membrane, displayed a relatively linear morphology, the C-terminal end which is close to the membrane had multiple locations where the filament was sharply bent. When the amino acid sequences of the CBMs were analyzed, they found modifications of amino acid residues in several of the CBMs near the C-terminal end, suggesting the possibility that these modifications prevent Ca^{2+}-binding.

The group generated mutants of the classical cadherin, E-cadherin, by replacing one of its CBMs with a modified CBM from Fat4. TEM revealed that these E-cadherin mutants displayed a bent arrangement in the location where the CBM was switched. Gel filtration experiments measuring elution speed showed that both wildtype E-cadherin run in Ca^{2+}-free conditions and the CBM-replaced E-cadherin mutants moved slower than the

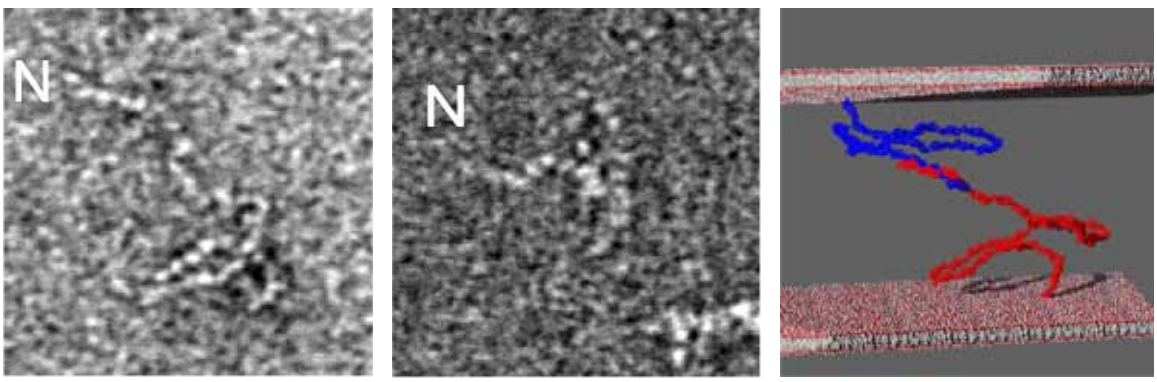
Tsukasaki Y, et al. Giant cadherins Fat and Dachsous self-bend to organize properly spaced intercellular junctions. *Proc Natl Acad Sci U S A* (2014)

wildtype E-cadherin run in presence of Ca^{2+}; these results supported their hypothesis that the modified CBM found in Fat and Dachsous is unable to bind Ca^{2+}, thus leading to a nonlinear ectodomain.

To further understand the morphological changes that occur in CBM-replaced E-cadherin, the group carried out computer simulations using the elastic network model based on the known X-ray crystal structure of E-cadherin. The simulations showed that, in CBM-replaced E-cadherin, the segments with modified CBM exhibited a wide range of bent configurations, consistent with TEM observations. They applied the same method for Fat4 and Dachsous1, and successfully created atomic models that matched morphologies seen under TEM. In a separate series of experiments, they also found that the four most N-terminal ectodomains appeared to be sufficient for Ca^{2+}-mediated heterophilic binding between Fat4 and Dachsous1, and that this binding is unaffected even when the C-terminal end is bent.

Tsukasaki et al. next analyzed the morphology of Fat4 and Dachsous1 in vivo, by transfecting a cell line with Fat4 or Dachsous1 cDNAs. When these separately transfected cells were co-cultured, they observed the formation of Fat4-Dachsous1 bindings at the cell boundaries between the two cell types just above the tight junctions. Closer analysis with TEM revealed that the intercellular spaces in which the Fat4-Dachsous1 binding complexes were found were approximately 47 nm wide, matching the dimensions of the molecules calculated from the elastic network model. Observations of Fat4-Dachsous1 binding in the mouse embryonic cortex also showed the span between cell boundaries were similar to those observed in the co-culture of transfected cells.

“It is a mystery as to why giant cadherins have gone as far as losing their Ca^{2+}-binding ability to squeeze into intracellular spaces by folding,” says Takeichi. “What is interesting is that the size of Fat and Dachsous, and the positions of modified CBMs within the ectodomain have been conserved across species. No doubt the bending strategy revealed in our study is closely linked to the physiological function of these molecules.”



TEM images of Fat4 (left) and Dachsous1 (middle). In both molecules, the N-terminal end had an elongated configuration, while the C-terminal end had a bent configuration. The right panel shows a simulated 3D structure of Fat4 (red) and Dachsous1 obtained from the elastic network model.

Protocadherin mediates collective axon extension of neurons

Shuichi HAYASHI

Formation of the complex neural network involves neurons extending a single axon to target and synapse with a dendrite sprouting from another neuron, which will in turn synapse with the next neuron. Some axons can extend up to one hundred times the length of the cell body to reach their target. The axons of neurons making up the amygdala, which is located deep in the middle of the brain mass, migrate along the hippocampus, making a long journey to reach the hypothalamus. These extending axons move collectively by forming fascicles (or bundles) consisting of several hundred to several thousand axons derived from the same neuronal group, but the underlying mechanism regulating fascicle formation and collective axonal migration remained unclear.

In a new study published in *Developmental Cell*, research scientist Shuichi Hayashi of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader) and colleagues identify one factor that plays an essential role in the collective axon extension of amygdala neurons. Using the developing mouse brain as a model, their findings indicate that protocadherin-17 (Pcdh17), a member of the cadherin superfamily of cell adhesion molecules, mediates axon-axon interactions and recruits actin regulators to the interaxonal contact sites which modify the surrounding actin dynamics to promote cell migration.

Protocadherins are broadly classified as being either clustered or non-clustered. Past studies suggest that clustered protocadherins have some function in the self-repelling mechanism of dendrites to prevent binding between dendrites from the same neuron, while non-clustered protocadherins have been implicated as a crucial player in axon elongation during neural network formation in the brain. In this study, Hayashi et al.

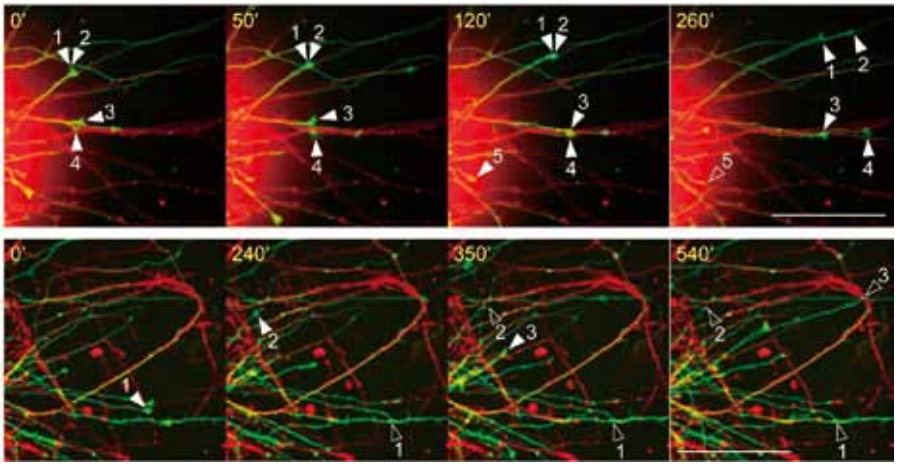
Hayashi S, et al. Protocadherin-17 mediates collective axon extension by recruiting actin regulator complexes to interaxonal contacts. *Dev Cell* 30.673-87 (2014)

focused their attention on a non-clustered protocadherin, Pcdh17, and its expression in amygdala neurons. Pcdh17 expression was detected in several subsets of amygdala neurons. Upon close examination, they found that Pcdh17 expression in these neuronal subsets was localized around the interaxonal adhesion sites, leading them to consider the role of Pcdh17 in the collective elongation of axons in amygdala neurons.

They next generated a *Pcdh17* knockout mouse line, and an analysis of its phenotype revealed that loss of Pcdh17 results in abnormal axonal elongation; the axon fascicle extending from the amygdala to the hypothalamus was thinner. The thinner fascicle was due to a collapse of the growth cones, the motor center located at the tip of growing axons. Labeling the Pcdh17 expressing and non-Pcdh17 expressing neurons with different colored fluorescent proteins, the group observed growth cone migration using live-imaging and found that, in wild-type conditions, when the elongating axons came into contact with an axon from other neurons of the same subtype, it continued to elongate along the other axon, whereas in the Pcdh17 mutant, the axon stopped elongating when it came into contact with another neuron of the same subtype.

So then, how is the migration of axons regulated? Hayashi et al. confirmed that, similar to other cadherins which mediate intercellular interactions, the extracellular domain of Pcdh17 facilitates the binding of axons of similar neuronal types. Using a pull-down assay, they next looked into which molecules bind to the intracellular domain of Pcdh17 and identified several factors that make up the WAVE complex, which is involved in actin polymerization. They also determined that Pcdh17 works together with the WAVE complex to recruit other downstream factors that plays a role in cell motility of the growth cone as well as at the interaxonal adhesion sites. The role of Pcdh17 mediating cell migration was further verified by observing the behavior of Pcdh17-transfected cultured cells that showed similar directional movements to neuronal growth cones. Thus, the results showed that Pcdh17 promotes cell migration by localizing at interaxonal adhesion sites and recruiting the WAVE complex and further downstream factors, which enhance the efficiency of axon elongation.

“The function of cadherins largely differs depending on the molecules it coordinates with,” explains Takeichi. “Neuronal axons can extend on their own, but when they form a fascicle, the contact enhances axon migration. Recent research shows that malfunction of protocadherins can lead to autism and schizophrenia. As the amygdala is a part of the brain that functions in emotion and memory, future studies looking at the relationship between protocadherins and amygdala will be very interesting.”



Still frames taken from live-imaging movie of axon elongation of amygdala neurons. Under normal conditions, when an axon comes into contact with another axon, it will continue elongating along the other neuron (top row). In Pcdh17 mutants, an axon stops elongating when it comes into contact with another axon (bottom row). Neurons express Pcdh17 are labeled green, non-Pcdh17 expressing neurons are labeled red.

Fat body protein shapes tracheal system

Bo DONG

The *Drosophila* tracheal system is a branched network of epithelial tubes extending throughout the body to transport oxygen to the tissues. During tracheal development, the tracheal lumen is filled with apical extracellular matrix (ECM), which is later cleared out and replaced by gases prior to larval hatching. Components of the apical ECM, such as proteins and chitin, which is also an important component of the exoskeleton protecting the fly's exterior, play important roles in tracheal morphogenesis by regulating the length and diameter of the tubes. One ECM protein required for tracheal development is a chitin deacetylase, serpentine (Serp). Past studies have reported that overexpression or loss of *serp* leads to overelongated and convoluted tracheal tubes. While tracheal cells are the major source of ECM proteins, including Serp, secreted into the lumen, it remains unclear whether and to what extent other tissues contribute to tracheal tube development.

Now, new work by Bo Dong and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader) reveals the *Drosophila* fat body, a mesoderm-derived tissue that plays physiological roles similar to the vertebrate liver, as a surprising new source of luminal Serp during tracheal morphogenesis. Published in the journal *Development*, their findings demonstrate that a non-epithelial tissue plays a role in tracheal morphogenesis and provides additional evidence of the fat body supporting the developmental process of various organs.

The insect fat body functions as a site of synthesis and storage of sugars, lipids and proteins, which are secreted into the hemolymph to be delivered to tissues and organs of the body as needed to maintain homeostasis. Dong et al. first confirmed the presence Serp in the fat body as well as in the tracheal system of fly embryos, consistent with previous reports. Then, using two different

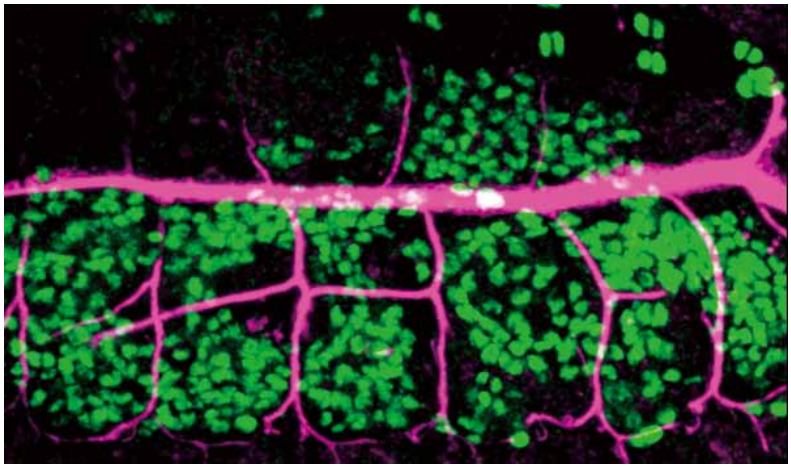
Dong B, et al. A fat body-derived apical extracellular matrix enzyme is transported to the tracheal lumen and is required for tube morphogenesis in *Drosophila. Development* 141.4104-9 (2014)

mutant fly strains (*rab9* and *shrub*) in which glitches in vesicular mechanisms affect Serp localization resulting in phenotypes similar to *serp* mutants (i.e. overelongated tracheal tubes), they found that both mutants showed higher levels of Serp in the fat bodies and lower Serp levels in the tracheal lumen than seen in wildtype, a pattern that was not observed with other apical ECM proteins. Additional experiments specifically blocking endocytic or exocytic pathways of fat bodies indicated that the source of Serp accumulation in the fat bodies of the mutants was cell-intrinsic synthesis, ruling out the possibility that Serp was being translocated there from an external source.

Under normal conditions, Serp does not remain localized in the fat body, so the group next examined whether Serp synthesized by the fat body can be delivered to the lumen of the developing tracheal tube. They ectopically expressed a secreted luminal marker protein containing the chitin-binding domain of Serp (Serp-CBD-GFP) in the fat body and found that the marker accumulated in the tracheal lumen. In contrast, when a functionally inert secreted protein marker with a molecular size similar to Serp-CBD-GFP was expressed, it was absent from the lumen. Further, the authors used the technique of IR-laser induced local heat shock (Miao and Hayashi, 2014) to demonstrate that Serp-GFP could be translocated to the lumen even after the developing tracheal tube acquired epithelial barrier function, suggesting that Serp is taken up by tracheal cells from the hemolymph and deposited in the lumen via transcytosis.

Finally, the group looked at whether fat body-derived Serp plays a functional role in tracheal development. When Serp was ectopically expressed in the fat body of *serp* mutants, they observed that the overelongated tube phenotype could be rescued, suggesting that Serp secreted from the fat body plays a role in tracheal morphogenesis. Closer examination also revealed that the function of fat body-derived Serp appears to overlap with that of tracheal cell-derived Serp and supports tracheal tube development when the tracheal source is limited.

“To find that the fat body influences tracheal tube morphogenesis was unexpected,” says Hayashi. “We speculate that the fat body plays a role in balancing the supply of a key enzyme to the trachea and epidermis so that differentiation of those epidermal organs is controlled under the same temporal schedule.”



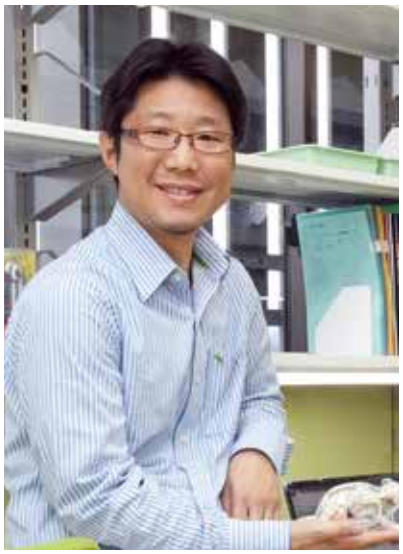
Tracheal cells secrete Serp protein into the lumen (magenta). Fat body cells (green) surround the trachea and serve as a secondary source of Serp.

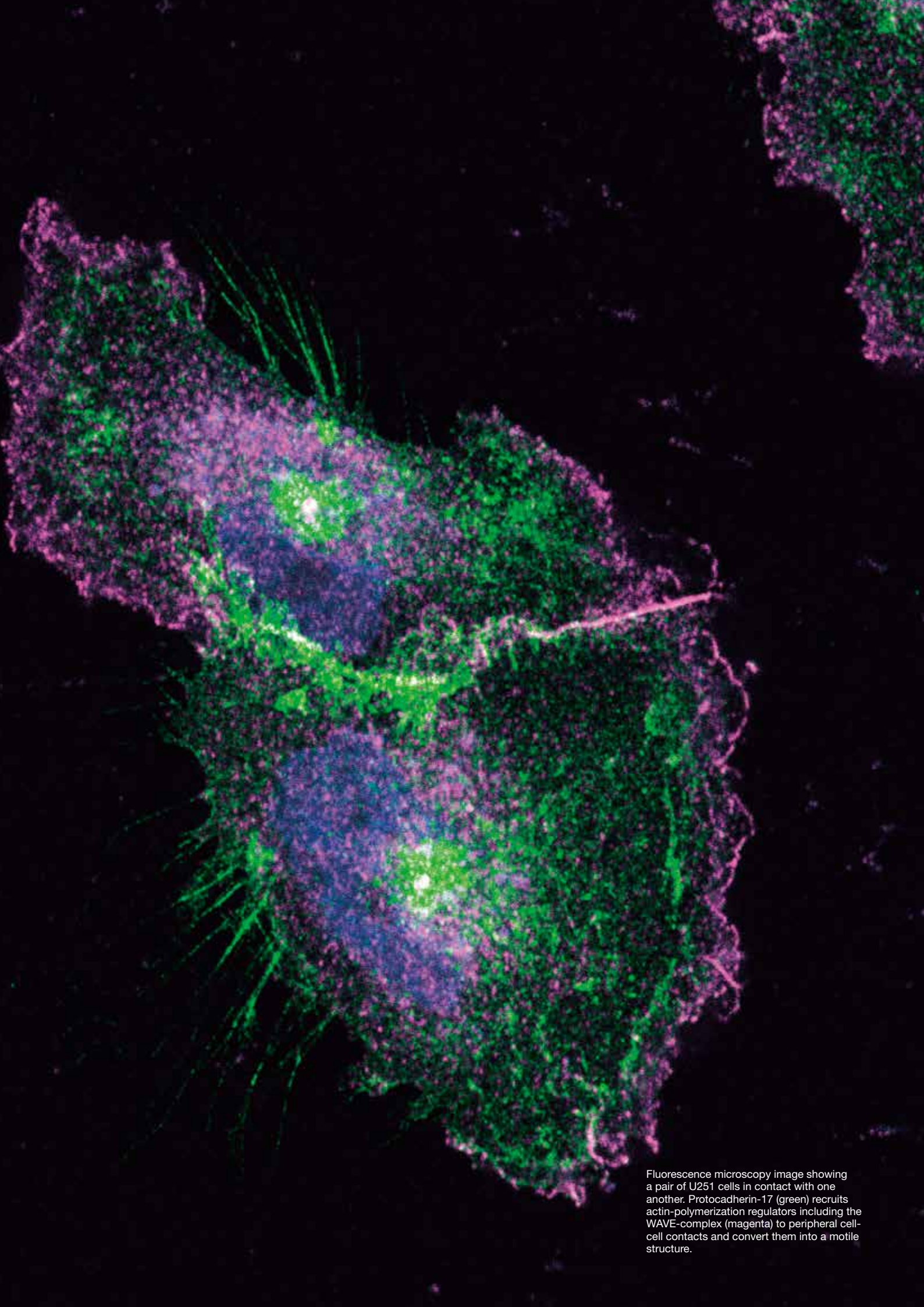
2014 Awards

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
Mitsuru Morimoto	Team Leader	Lung Development	The Young Scientists' Prize, The Commendation for Science and Technology	The Minister of Education, Culture, Sports, Science and Technology
Mitsuru Morimoto	Team Leader	Lung Development	International Session Award	The 55th Annual Meeting of the Japanese Respiratory Society
Erina Kuranaga	Team Leader	Histogenetic Dynamics	Editor-in-Chief Prize, Most Cited Paper	Development, Growth & Differentiation (Wiley Blackwell)
Masatoshi Takeichi	Team Leader	Cell Adhesion and Tissue Patterning	AAAS Fellow	American Association for the Advancement of Science (AAAS)



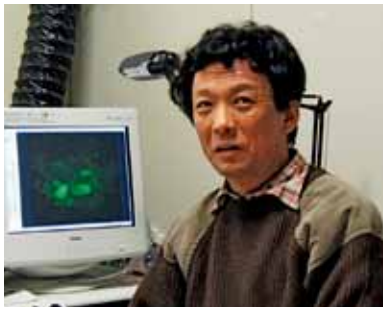


Fluorescence microscopy image showing a pair of U251 cells in contact with one another. Protocadherin-17 (green) recruits actin-polymerization regulators including the WAVE-complex (magenta) to peripheral cell-cell contacts and convert them into a motile structure.

Pluripotent Stem Cell Studies

Hitoshi NIWA M.D., Ph.D.

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



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

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

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

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Publications

Adachi K, et al. Context-Dependent Wiring of Sox2 Regulatory Networks for Self-Renewal of Embryonic and Trophoblast Stem Cells. *Mol Cell* 52.380-92 (2013)

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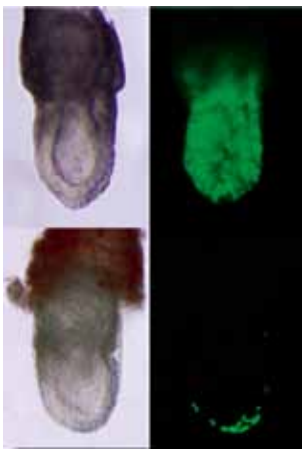
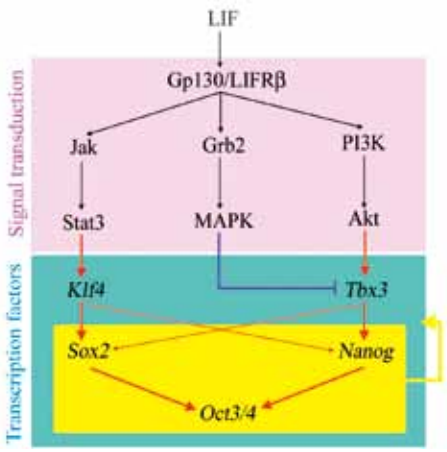


Chimeric embryos generated by injection of Gata6GR ES cells into blastocysts. These ES cells carry the constitutively-active Egfp transgene and contribute to primitive ectoderm (upper panels), whereas they contribute to parietal endoderm after induction of Gata6 activity with dexamethasone (lower panels).



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

Organogenesis and Neurogenesis

Yoshiki SASAI M.D., Ph.D.

http://www.cdb.riken.jp/en/research/laboratory/takeichi2.html

Yoshiki Sasai received his M.D. from the Kyoto University School of Medicine in 1986, subsequently performing internships in general practice and emergency medicine. He completed the Ph.D. course at the same institution in 1992, for work on neural specific transcriptional regulators. In 1993, he took a postdoctoral fellowship in the De Robertis lab at the UCLA School of Medicine, remaining there until 1996 when he was appointed associate professor at the Kyoto University School of Medicine. He assumed a professorship at the Kyoto University Institute for Frontier Medical Sciences in 1998, and was appointed group director at the CDB in 2000. He serves on the editorial boards of *Cell*, *Neuron*, *Developmental Cell*, *Genesis*, and *Developmental Dynamics*.

In memoriam Yoshiki Sasai (1962–2014)

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Publications

Kadoshima T, et al. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc Natl Acad Sci U S A* 110.20284-9 (2013)

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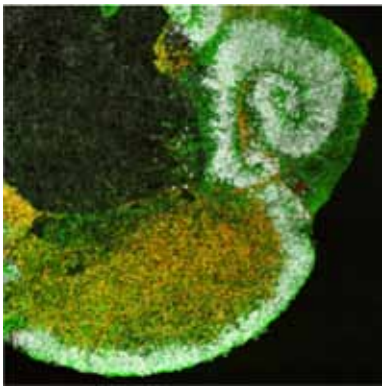
The complexity of the fully formed brain defies description, yet this organ arises from a nondescript clump of cells in the embryo. The specification of the dorsal-ventral (back-belly) axis is significant in neural development in that the central nervous system forms on the dorsal side of the body in all vertebrate orders. This process is dictated by the effects of a number of signaling factors that diffuse from organizing centers and direct dorsal ectoderm to maintain a neural fate. These molecules, which include Noggin, Chordin, Follistatin and their homologs, participate in elaborate signaling networks in which factors collaborate and compete to initiate the embryonic nervous system.

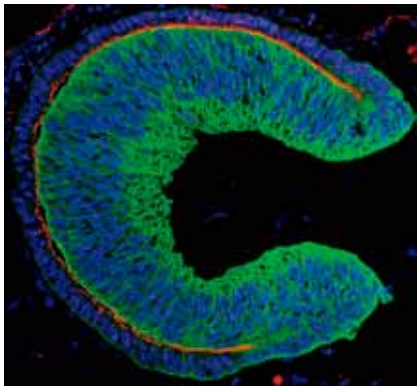
Using the African clawed frog, Xenopus laevis , as a model in molecular embryological studies, our group is engaged in clarifying the structure and extent of the signaling networks involved in setting up the dorsal-ventral axis and determining neural fate in the ectoderm. These studies focus on molecules that operate at early stages in embryonic development to lay down a complex of permissive, instructive and inhibitory patterns that determines the growth and differentiation of the brain in its earliest stages of development.

The group is now also actively developing effective methods of inducing neuralization in mammals, work which it is hoped will contribute to basic research by providing in vitro experimental systems for use in the analysis of mammalian neurogenesis. In addition, this approach has potential for applications in the treatment of neurodegenerative disorders, such as Parkinson disease. Using a system developed in our lab, we have succeeded in inducing mouse and primate embryonic stem (ES) cells to differentiate into a range of specialized neuronal types. The application of similar techniques to human ES cells represents a field of study that, although it remains at quite an early stage, shows immense clinical promise.

By studying very early neurogenesis, and the mechanisms of neuronal differentiation, our lab aims to understand the molecular basis underpinningthe formation of so intricate a system as the mammalian neural network, in the hopes that, by looking at the elegant simplicity of the embryonic brain, it may one day be possible to understand the cellular bases of the mind's activity.



Self-formation of layered cortical tissue from human ES cells

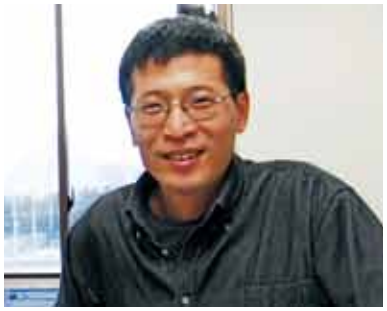


Self-organized formation of optic cup from human ES cells

Early Embryogenesis

Guojun SHENG Ph.D.

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



Guojun Sheng received his B.S. in microbiology from Fudan University in Shanghai, China in 1990. He entered the Rockefeller University the next year, attending the summer course in embryology at the Woods Hole Marine Biological Laboratory in 1996 and receiving his Ph.D. under Claude Desplan in 1997. He spent the period from 1998 to 2000 in Claudio Stern's laboratory in Columbia University, moving with Dr. Stern to University College London in 2001. He was appointed team leader at the RIKEN CDB in 2004.

The lab studies how mesoderm cells form and differentiate during early vertebrate development, using the chick as the main model organism. Our research currently focuses on two aspects: 1) the formation and early regionalization of mesoderm cells during gastrulation; 2) differentiation of the ventral-most mesoderm cell types.

Mesoderm cells are derived from the epiblast during gastrulation through a process called epithelial to mesenchymal transition (EMT). During EMT, mesoderm cells leave epithelial-shaped epiblast sheet and become migratory. We are interested in understanding the molecular and cellular mechanisms governing this EMT. We are also interested in understanding how this process is linked to mesoderm fate specification in the epiblast and to mesoderm regionalization along the dorsal-ventral axis during gastrulation.

After their formation, mesoderm cells give rise to several well-defined lineages: axial, paraxial, intermediate, lateral plate and extraembryonic. The extraembryonic lineage contains three cell types: blood, endothelial and smooth muscle. We are investigating how these three cell types are specified and how they are organized to form functional hematopoietic and vascular systems.

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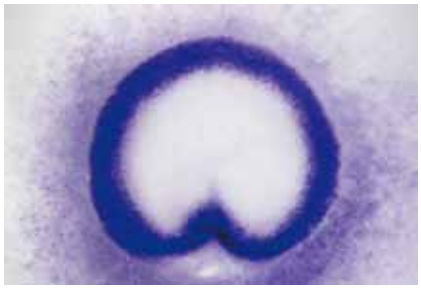
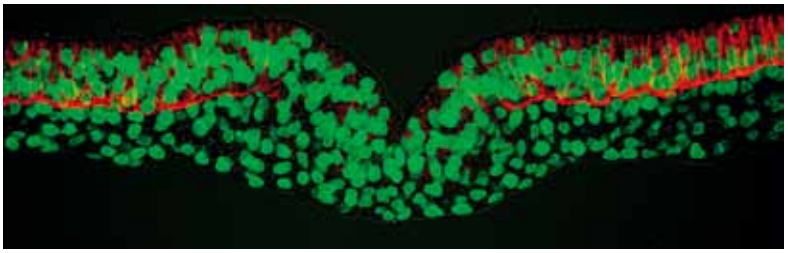


An anamniote-like, circumblastoporal ring of mesoderm precursors (blue: brachyury) can be induced in the chick embryo by subgerminal cavity injection of FGF growth factor, suggesting that the primitive streak is not an essential component of mesoderm formation in amniotes.



A cross-section of the chick primitive streak with epiblast cells in the middle undergoing EMT. Red: beta-Dystroglycan (basolaterally localized in lateral epiblast cells and downregulated in medial epiblast cells); Green: nuclei.

Physical Biology

Tatsuo SHIBATA Ph.D.

Tatsuo Shibata received his B.Sc. in Physics from Kyoto University, and Ph.D. from the Graduate School of Arts and Sciences, University of Tokyo in 1999, after which he worked as a postdoctoral research fellow at the Kyoto University Research Institute for Mathematical Sciences for two years. In 2001, he moved to Germany to do a second postdoc at the Fritz-Haber-Institut. He returned to Japan in 2002, taking an associate professorship at Hiroshima University. In 2007, he was additionally appointed as a researcher under the PRESTO program coordinated by the Japan Science and Technology Agency (JST). In October of 2010, he took his current position of research unit leader at the RIKEN CDB.

As part of the reorganization of the CDB on November 21, 2014, the Physical Biology lab was transferred to the RIKEN Quantitative Biology Center.

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Hiraiwa T, et al. Relevance of intracellular polarity to accuracy of eukaryotic chemotaxis. *Phys Biol* 11.056002 (2014)

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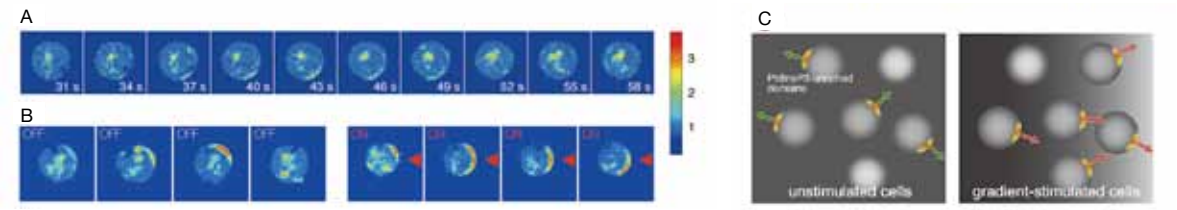
Advances in measurement technologies have afforded us glimpses into dynamic functional processes, such as morphogenesis and information processing, in cells and tissues involved in development and regeneration. The truly organic dynamism of the biological phenomena exhibited by living cells, individually or in groups, emerges from the coordinated interaction of numerous molecular and genetic factors, and the need for integrated, systems-based approaches to the study of design and operating principles in such “living” phenomena is becoming increasingly clear. This will require not only technologies for the measurement of such elements but the development of applicable mathematical methods as well. In Laboratory for Physical Biology, we will seek to use concepts and methodology from mathematical sciences such as physics in the study and elucidation of these emerging questions in biology.

One example of such a phenomenon is seen in cellular chemotaxis, in which cells recognize concentration gradients of attractant molecules and respond by directional movements necessary for functions such as the exploration of the environment by single-celled organisms, and morphogenesis in metazoa. Chemotactically responsive cells are able to detect differentials in the concentration of an attractant molecule of only a few percent, which, given cell sizes of ranging in the tens of micrometers, translates to a real difference of just a few dozen molecules. Cells are capable of interpreting this minute difference as a gradient that guides the direction of its movement, raising the question of how cells are able to detect and follow such weak and noisy signals. We now know that within their tiny intracellular spaces, cells comprise many interacting molecules that work in a highly orchestrated fashion, and thus give rise to emergent order enabling their orientation. Using quantitative fluorescence imaging data and the analysis of mathematical models, we seek to gain a better understanding of such mechanisms.

Mathematical modeling of the essential aspects of observed phenomena of interest is a useful approach to evaluating whether we have sufficient knowledge of associated molecules, reactions, and cellular interactions to explain them. The abstracted mathematical idea of particular phenomena may further reveal general principles that underlie the living systems more broadly across diverse taxa. We seek to contribute to the thorough exploration of these fascinating problems in biology through concepts and methods adapted from the mathematical sciences.

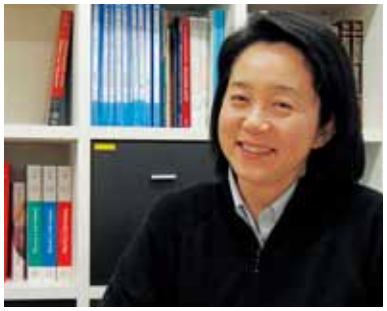


(A) 300msec cAMP stimulus induce asymmetric activation of phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) indicating an excitable reaction. (B) Spontaneously formed asymmetry of PtdIns(3,4,5)P3 (left panels) in a random direction, which is biased to the external gradient direction when it is applied (right panels). (C) Biased excitability is the principle of gradient sensing. (Nishikawa, et al. 2014).

Retinal Regeneration

Masayo TAKAHASHI M.D., Ph.D.

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



Masayo Takahashi received her M.D. from Kyoto University in 1986, and her Ph.D. from the same institution in 1992. After serving as an assistant professor 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 since 2001 served as an associate professor at the Translational Research Center in the same hospital. She joined the CDB as a team leader of the retinal regeneration research team in 2006. Her clinical specialty is retinal disease – macular diseases and retinal hereditary diseases in particular. Her aim is to understand these diseases at a fundamental level and develop retinal regeneration therapies.

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

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

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

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Publications

Assawachananont J, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports* 2.662-74 (2014)

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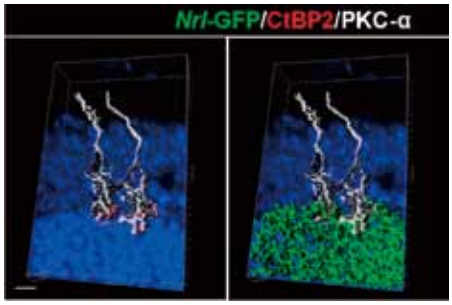
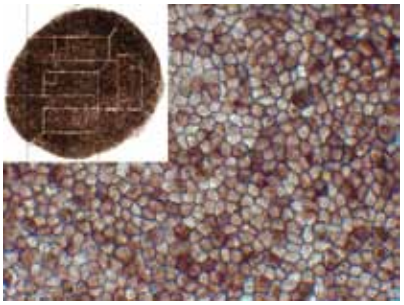
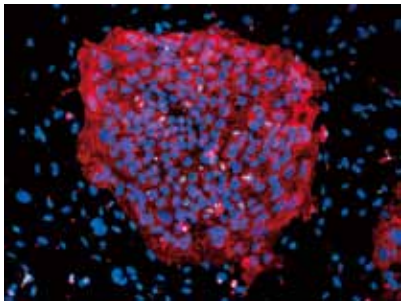


Mouse iPSC-derived photoreceptors are morphologically able to form synapses after transplantation into host animals.



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



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

Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph.D.

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

Masatoshi Takeichi is director of the RIKEN Center for Developmental Biology as well as director 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. Dr. Takeichi served as CDB Director from 2000 to 2014.

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Publications

Tsukasaki Y, et al. Giant cadherins Fat and Dachsous self-bend to organize properly spaced intercellular junctions. *Proc. Natl. Acad. Sci. USA* 45. 16011-16016 (2014)

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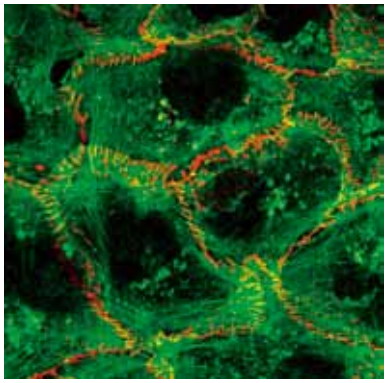
Animal cells organize into tissues with complex architecture. Our lab is exploring the molecular mechanisms by which individual cells assemble into a tissue-specific multicellular structure, such as epithelial sheets and neural networks. We are also interested in the molecular basis of how tissue architecture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

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

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

3) In addition, we have recently begun analyzing the functions of microtubule minus end-associated proteins, Nezha/CAMSAPs. These proteins regulate microtubule assembly patterns, centrosomal function, and organelle positioning. We are exploring molecular mechanisms underlying such regulatory activity, as well as 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.



Double-immunostaining for F-actin (green) and Kusabira Orange-tagged E-cadherin (red) introduced into A431D cells. In these cells, E-cadherin dynamically moves along cortical actin filaments, resulting in the unique distributions shown here.

Organ Regeneration

Takashi TSUJI Ph.D.

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

Takashi Tsuji received his Masters 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 a full professor in the same university. During his academic career, he received numerous prestigious grants and awards, and participated in numerous industry collaborations. He joined the CDB as a Group Director in 2014, and has served as Team Leader since the restructuring of the Center in November 2014.

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 three-dimensional tissue-like structures from pluripotent stem cells, such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), we seek both to elucidate the mechanisms by which such fields induce organogenesis and to develop new technologies for use in regenerative medicine. We are also interested in using 4D cell dynamics imaging techniques to quantitatively analyze organ-specific morphogenesis, and to analyze, model, and simulate molecular pathways involved in their control, in the hopes of gaining new insights into the fundamental principles underlying organogenetic processes. Building on these fundamental studies, we further seek to conduct applications research towards the development of technologies for use in therapeutic organ regeneration.

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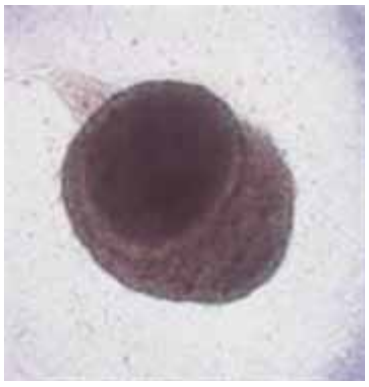

Bioengineered organ germ

Bioengineered hair follicle

Bioengineered tooth

Systems Biology

Hiroki R. UEDA M.D., Ph.D.

Hiroki R. Ueda received his M.D. from the Faculty of Medicine of the University of Tokyo in 2000, and received his Ph.D. from the University of Tokyo Graduate School of Medicine in March 2004. While an undergraduate, he worked as a research assistant on a biological simulation system project at Sony Computer Science Laboratories. As a graduate student he next went on to work, first as a researcher (2000) and then as a group leader (2002), at Yamanouchi Pharmaceuticals, on a project studying biological clock mechanisms in fly and mouse. He was appointed team leader of Laboratory for Systems Biology in 2003, and Leader of the Functional Genomics Unit at the CDB in 2004. He was also appointed as Professor (Invited) of Osaka University in 2006. In 2009, he was promoted project leader of the Laboratory for System Biology under CDB's Center Director's Strategic Program.

The Systems Biology lab closed in September 2014. Dr. Ueda is now affiliated with RIKEN QBiC and the University of Tokyo.

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Susaki E. A, et al. Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis. *Cell* 157.726-39 (2014)

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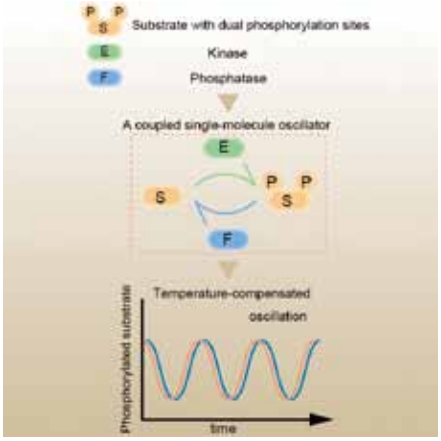
Recent large-scale efforts in genome-sequencing, expression profiling and functional screening have produced an embarrassment of riches for life science researchers and biological data can now be accessed in quantities that are orders of magnitude greater than were available even a few years ago. The growing need for interpretation of data sets, as well as the accelerating demand for their integration to a higher level understanding of life, has set the stage for the advent of systems biology, in which biological processes and phenomena are approached as complex and dynamic systems. Systems Biology is a natural extension of molecular biology, and can be defined as "biology after the identification of key genes." We see systems-biological research as a multi-stage process, beginning with the comprehensive identification and quantitative analysis of individual system components and their networked interactions, and leading to the ability to drive existing systems toward a desired state and design new ones based on an understanding of structure and underlying principles.

Over the last several years, the Laboratory for Systems Biology (LSB) has worked to establish experimental systems biology at the molecular-to-cellular level and apply them to system-level questions of complex and dynamic biological systems, such as the mammalian circadian clock. In October 2009, our laboratory was re-designated as a Project Lab in the Center Director's Strategic Program for Systems Biology research to promote challenging research endeavors. Based on the achievements over the past years, we strongly feel that it is now the time for us to take the next step forward toward experimental systems biology at the cellular-to-organism level.

Over the next several years, we intend to develop an efficient experimental platform to identify, monitor, and perturb cellular networks within organism. To this aim, we will attempt to invent and combine several key technologies ranging from (i) rapid engineering of the genome of ES cells, (ii) generation of "100% chimera" animals for F0 phenotyping, and (iii) phenotype analysis of a small number of the generated animals (ideally with a single animal). Full utilization of these technologies will formulate cellular-to-organism-level systems biology, which will provide new strategies and concepts for the diagnosis, treatment, and prevention of biological-time-related disorders, including rhythm disorder, seasonal affective disorder, and sleep disorder.

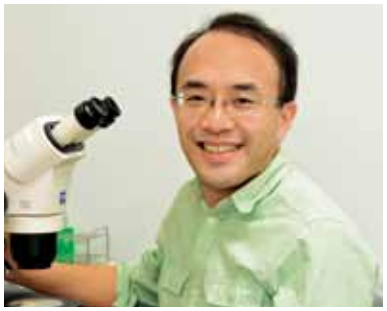


Post-translational processes such as protein phosphorylation are vital for circadian rhythms in many organisms. In cyanobacteria, circadian proteins can be incubated with ATP to form an in vitro post-translational oscillator (PTO) that operates in the absence of transcription and translation. It is still unknown whether components of the mammalian clock may also be able to function as a PTO. In a recent paper, Jolley, Ode, and Ueda developed a mathematical model to examine the possibility of oscillations in a simple system with only three components. They found that two essential design motifs are necessary for sustained post-translational oscillation: a preferred ordering of phosphorylation states ("single-molecule oscillators") and synchronization of these autonomous oscillators by enzyme sequestration.

Epithelial Morphogenesis

Yu-Chiun WANG Ph.D.

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



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.

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

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

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

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Khan, Z. et al. Quantitative 4D analyses of epithelial folding during *Drosophila* gastrulation. *Development* 141.2895-900 (2014)

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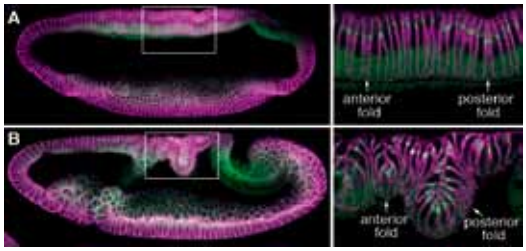
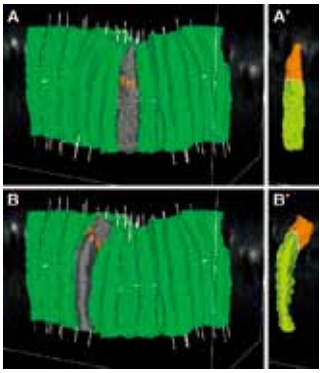


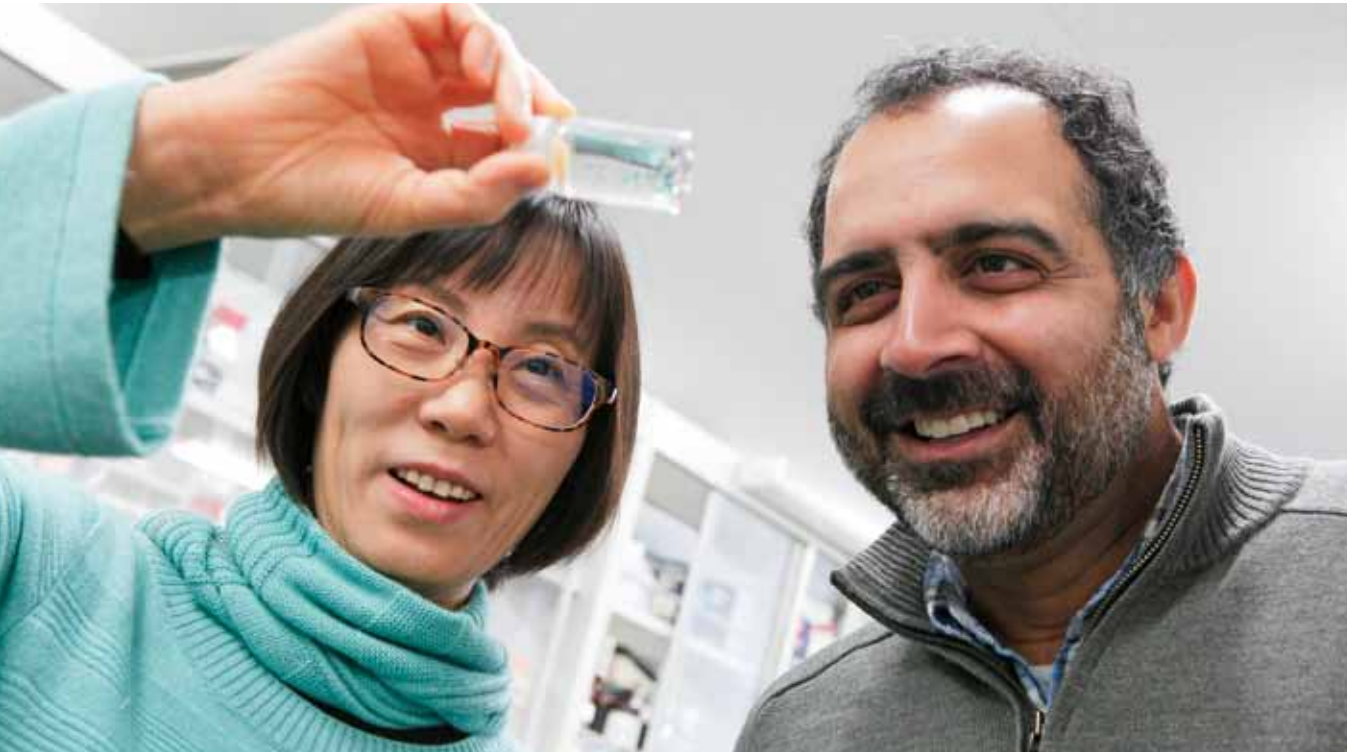
Dorsal fold formation during *Drosophila* gastrulation.
The dorsal epithelium of the *Drosophila* gastrula forms two epithelial (anterior and posterior) folds, shown here with the mid-sagittal optical sections at an early (A) and a late (B) stage. Adherens junctions are labeled in green; plasma membrane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



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

Plastic cast of airway

Inklings of insight into inner ear development



Xiaorei SAI, Raj LADHER

The inner ear is located in the inside of the head, however at the earliest stages of embryonic development, it forms as a thickening of the ectoderm (epithelia) on the outer surface of the embryo, referred to as the otic placode. This placode invaginates into the mesenchyme of the future head region, eventually budding off to form an otic vesicle, the precursor of inner ear organs. Invagination of the otic placode, similar to many epithelial invagination processes, involves two phases: first, there is an expansion of the basal membrane of otic cells, followed by a constriction of the apical side of otic cells. Whereas the molecular mechanisms that drive basal membrane expansion in the first phase of otic invagination are beginning to be understood, the mechanisms controlling constriction of the apical side in the second phase remain relatively unknown.

Now, new work published in *Developmental Biology* by research scientist Xiaorei Sai of the Laboratory for Sensory Development (Raj K. Ladher, Team Leader) and colleagues has uncovered a molecular pathway coordinating the apical constriction of otic cells during the second phase of otic invagination. Using chicken embryos as a model, they demonstrate that localized activation of Ras homolog gene family member A (RhoA) protein at the apical cell junctions triggers a signaling pathway leading to recruitment and activation of non-muscle myosin-II, an actin-binding protein, to initiate apical constriction of the otic placode.

A previous study from this laboratory (Sai and Ladher, 2008) showed that basal activation of non-muscle myosin-II instigated basal expansion of the otic placode in phase 1 of otic invagination; myosin-II is activated by basally localized FGF signaling, which leads to depletion of actin filaments on the basal side, and consequently, basal expansion. Studies of similar invagination processes, such as gastrulation and lens placode formation, by other groups have indicated that activation of myosin-II is also important for apical constriction, where activated myosin-II triggers a signaling cascade leading to constriction of actin filaments on the apical side. Thus, these findings suggest

Sai X, et al. Junctionally restricted RhoA activity is necessary for apical constriction during phase 2 inner ear placode invagination. *Dev Biol* 394.206-16 (2014)

that activation of myosin-II at apical and basal sides of the otic placode involve different molecular cues, resulting in contrasting activities for myosin-II on actin filaments.

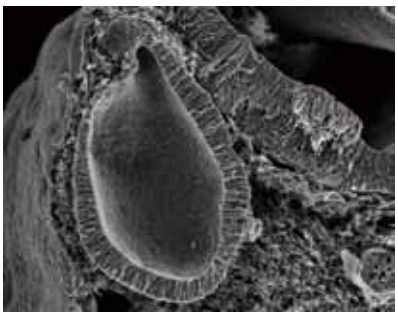
So what are the molecular cues at work at the apical side? Sai et al. focused their sights on the RhoA protein, a member of the Rho family of small GTPases, which is known to regulate actin cytoskeleton dynamics and has also been implicated in promoting apical constriction of epithelial cells during invagination processes in other model systems. In these systems, RhoA triggers Rho-associated coiled-coil protein kinases (ROCKs) to phosphorylate myosin light chain (MLC) to activate myosin-II, leading to constriction of actin filaments. In the case of the otic placode, Sai et al. observed a rise in RhoA expression during phase 2 of otic invagination, with high levels detected in the apical junction complex. RhoA activation assays on otic placode extracted from embryos during this same period also confirmed the presence RhoA activity. When RhoA activity was knocked down using small RNAi, they detected reduced levels of active apical MLC as well as actin in the apical junctions, and a larger apical surface area than control embryos suggested that minimal or no constriction had taken place. Thus, these results highlight the importance of RhoA for apical constriction. They also looked into whether ROCKs, potential RhoA downstream factors, also played a role in phase 2 constriction by inhibiting ROCK expression and found that otic invagination was blocked in addition to disruption of the localization of actin and activated MLC to apical junctions, indicating ROCKs were also needed for apical constriction.

The team next attempted to determine the molecule triggering RhoA activity. Rho family small GTPases are generally switched on by guanine exchange factors (GEFs), so they performed a screening for apically localized GEFs with using antibodies. The ArhGEF11 protein was deemed a putative RhoA GEF since its expression became concentrated in the apical area as otic placode invagination progressed, overlapping with expression of junctional marker ZO-1. Knocking down normal ArhGEF11 activity levels led to reduced activity of myosin-II and localization of actin to apical junctions. ArhGEF11 expression was not affected when RhoA was knocked down, thereby indicating that ArhGEF11 was upstream of RhoA and also involved in apical constriction.

Studies on neural tube formation had reported that a planar cell polarity signal, Celsr1, was needed to activate myosin-II through RhoA and its upstream GEF. Thus, the team speculated that Celsr1 may be an upstream regulator of RhoA and its GEF, ArhGEF11, to enhance apical constriction in the otic placode. Expression experiments showed that while weak Celsr1 expression is detected in the ectoderm just as the otic placode is beginning established, during the phase I of otic morphogenesis, its expression is upregulated and localized to the apical junction, reaching a peak during the phase 2. When Celsr1 expression levels were reduced in otic placodes during the phase 2, they found reduced activity and localization of actomyosin at the apical cell junction, resembling what was seen in ArhGEF11 knockdown experiments.

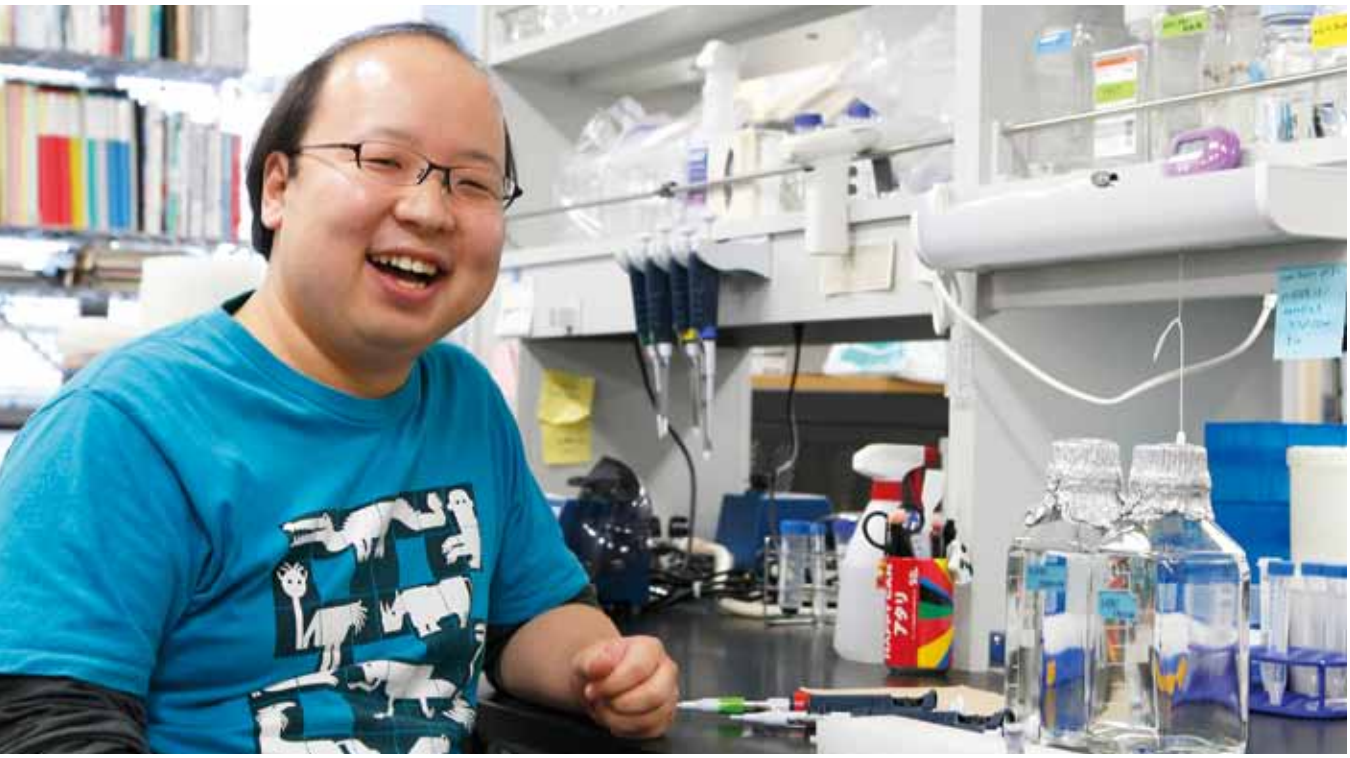
Based on their data, they proposed a model for apical constriction seen in phase 2 of otic invagination, where the planar cell polarity protein Celsr1 is upregulated in the apical otic placode as otic morphogenesis begins, and accumulates at the apical cell junctions. Celsr1 then recruits ArhGEF11 to the apical junction, which in turn activates RhoA, a key factor needed to trigger ROCKs to activate myosin-II, leading to constriction of actin filaments anchored to the apical junction.

“It is clear that epithelial remodeling in the neural tube and during inner ear invagination share a common morphogenetic toolbox,” says Ladher. “Thus findings in one of these processes could be used and adapted to understand conditions with clinical significance, such as neural tube defects, body wall closure defects and wound healing.“



Scanning electron micrograph showing the inner ear after 50 hours of development. The inner ear, which formed on the surface ectoderm, has now completed its invagination, and is embedded within the head mesenchyme

Cellular communication is key for upper-layer neurogenesis



Kenichi TOMA

The neocortex is a six-layered brain region unique to mammals and is made up of a complex network of multiple cell types. This region is particularly large in primates, including humans, and is associated with higher order brain functions such as perception, thinking, memory, and language. Neurons of the neocortex are derived from progenitor cells that lie in the ventricular zone, and these progenitors divide and differentiate to generate deep-layer (DL) projection neurons and then upper-layer (UL) projection neurons in a timely manner, with the later-born neurons migrating to upper layers. The transition from generating one neuronal subtype to another is critical for creating the layered organization of the neocortex, but the mechanisms involved in this regulation are still shrouded in mystery.

Now, a study by research scientist Kenichi Toma of the Laboratory for Neocortical Development (Carina Hanashima, Team Leader) and colleagues, published in the *Journal of Neuroscience*, identifies the cues involved in triggering the onset of UL neurogenesis in the developing neocortex. Using a series of transgenic mouse lines, they found that induction of UL neurogenesis requires sequential derepression of transcription factors, triggered by *Foxg1*, and also a feedback signal from earlier-born, differentiated DL neurons. Thus, the neural progenitors use a combination of intrinsic gene regulatory mechanisms and extrinsic cues from surrounding cells to switch from DL to UL neurogenesis.

In neocortical development, Cajal-Retzius (CR) cells, which occupy the most superficial layer of the cortex, are generated first, followed by DL neurons that are generated from embryonic day (E)11.5, and UL neurons born at E14.5. These neurons then undergo a process of maturation to form a six-layered structure. Previous work from the laboratory has shown that expression of a transcription factor, *Foxg1*, triggers progenitors to enter

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

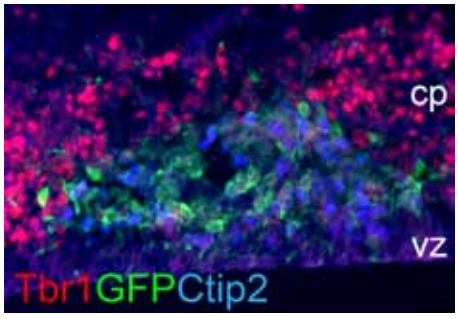
DL neurogenesis from CR cell production (see CDB News: April 5, 2013). The regulatory mechanisms involved in the switch from DL neurogenesis to UL neurogenesis, however, remained unclear.

In the current study, the team examined development of UL neurons in detail using transgenic mouse lines previously developed in the lab, in which timing of *Foxg1* expression could be manipulated. Under normal conditions, the expression of this gene first appears around E8.5. When they delayed the expression onset to a later developmental stage (at E14.5), the period of CR neuron generation was prolonged concomitantly, until the expression of Foxg1 rapidly induced DL neurogenesis. Using different markers to label the cohort of neurons produced at E14.5 and those produced the next day, the team found that at E18.5 most of the earlier-born neurons had differentiated into DL neurons, while the later-born neurons had primarily differentiated into UL neurons. The further 1-day delay of *Foxg1* induction to E15.5 also gave similar results, suggesting that irrespective of the progression of development, the onset of UL neurogenesis cannot bypass DL neurogenesis.

Maintaining the layered structure of the neocortex also requires regulation of transcription factors. Each differentiated layer subtype expresses specific transcription factors, and is segregated from the other layer subtypes through mutual repression of four transcription factors. However, how this repression network is triggered to confer fate specification in neurogenesis was not well understood. Toma et al. speculated that *Foxg1* was the trigger to tip the balance, and looked into the gene expression levels of the four transcription factors after *Foxg1* was induced. *Tbr1* was the only transcription factor out of the four that showed a marked decrease in expression levels after *Foxg1* induction. The cells in which *Tbr1* was suppressed after *Foxg1* induction also showed a rise in expression of DL neuron marker, and went on to differentiate into DL neurons, indicating that induction of *Foxg1* leads to suppression of *Tbr1* in progenitor cells signaling them to commit to DL neuronal fate.

So after induction of DL neurogenesis, how do the progenitors time the switch over to UL neurogenesis? Under culture conditions, progenitors need to be maintained under relatively high density conditions to induce differentiation of UL neurons. Fixing on this point, the team examined whether onset of UL neurogenesis could be regulated by an extrinsic signal originating from DL neurons themselves. They generated a mouse line in which DL neurons produced between E11.5 to E13.5 were specifically ablated, and found that, despite a large drop in DL neuronal population immediately after ablation, their numbers had recovered substantially by E18.5. UL neurons were also generated, although slightly fewer than seen under normal conditions. Closer examination revealed a prolonged period of DL neurogenesis, which in turn led to a delay in the onset of UL neurogenesis. These results indicate that the differentiated DL neurons send a signal to the progenitors to halt DL neurogenesis and initiate UL neurogenesis.

"The mechanisms uncovered in this study are extremely useful systems for generating the necessary neuronal subtypes in sequential order, as well as maintaining the relative balance of their number regardless of the size differences, for example, between mouse and human brains," says Hanashima. "Our next step is to reveal what molecular signal from DL neurons is relayed to progenitors to facilitate the switch to UL neurogenesis, bringing us closer to understanding the mechanisms of neocortical neurogenesis."



Coelectroporation of Foxg1 and GFP constructs (shown in green) in brain cells of *Foxg1* knockout mouse, represses Tbr1 expression (red) and triggers the expression of DL neuron marker Ctip2 (blue)

When flies are low on sugar

Hiroko MATSUDA (Front:left), Takayuki YAMADA (Back:second from the right)

Sugars such as glucose circulating in the blood and stored in tissues are important sources of energy and carbon necessary for living organisms to maintain metabolic homeostasis. In many invertebrates, including insects, trehalose is the predominant sugar found circulating in the blood (or hemolymph). The unique chemical properties of trehalose are thought to protect organisms from environmental stresses such as desiccation and changes in temperature. Trehalose levels in the hemolymph are known to be regulated by a pair of endocrine hormones, similar to how insulin and glucagon regulate glucose levels in humans, to control the glycometabolism of an organism in response to availability of nutrients. However, the precise function of trehalose during the process of development remains largely unknown.

In a new study carried out by research scientist Hiroko Matsuda and technical staff Takayuki Yamada in the Laboratory for Growth Control Signaling (Takashi Nishimura, Team Leader) and others, published in the *Journal of Biological Chemistry*, they use the *Drosophila* system to analyze the physiological role of trehalose. Their findings reveal that trehalose plays an important role in regulating glycometabolism and growth as well as on the viability of the fly, especially when there are changes in external factors such as nutrient availability.

In *Drosophila*, glycogen, the main storage form of glucose, is stored in tissues, whereas trehalose is found stored and circulating in relatively high concentrations in the hemolymph. When needed, these storage sugars are broken down into glucose, which is used by tissues to produce energy in the form of ATP. In this study, to understand the role of trehalose during development, Matsuda et al. generated *Drosophila* mutants that lacked the trehalose-synthesizing enzyme, trehalose-6-phosphate synthase (*Tps1*), and compared their developmental physiology with wildtype flies.

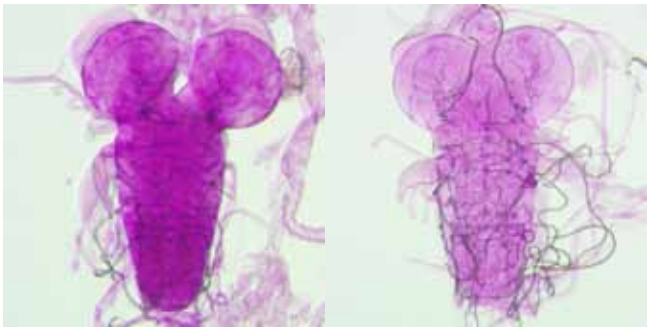
Matsuda H, et al. Flies without Trehalose. *J Biol Chem* (2014)

The group first examined the levels of trehalose in wildtype flies at different developmental stages. Trehalose was undetected in early stage embryos, but as development progressed from late embryonic to larval stages, trehalose levels began to rise significantly, and during the pupal period, saw a sharp decline. These observations suggested that trehalose was synthesized during embryonic and larval development, and then consumed during pupal development. They also confirmed that the main site of trehalose synthesis was the fat body, an organ with functions similar to the mammalian liver. When development of *Tps1* mutants was examined, as expected, trehalose production and trehalose was not detected. When grown under normal nutrient conditions, the mutants displayed relatively normal development until the pupal stage, albeit slightly smaller in size than wildtype; but, they died before eclosion. Thus, while trehalose is important for viability of the fly, their findings suggest that it is not essential for larval development up to the pupal stage, at least under normal nutrient conditions.

So Matsuda et al. next investigated how dietary stress (starvation) would affect the larval period of *Tps1* mutants. When the mutant larvae were placed on a water-only diet, most died after the first day of starvation, while wildtype larvae survived at least three days on the same diet. The starved *Tps1* mutant larvae showed no major changes in the levels of ATP and of hormones that regulate energy metabolism, and also began producing energy by breaking down the proteins and stored fat in the body, as is seen in starved wildtype larvae. However, a closer examination revealed a significant reduction in glycogen levels in the central nervous system of the *Tps1* mutants. Many cells in the brain also underwent apoptosis. Thus, the lack of trehalose synthesis in *Tps1* mutants results in a localized depletion of energy sources in brain cells which use glucose as its primary source of energy, causing defects in the brain and likely lethality.

The group further tested the effects of nutrient availability on the development of *Tps1* mutants, placing them under a number of different dietary conditions. Under a low-sugar diet, the mutant larvae died before the pupal stage. In contrast, under a low-protein (normal sugar) diet, most of the mutant larvae reached the pupal stage, but with a delay in the timing of puparium formation and a markedly smaller pupa sizes. An analysis of the developmental physiology revealed that *Tps1* mutants had reduced insulin/insulin-like growth factor signaling activity, which is involved in growth control of the fly.

“Our study showed that *Tps1* mutants can survive to the pupal stage if dietary sugar is available, but die before eclosion, so we would like to close in on the exact role trehalose plays during the pupal period,” says Nishimura. “It would also be interesting to understand how the *Drosophila* uses the two storage sugars, glycogen and trehalose, for the same or different purposes.”

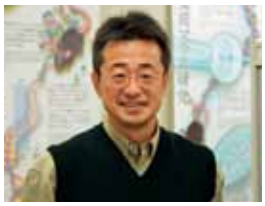


Central nervous system of larval stage *Drosophila*. After starvation, glycogen levels of the *Tps1* mutant (right) are markedly reduced compared with wildtype (left).

Under low protein conditions, the pupa sizes *Tps1* mutants (right) are smaller than wildtype (left).

Animal Resources and Genetic Engineering

Yasuhide FURUTA Ph.D.



Genetically engineered mice are one essential tool in modern biomedical research. The quality and efficacy of research are greatly dependent on how efficiently mutant mice can be generated, propagated, and housed. The major role of the Laboratory for Animal Resources and Genetic Engineering (LARGE) is to provide comprehensive services to develop mutant mouse models that are useful for research in animal development and regeneration, and to maintain and enhance the experimental rodent resources at the CDB. LARGE comprises two complementary units that work to accomplish these missions.

As part of the reorganization of the CDB in November 2014, the LARGE Unit was transferred to the RIKEN Center for Life Science Technologies.

Genetic Engineering Unit

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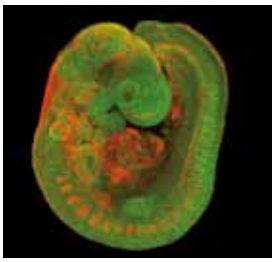
The Genetic Engineering Unit works with research labs within the CDB, as well as others in Japan and throughout the Asia-Pacific region to develop genetically engineered mice useful for the study of development and regeneration. In these joint development projects, we receive sequence information of the subject genes from our collaborators, and perform all subsequent stages of mutant mouse production from constructing the targeting vectors to generating chimeras, making about one hundred new mutant mouse lines every year. In addition, we develop new tools and technologies for bioimaging to aid in the visualization of mouse development at tissue, cell, and organelle levels.

Chimeric mice exhibiting mixed coat color



An E9.5 embryo expressing the R26p-Fucci2 cell cycle probes

Publications

Miyazaki H, et al. Singular localization of sodium channel beta4 subunit in unmyelinated fibres and its role in the striatum. *Nat Commun* 5.5525 (2014)

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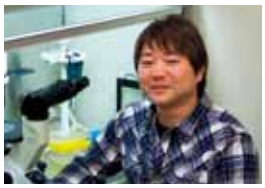
Ishiguro K, et al. Meiosis-specific cohesin mediates homolog recognition in mouse spermatocytes. *Genes Dev* 28.594-607 (2014)

Animal Resource Unit



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Daisuke YAMAMOTO

Assistant
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Yuki TSUJI

The Animal Resource Unit maintains and cares for the CDB's laboratory mouse and rat resources in a Specific Pathogen Free (SPF) environment. We also handle transfer of mutant mice both within the CDB and to/from other domestic and overseas institutions. In addition, we provide pregnant females, fertilized mouse eggs, and services for colony expansion and strain cryopreservation. We also carry out research projects aimed at developing new technologies in reproductive biology. More recently, we have established colonies of a metatherian species, the gray short-tail opossum (Monodelphis domestica), and a reptilian species, the gecko (Paroedura picta), establishing optimal environment and breeding conditions, with the aim for distribution of these new model animals to the research community in the near future.

Pronuclear injection of DNA for generation of transgenic mice

Publications

Hayashi S, et al. Protocadherin-17 mediates collective axon extension by recruiting actin regulator complexes to interaxonal contacts. *Dev Cell* 30.673-87 (2014)

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Electron Microscope

Shigenobu YONEMURA Ph.D.

Detailed knowledge of the shapes of individual cells and fine subcellular structures is vital to the understanding of biological structures and organization. In developmental processes in particular, changes in cell morphology and behavior play fundamental roles. The Electron Microscope Laboratory provides technical assistance and expert guidance in the use of transmission and scanning electron microscopy (TEM and SEM) and related technologies in morphological analyses.

As part of the reorganization of the CDB in November 2014, the Electron Microscope Lab was transferred to the RIKEN Center for Life Science Technologies.

Shigenobu YONEMURA Ph.D.

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Kazuyo MISAKI
Suzuka TAKAHASHI

Visiting Scientist
Masatsune TSUJIOKA

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Electron Microscope

Our technical support activities aim to assist scientists with all procedures related to conventional transmission and scanning electron microscopy, including the preparation of specimens of any type, assistance in the use of equipment, and the recording and printing of images. We also provide instructions equipment use, specimen preparation, and interpretation of images. In all cases, we seek to provide researchers with specific advice as to the appropriate electron micrograph analysis before and during the observation, in order to facilitate the efficient use of electron microscopy in the CDB's research activities.

Our lab additionally conducts research into cytoskeletal elements, and the biophysical aspects of morphological rearrangements in epithelial cells.

Publications

Yonemura S. Differential sensitivity of epithelial cells to extracellular matrix in polarity establishment. *PLoS One* 9.e112922 (2014)

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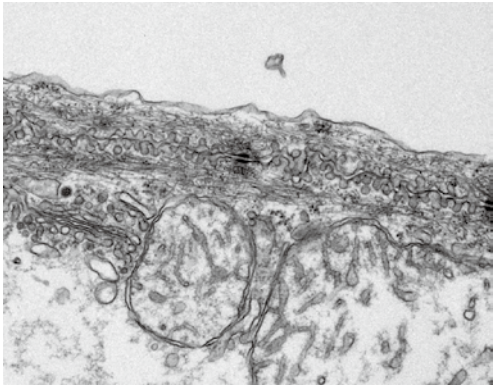
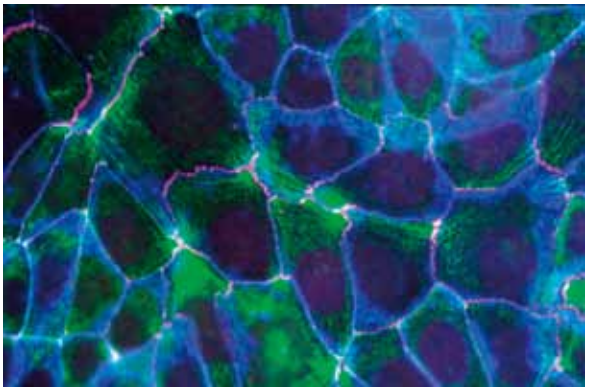


Cells organizing the notochord of zebrafish embryo



Alpha-catenin molecules stretched by forces produced by Myosin II (green) are selectively labeled with alpha18 antibody(red).

Bio-Imaging

Shigeo HAYASHI Ph.D.

The role of the Bioimaging Laboratory is to assist in the operation and maintenance of the Center's core imaging facility and support scientists through the provision of superior optical imaging technologies, analysis and presentation of results. The lab will manage central optical microscopy systems, image analysis software and the associated IT environment, and promote a secure environment for the effective use of these resources.

As part of the reorganization of the CDB in November 2014, the Bio-imaging lab was transferred to the RIKEN Center for Life Science Technologies.

Yuko KIYOSUE Ph.D.

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Tomoko HAMAJI
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YuPing WU
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Yuki TANO

Optical Image Analysis Unit

This unit runs common use CDB Imaging Facility. Bio-imaging is an interdisciplinary process that integrates molecular cell biology and biochemistry, as well as technology from optics, engineering, and computer sciences. It has enabled scientists to visualize biological processes at the cellular and molecular levels and today, has become an indispensable field for research on biological systems. We aim to design an imaging environment that can adapt to changing scientific demands and can contribute to the progress of biology. We hope to accelerate biological research by matching research concepts with the latest technologies from the different specialized fields.

Our laboratory studies mechanisms of life woven by cellular skeleton molecules. We aslo aim to develop our basic reasearch to contribute to the development of treatments for disease and health promotion.

Publications

van der Vaart B, et al. CFEOM1-Associated Kinesin KIF21A Is a Cortical Microtubule Growth Inhibitor. *Dev Cell* 27. 145-60 (2013)

Shimozawa T, et al. Improving spinning disk confocal microscopy by preventing pinhole cross-talk for intravital imaging. *Proc Natl Acad Sci U S A* 110.3399-404 (2013)

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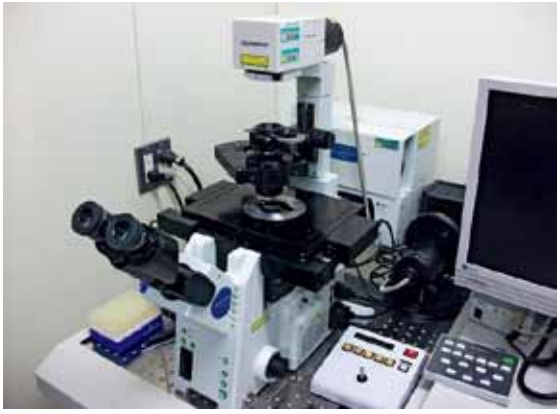

Inverted microscope maintained by the Optical Image Analysis Unit

Genomics

Fumio MATSUZAKI Ph.D.

The Genomics Laboratory works to support a wide range of genomic and epigenomic research and functional genomics research, providing genome-scale, high-throughput services in sequencing gene expression analysis to all CDB labs. All projects can be initiated and followed using an internal website designed to ensure a smooth workflow and timely reporting of results.

As part of the reorganization of the CDB in November 2014, the Genomics lab was transferred to the RIKEN Center for Life Science Technologies.

Shigehiro KURAKU Ph.D.

Genome Resource and Analysis Unit

The Genome Resource and Analysis Unit (GRAS) provides a broad range of biologist-oriented support for Sanger and massively parallel deep sequencing, sequence informatics and gene expression profiling. In parallel, we conduct our original research projects on vertebrate comparative genomics, focusing on cross-species comparisons of developmental programs. Above all, GRAS aims to create an integrative research support station with active communication between laboratory staff and bioinformaticians, and take full advantage of evolving massively parallel sequencing technologies to apply them to transcriptomic, epigenetic and genomic projects in developmental biology arising in the whole institute.

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Miyuki NORO

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Kazu ITOMI
Kana MIYAGISHI
Osamu NISHIMURA
Kaori TANAKA
Chiharu TANEGASHIMA
Kaori TATSUMI
Kenichiro UNO

Assistant
Sayo SAITO

Student Trainee
Fumio MOTONE

Publications

Kuraku S, et al. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Res* 41.W22-8 (2013)

Smith J. J, et al. Sequencing of the sea lamprey (Petromyzon marinus) genome provides insights into vertebrate evolution. *Nat Genet* 45.415-21, 21e1-2 (2013)

Kuraku S. Impact of asymmetric gene repertoire between cyclostomes and gnathostomes. *Semin Cell Dev Biol* 24.119-27 (2013)

Oisi Y, et al. Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* 493.175-80 (2013)

Wang Z, et al. The draft genomes of soft-shell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan. *Nat Genet* 45.701-6 (2013)

Adachi K, et al. Context-dependent wiring of sox2 regulatory networks for self-renewal of embryonic and trophoblast stem cells. *Mol Cell* 52.380-92 (2013)

Our main sequencing platform, illumina HiSeq 1500, in operation since spring 2012

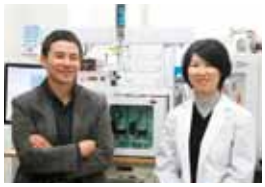
Microfluidics-based equipment for single cell gene expression profiling

Proteomics

Shigeo HAYASHI Ph.D.

The identification of proteins from trace amounts of biologically important protein complexes help researchers to discover novel molecular pathways and has become an indispensable approach to the study of development and regeneration. A better understanding of the protein components of cells and tissues may yield new insights into the molecular structure and function that underlies the spectrum of biological phenomena and improve our ability to manipulate and recapitulate them.

The Proteomics lab was closed as part of the reorganization of the CDB in November 2014.



Shigeo HAYASHI Ph.D.

Staff

Research Specialist
Reiko NAKAGAWA

Mass Spectrometry Analysis Unit

The Mass Spectrometry Analysis Unit uses LC-MASS spectrometry, a technology that enables the high-speed analysis of protein components from even minute biological samples. Its support activity takes the forms of protein identification services and analysis of protein modifications such as phosphorylation, ubiquitination, and proteolysis. The unit supports laboratories within the CDB, and collaborations with a number of laboratories at other institutions. The lab receives gel slices containing proteins from researchers and conducts all subsequent steps, including de-staining of gels, in-gel digestion of proteins, peptide extraction, and operating the LC-MS/MS.

Publications

Nishibuchi G, et al. N-terminal phosphorylation of HP1alpha increases its nucleosome-binding specificity. *Nucleic Acids Res* 42.12498-511 (2014)

Kamata K, et al. The N-terminus and Tudor domains of Sgf29 are important for its heterochromatin boundary formation function. *J Biochem* 155.159-71 (2014)

Nishibuchi G, et al. Physical and functional interactions between the histone H3K4 demethylase KDM5A and the nucleosome remodeling and deacetylase (NuRD) complex. *J Biol Chem* 289.28956-70 (2014)

Izumi H, et al. p54nrb/NonO and PSF promote U snRNA nuclear export by accelerating its export complex assembly. *Nucleic Acids Res* 42.3998-4007 (2014)

Mass Spectrometer Orbitrap Velos Pro

PAL HTS-xt autosampler

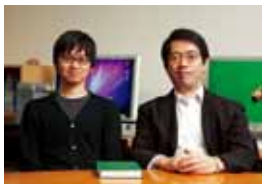
MASCOT proteome analysis server

Human Stem Cell Technology

Yoshiki SASAI M.D., Ph.D.

The Division of Human Stem Cell Technology (DHSCT) was established to provide support services to any lab with an interest in using human embryonic stem cells (hESCs) and their derivatives in their research. The DHSCT provides technological expertise, training and support in hESC culture, maintenance, distribution and management, as well as monitoring and analysis of global trends in stem cell research and regulation.

The Human Stem Cell Technology Division was closed as part of the reorganization of the CDB in November 2014.



Deputy Unit Leader
Masatoshi OHGUSHI Ph.D.

Staff

Research Specialist
Hiroyuki KITAJIMA

Visiting Scientist
Taisuke KADOSHIMA
Daiki NUKAYA

Technical Staff
Masayo FUJIWARA
Michiru MATSUMURA-IMOTO
Maki MINAGUCHI

Junior Research Associate
Chikafumi OZONE

Part-Time Staff
Yuko MARUYAMA

Human Stem Cell Technology Unit

Stem cells - both embryonic and somatic - can be challenging to study and manipulate in vitro. The Division of Human Stem Cell Technology Unit was established to provide a full spectrum of support services to labs within the CDB and throughout Japan with an interest in using human embryonic stem cells (hESCs) and their derivatives in their research. The unit provides expertise, training, and support in hESC culture, maintenance, distribution and management, for users in the life sciences community. We seek to contribute to achieving the goals of translational research, in line with the CDB mission to establish a solid scientific foundation for regenerative medicine.

Publications

Nakano T, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 10.771-85 (2012)

Ohgushi, M., et al. Molecular Pathway and Cell State Responsible for Dissociation-Induced Apoptosis in Human Embryonic Stem Cells. *Cell Stem Cell* 7, 225-239 (2010)

Science Policy and Ethics Studies Unit

Masatoshi TAKEICHI Ph.D.
(Acting Unit Leader)

The field of stem cell research has been subject to legal, social, and ethical tensions across a broad range of issues, from the research use of human embryos to the optimization of pathways for the translation of basic research into clinical applications. We seek to compare different science policy approaches to these issues and identify regulatory frameworks best suited to the development and promulgation of stem cell applications. We will further explore approaches to the clinical translation of human stem cell research.

Publications

Sipp D. Direct-to-consumer stem cell marketing and regulatory responses. *Stem Cells Transl Med* 2.638-40 (2013)

Sipp D. and Turner L. U.S. Regulation of Stem Cells as Medical Products. *Science* 338.1296-1297 (2012)

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 latenight bars promise a great evening out any night of the week. The neighboring Motomachi district offers a mix of upscale department stores and funky shopping arcades, standing in contrast to the colorful Chinatown that's right next door. A short walk north from city center lies the old foreign settlement of Kitano, whose clapboard houses and well-kept parks are a perfect retreat from the dynamism downtown.

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, and hiking trails crisscross the forested mountains that span the entire length of the city, beckoning hikers and picnickers to enjoy a day in the fresh air. The city is also dotted with parks that come into bloom at the start of cherry blossom season, and its many rivers, beaches and scenic areas make it easy to take enjoyable day trips, inside the city or out.

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

RIKEN in Kobe
Budget and Staff
CDB Symposium
Seminars
About RIKEN
RIKEN Campuses

RIKEN in Kobe

The RIKEN Center for Developmental Biology was the first research center established by RIKEN in the city of Kobe, 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 Center for Life Science Technologies (CLST), and the Osaka-based RIKEN Quantitative Biology Center (QBiC). The CLST was established in 2013 through the fusion of research programs at the former RIKEN Yokohama and Kobe Institutes, and focuses on technology research and development to support the medical and pharmaceutical sectors, while QBiC focuses on measurement, analysis, and modeling technologies and techniques to model cell dynamics.

Kobe is also home to the RIKEN High-Performance Computer Infrastructure Program for Computational Life Sciences (HPCI), and the Advanced Institute for Computational Sciences (AICS). AICS and HPCI are both associated with the national K Supercomputer project, working respectively to generate cutting-edge scientific results and technological breakthroughs through collaboration and integration of computational and computer sciences, and to promote computational science and technology in the life sciences.

Administrative support at RIKEN Kobe and CDB

Kobe Branch

- RIKEN Kobe Administrative Division
  - General Affairs
  - Human Resources
  - Finance
- Information Networks Office – Kobe branch
- RIKEN Kobe Safety Center
- CDB Planning Section
  The Planning Section coordinates funding and external review activities, as well as providing support for international communications, scientific meetings, and the Center's non-Japanese staff.
- 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.

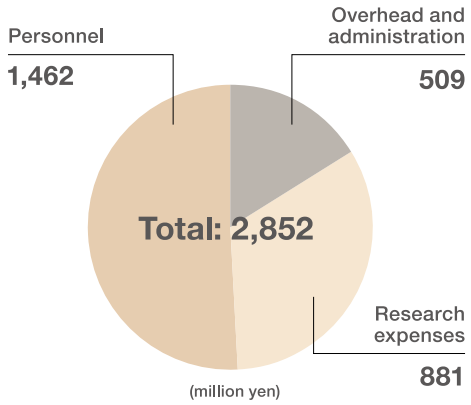
Kobe Administrative Services

Since the reorganization 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, and finances and accounting. Within the CDB, the Developmental Biology Planning Office coordinates important activities including budget and funding management, and administrative support for laboratory performance reviews and renewals. The Information Networks Office maintains both network access and the Center's multiple intranet services. The Library Office manages the CDB research literature collections and interlibrary loans.

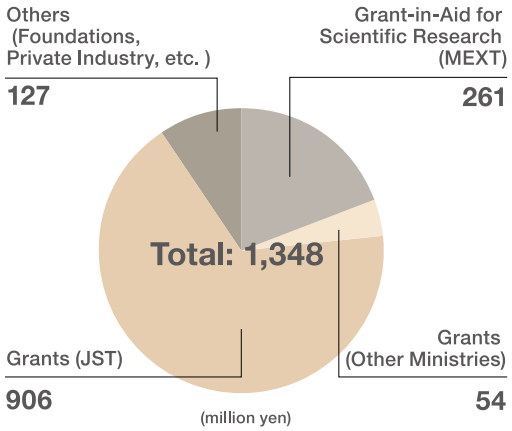
Safety Center

The Kobe Institute 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, and administers the institute's nursing station.

2014 CDB Budget



In additional to the dedicated funds outlined above, individual labs and investigators are encouraged to compete for external funding as well, 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.



2014 CDB Staff

Laboratory heads	21
Deputy leaders	2
Research scientists	64
Research associates	12
Technical staff	68
Assistants	15
Visiting scientists	81
Student trainees	15
Part-time staff	35
Other	3
Total	326
Research Promotion Division	*69

*Administrative staff for RIKEN Kobe branch








2014 CDB Symposium

Regeneration of Organs: Programming and Self-organization

March 10–12, 2014

The RIKEN CDB hosted its twelfth annual symposium on March 10 to 12 in the CDB auditorium. This year's symposium was themed, "Regeneration of Organs: Programming and Self-organization," and drew an audience of 180 scientists and students from 15 different countries to take part in a program of talks and discussions exploring inroads into our understanding of the phenomena of regeneration and organogenetic processes.

The three-day program featured 30 invited and selected talks and over 60 poster presentations that covered a range of topics related to regeneration including molecular mechanisms underlying regeneration, self-organization in regeneration, self-organization from a mathematical perspective, and mechanisms determining shape and size of organs and organisms. The co-organizers this year were Yoshihiro Morishita and Hitoshi Niwa from the CDB, Kiyokazu Agata of Kyoto University (Japan), and Elly Tanaka of Center for Regenerative Therapies Dresden (Germany).

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

Session 1

Yoshihiko Umesono (The University of Tokushima, Japan)
Elly M. Tanaka
(Center for Regenerative Therapies Dresden, Germany)
Akira Satoh (Okayama University, Japan)
Kenneth Poss (Duke University Medical Center, USA)
Ashley W. Seifert (University of Kentucky, USA)
Feng Chen (Stanford University, USA)

Session 2

Andras Simon (Karolinska Institute, Sweden)
Daniel Goldman (University of Michigan, USA)
Koji Tamura (Tohoku University, Japan)
Alan Rodrigues (Harvard Medical School, USA)
Takashi Takeuchi (Tottori University, Japan)
Ryo Nakamura (The University of Tokyo, Japan)

Session 3

Hans Meinhardt
(Max Planck Institute for Developmental Biology, Germany)
Naama Barkai (Weizmann Institute of Science, Israel)
Tatsuo Shibata (RIKEN CDB, Japan)
Yoshihiro Morishita (RIKEN CDB, Japan)
Taiji Adachi (Kyoto University, Japan)
Yanlan Mao
(MRC Laboratory for Molecular Cell Biology, University College London, UK)

2015 CDB Symposium

Time in Development

March 23–25, 2015

The thirteenth annual symposium “Time in Development” will be held on March 23–25, 2015 in the CDB Auditorium. This symposium will focus on ‘time’. There are various time scales in developmental events, which are mutually related and tightly coordinated; the timing of cells to divide and differentiate, time as developmental stages and specific events (such as metamorphosis), time as the lifespan of a species, time along the course of evolution, or even the heterochronically shifted time that we perceive through comparison of the developmental time table of different animals. We aim to discuss and understand the various contexts of 'developmental time,' while reflecting on the concept of time used in our own research. Intrinsic genetic programs coordinate developmental time through regulatory networks of transcription factors. More complex timing programs involve systemic signaling that coordinates the developmental processes among tissues within an organism. The duration of developmental intervals can be regulated in response to nutritional or environmental cues. Developmental timing mechanisms ensure correct tissue organization, patterning, and size in a variety of organisms. How time is controlled during development, or alternatively, how development employs temporal cues (clocks/oscillators) to generate cell diversity and coordinated patterns are therefore central themes in development.

Invited Speakers

Victor R. Ambros
(University of Massachusetts Medical School, USA)

Adam Antebi
(Max Planck Institute for Biology of Ageing, Germany)

Constance Cepko (Harvard Medical School and HHMI, USA)
Claude Desplan (New York University, USA)
Yukiko Gotoh (The University of Tokyo, Japan)
Carina Hanashima (RIKEN CDB, Japan)
Naoki Irie (The University of Tokyo, Japan)
Ryoichiro Kageyama (Kyoto University, Japan)
Takao Kondo (Nagoya University, Japan)
Fred Nijhout (Duke University, USA)
Takashi Nishimura (RIKEN CDB, Japan)
Andrew Oates (MRC National Institute for Medical Research, UK)
Michael B. O’Connor (University of Minnesota, USA)

Patrick H. O’Farrell
(University of California, San Francisco, USA)

Linda Partridge
(Max Planck Institute for Biology of Ageing, Germany)

Olivier Pourquié
(Institute of Genetics and Molecular and Cellular Biology (IGBMC), France)

Yun-Bo Shi (National Institutes of Health, USA)

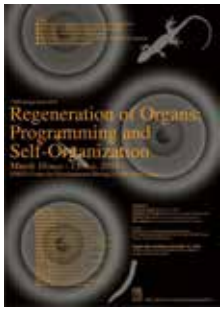
Ralf J. Sommer
(Max Planck Institute for Developmental Biology, Tübingen, Germany)

Claudio Stern (University College London, UK)
Stefan Thor (Linköping University, Sweden)

Pavel Tomancak
(Max Planck Institute of Molecular Cell Biology and Genetics, Germany)

Session 4
Pierre Léopold (Institut de Biologie Valrose (iBV), France)
Richard Adams (University of Cambridge, UK)
Tsuyoshi Hirashima (Kyoto University, Japan)
Kenneth D. Irvine (Rutgers University and HHMI, USA)
Ginés Morata (Universidad Autónoma de Madrid, Spain)
Shinichi Hayashi (Tohoku University, Japan)

Session 5
Manuel Serrano
(Spanish National Cancer Research Center (CNIO), Spain)
Kaoru Sugimoto (California Institute of Technology, USA)
Takashi Tsuji (Tokyo University of Science, Japan)
Karl R. Koehler (Indiana University School of Medicine, USA)
Atsuhiro Taguchi (Kumamoto University, Japan)
Yoshiki Sasai (RIKEN CDB, Japan)



Seminars

The RIKEN CDB makes special efforts to provide a full and diverse series of invited seminars by scientists from around the world. To date, the Center has hosted more than 720 such talks, in addition to numerous meetings, internal forums, and colloquia. The following speakers presented seminars in the period from January to December 2014.

Date	Title	Speaker
01-29	New therapies for retinal degenerative disorders	Akiko MAEDA
02-04	Regional allocation and origin of stem cells in the postnatal brain	Arturo ALVAREZ-BUYLLA
02-06	Artificial induction and disease-related conversion of the hepatic fate	Atsushi SUZUKI
02-17	The cell biology and biophysics of *Ciona* notochord morphogenesis, from an actomyosin perspective	Di JIANG
03-05	Planar Cell Polarity and septins compartmentalize cortical actomyosin to direct collective cell movement	Asako SHINDO
03-13	The two faces of Hippo : Mammalian Hippo signaling in tissue homeostasis and disease	Randy L. JOHNSON
03-13	The Hippo pathway in organ growth control	Georg HALDER
04-11	Patterning mechanisms responsible for generating neurovascular network during organogenesis	Yoh-suke MUKOUYAMA
04-17	Investigation of beta-Spectrin function in the Hippo signaling pathway	Yan YAN
05-22	Anisotropic stress orients remodelling of mammalian limb bud ectoderm	Hirotaka TAO
06-10	Coping with stress: Dynamic structural changes in αα-catenin regulate the cadherin-actin linkage	Noboru ISHIYAMA
07-22	Coordinated actomyosin kinetics in generating self-organized pattern formation in the cell cortex	Masatoshi NISHIKAWA
07-23	How to visualize published ChIP-seq raw data	Shinya OKI
07-24	Genetically speaking, BIG≠FAT	Akihiro MORI
09-08	Defining the strategies of stem cell self-renewal	Benjamin SIMONS

Date	Title	Speaker
09-09	Cyclic conversion of 5-methylcytosine to 5-hydroxymethylcytosine during the cell cycle in mouse embryonic stem cells	Masako TADA
09-16	A quantum jump in acorn worm diversity: deep-sea technology meets molecular phylogenetics	Nicholas D. HOLLAND
09-24	The *Drosophila* antimicrobial response at the time of the Cas9/CRISPR gene targeting revolution	Bruno LEMAITRE
10-30	Roles of Myosin II and Myosin III in regulation of hair cell apical morphology	Seham EBRAHIM
10-31	Know thy neighbor: The role of contact area geometry on cell-cell signaling	David SPRINZAK
10-31	Shaping the mammalian inner ear sensory organs by the vertebrate planar cell polarity pathway	Ping CHEN
11-12	The genomics of deafness: gene discovery and regulation	Karen B. AVRAHAM
11-13	Wounds: to heal or not to heal, that is the question	Sa Kan YOO
11-20	Epithelial spreading in zebrafish gastrulation	Carl-Philipp HEISENBERG
12-02	Unique mechanisms of successful fertilization in birds	Tomohiro SASANAMI
12-04	Spatial-temporal-spatial transformation in visual map plasticity: A novel rule in input dependent organization of circuit	Masateru HIRAMOTO
12-08	An evolving retinoic acid signaling pathway active during evolution	Vincent LAUDET
12-11	How birds create diverse feather colors — Avian melanocyte stem cells and regulation of feather pigment patterns	Sung-Jan LIN
12-17	Mammalian odorant receptors: deorphanization, accessory proteins and receptor gene choice	Hiroaki MATSUNAMI

About RIKEN

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

RIKEN was first organized in 1917 as a private research foundation, and reorganized in 2003 as an independent administrative institution under the Ministry of Education, Culture, Sports, Science and Technology.

RIKEN Website

The RIKEN website provides an in-depth resource for online visitors to the institute. The site contains important links to all materials, as well as databases and other electronicresources developed by RIKEN labs. We encourage those with an interest in learning more about RIKEN’s organization, activities and history to visit : **http://www.riken.jp/**

RIKEN Research

RIKEN publishes the monthly print and online newsletter RIKEN RESEARCH to draw the world’s attention to some of the institute’s best research in a timely and easy to understand fashion. This magazine provides a central resource for up-to-date information on key achievements of the numerous RIKEN institutes and research centers, along with related news and retrospectives on the history of institute. The core component of RIKEN RESEARCH is short, easy-to-understand ‘Research Highlight’ articles explaining for a broad scientific audience a sampling of the latest research articles published by RIKEN scientists. **http://www.riken.jp/en/research/rikenresearch/**

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Center for Advanced Photonics
Center for Sustainable Resource Science
Brain Science Institute
Nishina Center for Accelerator-Based Science
Advanced Center for Computing and Communication
Research Cluster for Innovation
Chief Scientist Laboratories
Associate Chief Scientist Laboratories
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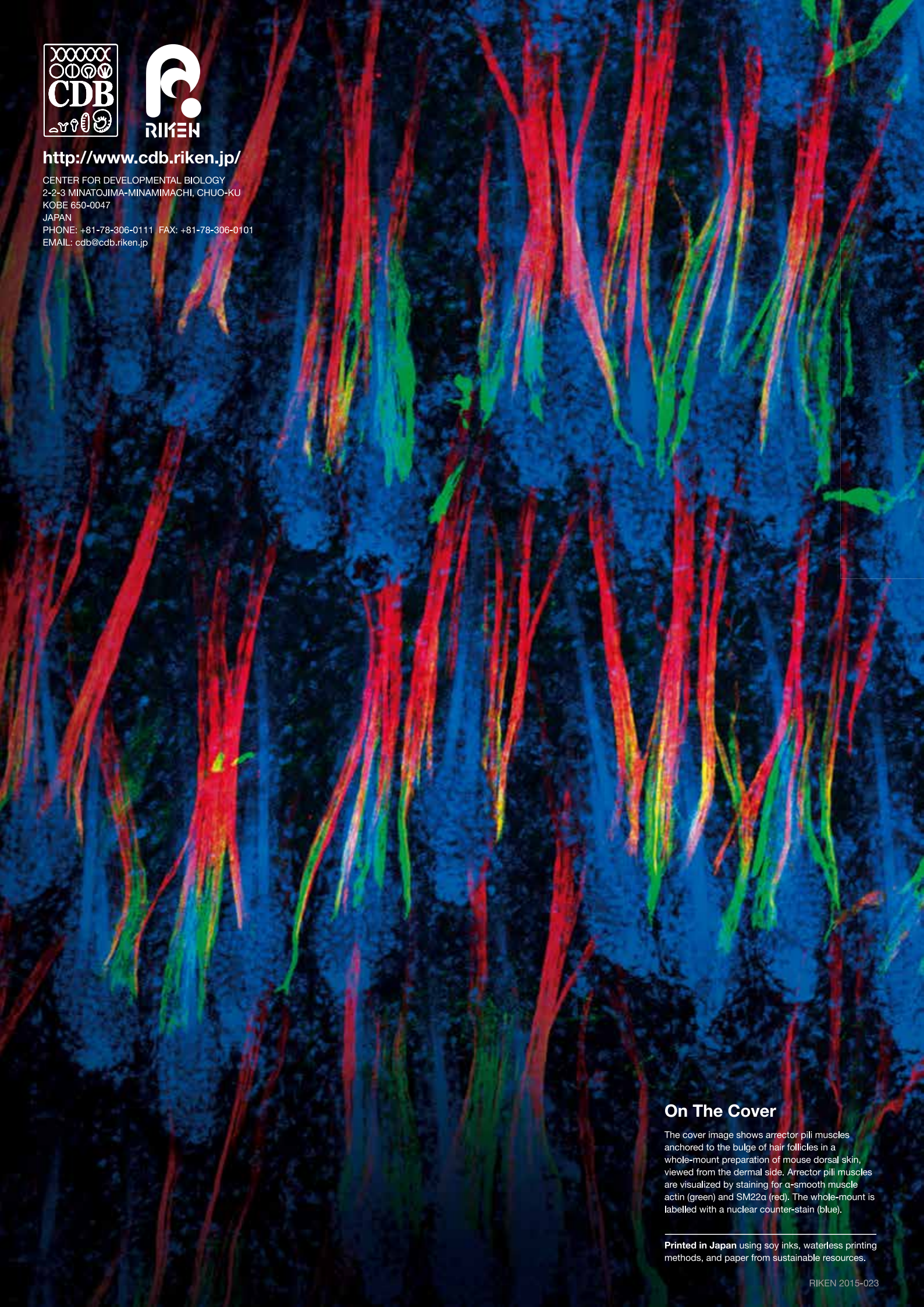
RIKEN

http://www.riken.jp/

RIKEN is a publicly funded research organization established to conduct comprehensive research in science and technology, and to disseminate the results of its scientific research and technological developments. RIKEN carries 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 kenkyuusho* (The Institute of Physical and Chemical Research). In 2003, the Institute was reorganized as an Independent Administrative Institution under the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and has continued to engage in wide-ranging research activities spanning the basic and applied sciences.



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On The Cover

The cover 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 labelled with a nuclear counter-stain (blue).

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

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