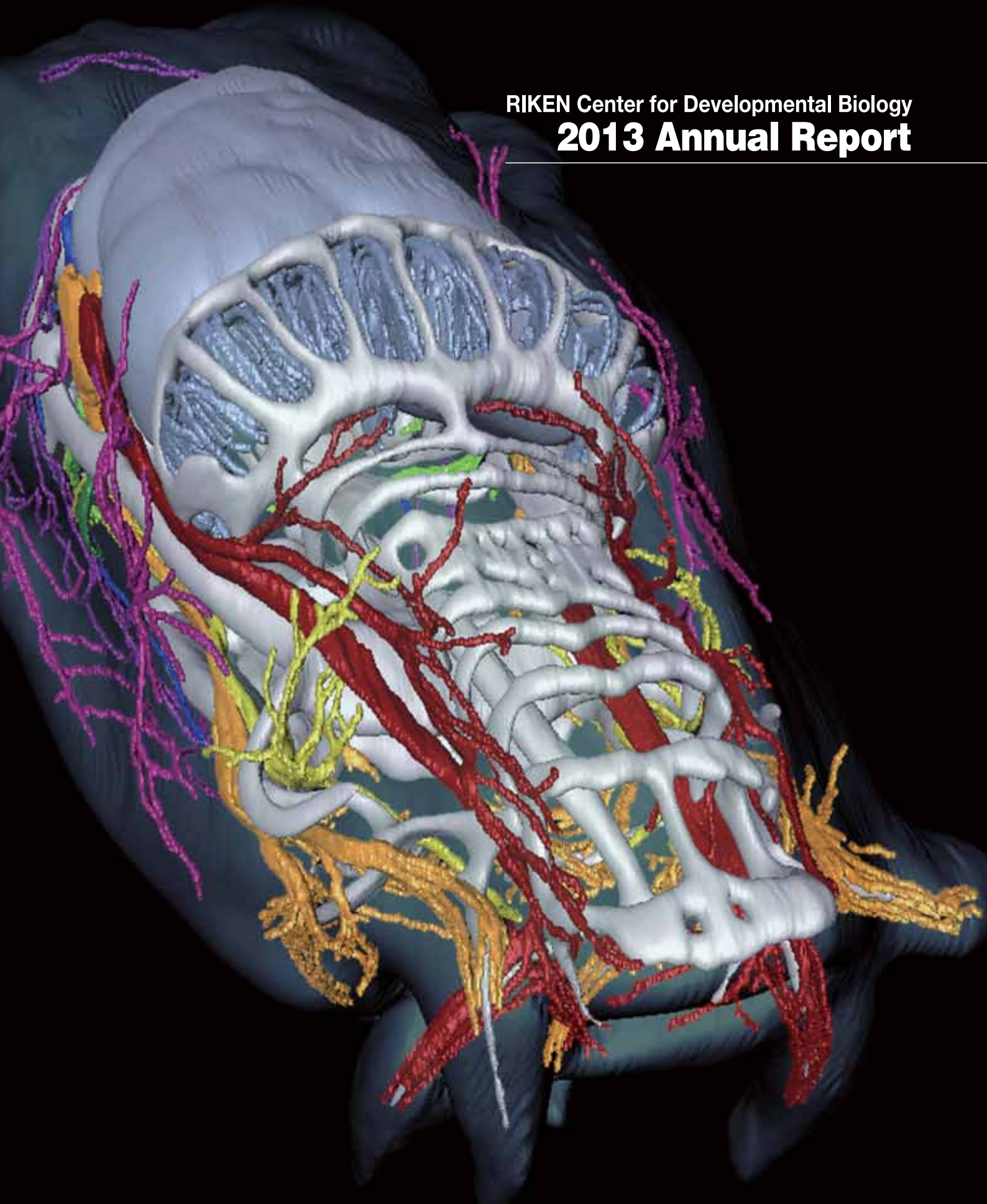


RIKEN Center for Developmental Biology
2013 Annual Report



RIKEN
Center for
Developmental Biology

2013 Annual Report



Contents

Message from the Director	2
Organizational Chart	4
2013 at the CDB	6
News Highlights 1	10
Labs	
Vertebrate Body Plan	24
Shinichi AIZAWA	
Reconstitutive Developmental Biology	25
Miki EBISUYA	
Neuronal Differentiation and Regeneration	26
Hideki ENOMOTO	
Tissue Microenvironment	27
Hironobu FUJIWARA	
Neocortical Development	28
Carina HANASHIMA	
Morphogenetic Signaling	29
Shigeo HAYASHI	
Developmental Epigenetics	30
Ichiro HIRATANI	
Sensory Circuit Formation	31
Takeshi IMAI	
Chromosome Segregation	32
Tomoya KITAJIMA	
Histogenetic Dynamics	33
Erina KURANAGA	

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

News Highlights 2 34**Labs****Evolutionary Morphology** 48

Shigeru KURATANI

Sensory Development 49

Raj LADHER

Cell Asymmetry 50

Fumio MATSUZAKI

Lung Development 51

Mitsuru MORIMOTO

Developmental Morphogeometry 52

Yoshihiro MORISHITA

Germline Development 53

Akira NAKAMURA

Chromatin Dynamics 54

Jun-ichi NAKAYAMA

Stem Cell Biology 55

Shin-Ichi NISHIKAWA

Growth Control Signaling 56

Takashi NISHIMURA

Pluripotent Stem Cell Studies 57

Hitoshi NIWA

News Highlights 3 58**Labs****Cellular Reprogramming** 72

Haruko OBOKATA

Mammalian Epigenetic Studies 73

Masaki OKANO

Organogenesis and Neurogenesis 74

Yoshiki SASAI

Early Embryogenesis 75

Guojun SHENG

Physical Biology 76

Tatsuo SHIBATA

Retinal Regeneration 77

Masayo TAKAHASHI

Cell Adhesion and Tissue Patterning 78

Masatoshi TAKEICHI

Systems Biology 79

Hiroki R. UEDA

Genomic Reprogramming 80

Teruhiko WAKAYAMA

Epithelial Morphogenesis 81

Yu-Chiun WANG

News Highlights 4 82**Supporting Laboratories** 92

RIKEN in Kobe 100

CDB Symposium 102

CDB Seminars 104

About RIKEN 106

RIKEN Campuses 107

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.





Message from the Director

Since our establishment in 2000, the RIKEN Center for Developmental Biology has maintained a firm commitment to solid science and pushing back the horizons of human understanding. In recent years, we have begun expanding our own horizons as well, with a greater focus on developing applications based on a strong foundation of knowledge derived from the study of embryonic development, regeneration, evolution, and cell and molecular biology. This broader focus is in line with the founding principle of responsible scientific citizenship that inspired the Center's launch, and which continues to guide us in our work.

Labs at the CDB made international headlines for a number of efforts in 2013. Perhaps the most visible as the announcement of the approval of a pilot safety study of the use of induced pluripotent stem cell (iPSC)-derived cell sheets in age-related macular degeneration, conducted jointly by the CDB and the

neighboring Institute for Biomedical Research and Innovation (IBRI). As the first clinical study in the world to gain regulatory approval, this project attracted a great deal of attention among patients, policymakers, and our fellow scientists, and we are hopeful that it will make a contribution to the development of new treatment options for this serious disease.

Our fundamental research efforts have also remained extraordinarily active and productive. CDB labs made important discoveries in the study of gastrulation, evolutionary morphological changes in both insects and vertebrates, and the regulation of the cytoskeleton and growth. Our scientists also developed new technologies for rendering the embryonic brain transparent, fluorescence in situ hybridization, and fully automated sleep staging, as well as the self-organized differentiation of human embryonic stem cells into tissue-like structures.



These activities have made the CDB the focus of national attention, and we were proud to welcome Japanese Prime Minister Shinzo Abe, who visited the Center in January to hear more about work in a number of our laboratories. The government's investments into basic science and potential clinical applications in regenerative medicine have been a key factor in enabling the CDB's research achievements.

We were delighted to welcome a number of new laboratories in 2014 as well. This spring, a pair of talented young scientists entered our strategic project for stem cell research, and in the fall two new team leaders joined our growing creative research program. This newest generation of CDB scientists is increasingly diverse and international, with more scientists from around the world, and more female laboratory leaders than ever before. We were also delighted to enter into a new international memorandum of understanding with the Korea Advanced Institute of Science and Technology (KAIST), raising the total number of such agreements to nine.

Here in Kobe, we continue to work closely with the academic and scientific communities in the greater Kansai area. As part of an organization-level restructuring, the former RIKEN Kobe Institute has been decentralized and re-designated as the Kobe Campus, but we maintain our strong ties and shared

interests with fellow RIKEN scientists at RIKEN Center for Life Science Technologies, Quantitative Biology Center, and the world-leading K supercomputer. We also work closely with high school students and educators through our ongoing workshops and educational programs, and engage with the public through events such as our popular annual Open House.

More than 10 years of active research stands between the completion of our facilities in 2002, and the end of 2013, and it has been a tremendously challenging and satisfying decade for all of us here. As we enter the New Year, we look forward to meeting even greater challenges and realizing even greater achievements, and we thank all of the many people around the world who have made our work to this point possible.

Masatoshi Takeichi

Director, RIKEN Center for Developmental Biology

A handwritten signature in black ink, appearing to read "Masatoshi Takeichi".

RIKEN Kobe Branch

Center for Developmental Biology

The Center for Developmental Biology (CDB) is a research center within the RIKEN Kobe Institute, which also comprises the Center for Molecular Imaging Science (CMIS), the Quantitative Biology Center (QBIC) the Kobe Research Promotion Division, which provides administrative services and support, and the institutional Safety Center. The CDB is home to a total of 32 laboratories in its Core Research Program (7 groups), Center Director's Strategic Programs (6 projects), Creative Research Promoting Program (16 teams), Regenerative Medicine Development Program (1 project) and Supporting Laboratories (6 labs). The CDB Director is assisted by a Deputy Director and is advised by the Advisory Council and the Institutional Review Board.

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.

Core Program

The Core Program aims to promote highly innovative developmental and regeneration studies in a strategic and multi-disciplinary manner. This program focuses on the main themes of the CDB: the mechanisms of development, organogenesis, and strengthening the scientific basis of regenerative medicine through the study of stem cells and regeneration.

- **Vertebrate Body Plan**
Shinichi AIZAWA Ph.D.
- **Morphogenetic Signaling**
Shigeo HAYASHI Ph.D.
- **Evolutionary Morphology**
Shigeru KURATANI Ph.D.
- **Cell Asymmetry**
Fumio MATSUZAKI Ph.D.
- **Stem Cell Biology**
Shin-Ichi NISHIKAWA M.D., Ph.D.
- **Organogenesis and Neurogenesis**
Yoshiki SASAI M.D., Ph.D.
- **Cell Adhesion and Tissue Patterning**
Masatoshi TAKEICHI Ph.D.

Center Director's Strategic Program

This program provides for concentrated focus on priority areas of research determined by the CDB Center Director. The initial 10-year projects will focus on stem cell and systems biology.

- **Reconstitutive Developmental Biology**
Miki EBISUYA Ph.D.
- **Developmental Morphogeometry**
Yoshihiro MORISHITA Ph.D.
- **Pluripotent Stem Cell Studies**
Hitoshi NIWA M.D., Ph.D.
- **Cellular Reprogramming**
Haruko OBOKATA Ph.D.
- **Physical Biology**
Tatsuo SHIBATA Ph.D.
- **Systems Biology**
Hiroki R. UEDA M.D., Ph.D.

Advisory Council

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

Director Masatoshi TAKEICHI

Deputy Director Yoshiki SASAI

Creative Research Promoting Program

The Creative Research Promoting Program provides solid support to encourage young researchers to carry out innovative and independent research plans. The teams are afforded a great deal of flexibility and control in regard to projects, budget use, and lab size. The program also emphasizes cooperation and international collaboration.

- **Neuronal Differentiation and Regeneration**
Hideki ENOMOTO M.D., Ph.D.
- **Tissue Microenvironment**
Hironobu FUJIWARA Ph.D.
- **Neocortical Development**
Carina HANASHIMA Ph.D.
- **Developmental Epigenetics**
Ichiro HIRATANI Ph.D.
- **Sensory Circuit Formation**
Takeshi IMAI Ph.D.
- **Chromosome Segregation**
Tomoya KITAJIMA Ph.D.
- **Histogenetic Dynamics**
Erina KURANAGA Ph.D.
- **Sensory Development**
Raj LADHER Ph.D.
- **Lung Development**
Mitsuru MORIMOTO Ph.D.
- **Germline Development**
Akira NAKAMURA Ph.D.
- **Chromatin Dynamics**
Jun-ichi NAKAYAMA Ph.D.
- **Growth Control Signaling**
Takashi NISHIMURA Ph.D.
- **Mammalian Epigenetic Studies**
Masaki OKANO Ph.D.
- **Early Embryogenesis**
Guojun SHENG Ph.D.
- **Genomic Reprogramming**
Teruhiko WAKAYAMA Ph.D.
- **Epithelial Morphogenesis**
Yu-Chiun WANG Ph.D.

Regenerative Medicine Development Program

The Regenerative Medicine Development Program provides support for labs engaged in the clinical translation of research relating to regenerative medicine, such as cell transplantation and tissue engineering.

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

Supporting Laboratories

The Supporting Labs provide a range of technical support functions available to all researchers working at the CDB. They also conduct research and development of new lab equipment and analytic software, and provide training on the use of research technologies.

- **Animal Resources and Genetic Engineering**
Yasuhide FURUTA Ph.D.
Genetic Engineering Unit
Yasuhide FURUTA Ph.D.
Animal Resource Unit
Hiroshi KIYONARI Ph.D.
- **Electron Microscope**
Shigenobu YONEMURA Ph.D.
- **Bio-imaging**
Shigeo HAYASHI Ph.D.
Optical Image Analysis Unit
Yuko KIYOSUE Ph.D.
- **Genomics**
Fumio MATSUZAKI Ph.D.
Genome Resource and Analysis Unit
Shigehiro KURAKU Ph.D.
Functional Genomics Unit
Hiroki R. UEDA M.D., Ph.D.
- **Proteomics**
Shigeo HAYASHI Ph.D.
Mass Spectrometry Analysis Unit
Shigeo HAYASHI Ph.D.
- **Division of Human Stem Cell Technology**
Yoshiki SASAI M.D., Ph.D.
Human Stem Cell Technology Unit
Yoshiki SASAI M.D., Ph.D.
Four-dimensional Tissue Analysis Unit
Mototsugu EIRAKU Ph.D.
Science Policy and Ethics Studies Unit
Douglas SIPP

2013 Highlights

Prime Minister Abe visits CDB

Japanese Prime Minister Shinzo Abe visited the Center on January 11, accompanied by RIKEN President Ryoji Noyori and Kyoto University Professor Shinya Yamanka, of induced pluripotent stem cell (iPSC) fame. The delegation met with group director Yoshiki Sasai, who introduced his techniques for pluripotent stem cell differentiation, and Project Leader Masayo Takahashi, who spoke about her plans to test an iPSC-based approach to the treatment of age-related macular degeneration.



The Making of a Vertebrate : 11th CDB Symposium

The Center held its 11th annual symposium on March 4–6, on the theme “The Making of a Vertebrate.” More than 180 scientists from around the world gathered to share their latest work and exchange views on novel insights into vertebrate embryology.



Clones cloned from clones

The Lab for Genomic Reprogramming reported new refinements to somatic cell nuclear transfer techniques that increase efficiency and enable cloning of mice across a great many generations. The researchers found that they were able to serially clone mice for at least 25 generations.



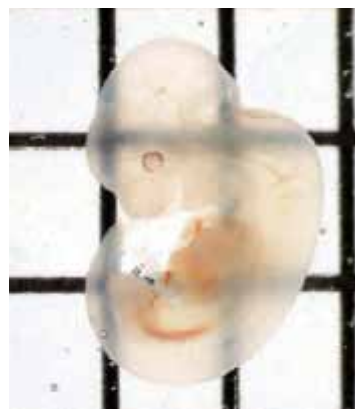
New Research Unit Leaders join the Center

The CDB welcomed two new Research Unit Leaders in April, both as part of the Center Director's Strategic Program in Stem Cell Biology. Miki Ebisuya will focus on reconstituting developmental phenomena in vitro, while Haruko Obokata will study new approaches to cellular reprogramming.



CDB and KAIST sign collaborative agreement

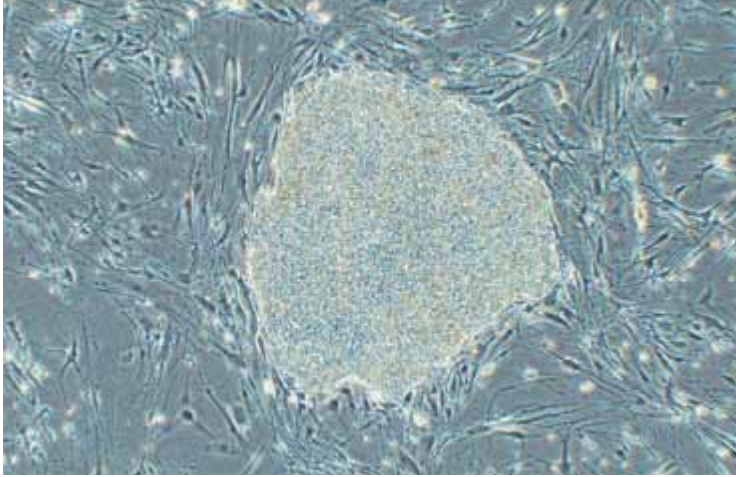
Also in April, research leaders from the CDB and the Korea Advanced Institute for Science and Technology (KAIST) signed a memorandum of understanding to promote exchange of scientists and research interactions. This is the tenth such agreement with an international institution entered into by the CDB.



SeeDB gives a new look into the embryonic brain

A new study by the Lab for Sensory Circuit Formation introduces a novel technique for rendering the embryonic brain transparent, making it possible to study intricate neural structures stained by fluorescent dyes. This simple and efficient method represents a powerful tool for a wide range of neuroscientists and developmental biologists.

iPSC-based pilot study gets go-ahead



The Laboratory for Retinal Regeneration received approval to begin enrolment for the world's first clinical study of an iPSC-based intervention. Working with the Institute for Biomedical Research and Innovation, the lab will test the safety of autologous iPSC-derived retinal pigment epithelium cell sheets in a small number of patients with wet-type age-related macular degeneration.

Studying protein isolation at the CDB Summer School

Groups of local high school students got a chance to learn some developmental biology firsthand during the Center's summer school courses in August. Team Leader Mitsuru Morimoto began by introducing the topic of cellular differentiation with a focus on his work in lung development, after which the students learned to extract and purify proteins from a number of tissues using a form of electrophoresis called SDS-PAGE.



Kobe Campus holds Open House

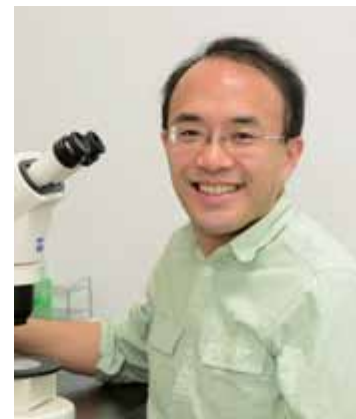


The RIKEN Kobe Campus held its 2013 Open House on October 19, welcoming more than 1500 visitors from Kobe and surrounding areas to hear about the latest scientific developments from labs at the CDB, CLST, and QBiC. A public lecture by CDB lab head Masayo Takahashi on her recently approved clinical study was one of the most popular events of the day.



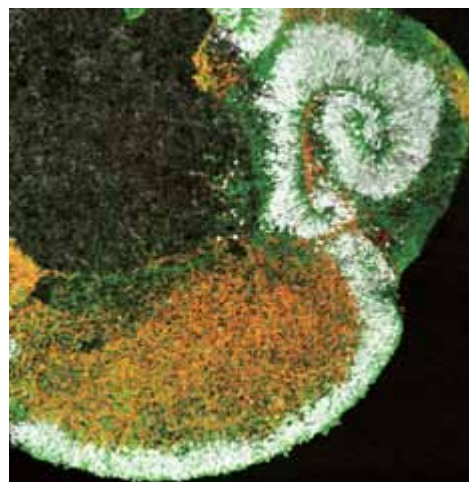
CDB welcomes two new Team Leaders

The Center was pleased to welcome Ichiro Hiratani and Yu-Chiun Wang as its newest team leaders in October. Hiratani will focus on studying the formation and epigenetic functions of heterochromatin during development, while Wang will investigate how epithelial cell polarity and morphology determine tissue structure.



Neocortex formed from hESCs

The Lab for Organogenesis and Neurogenesis reported their latest success in inducing self-organized three-dimensional structures from pluripotent stem cells in vitro, taking their technique to the next level by directing human embryonic stem cells to form highly organized neocortex-like structures in a 4D culture environment.



Decoy insulin receptor controls *Drosophila* body size



(Front) Yuki YAMAUCHI, Rinna NAKAMORI
(Middle) Junko SHINNO, Tomoka MURAI, Kanako HIGUCHI, Noriko NISHIMURA
(Back) Takashi NISHIMURA, Yuichiro KASHIHARA, Tomoyuki TAKAHASHI, Naoki OKAMOTO, Takayuki YAMADA

Insulin and insulin-like growth factors (IGFs) are known to play important roles in growth and metabolism. These hormones, which are conserved from insects to mammals, circulate in the blood and bind with specific cell surface receptors, triggering downstream signals within the cell. Although Insulin and IGFs are essential for regulating growth and metabolism, how these secreted molecules are regulated in the circulating blood has remained something of a puzzle.

Naoki Okamoto and colleagues in the Lab for Growth Control Signaling (Takashi Nishimura, Team Leader) have fitted a major new piece into this jigsaw, showing how a secreted decoy insulin receptor acts as a negative regulator of in-

sulin/IGF signaling in *Drosophila*, thus controlling growth. This work is published in *Genes & Development*.

In vertebrates, secreted IGFs associate with IGF-binding proteins (IGFBPs) in the bloodstream, which stabilizes the IGFs and prevents them from binding with cell surface receptors. In the fly, *Drosophila* insulin-like peptides (Dilps) play the roles of both insulin and the IGFs in vertebrates. Previous reports have shown that the IGFBP-like protein Imp-L2 binds directly to several Dilps in the hemolymph, thereby regulating insulin/IGF signaling. But despite the need to keep such a crucial pathway under tight control, no other molecules capable of regulating Dilps have been reported in *Drosophila*.



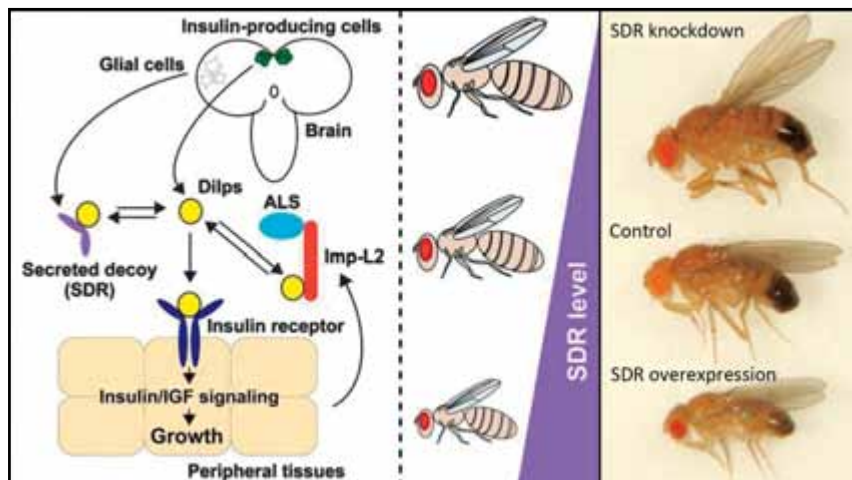
The Nishimura lab set out to identify novel molecules involved in developmental growth regulation by screening secreted protein RNAi knockdown phenotypes for effects on body size, and identified a gene whose loss of function resulted in a higher growth rate in larval stages and larger adult body sizes. This new gene included a sequence closely similar to that of the insulin receptor (InR) extracellular domain, which, along with its functional attributes, led them to name it *secreted decoy of InR (SDR)*. *SDR* mutants showed no defects in survival or fertility, but grew about 20% larger than wildtype flies. Close observation of wings in adult mutants revealed that the change in overall size was a result both of greater numbers of cells and increased cell size.

SDR has neither a transmembrane nor a cytoplasmic domain, pegging it as a likely secreted protein, which Okamoto et al confirmed by detecting high levels of the factor in hemolymph. The team next looked at gene expression patterns and conditional RNAi phenotypes in various tissues and found that it was highly expressed in the glial cells of the brain. After deciphering where *SDR* was made, the next step was to find out how it worked. Using the localization of dFOXO, which responds to insulin/IGF signaling on/off cues, as an index, they found that insulin/IGF signaling was accelerated in *SDR* mutants, and conversely inhibited on its overexpression. *In vitro* assays further showed direct bind-

ing between *SDR* and several Dilps, suggesting that its mode of action involves antagonistic binding with these Dilps, preventing their association with receptors.

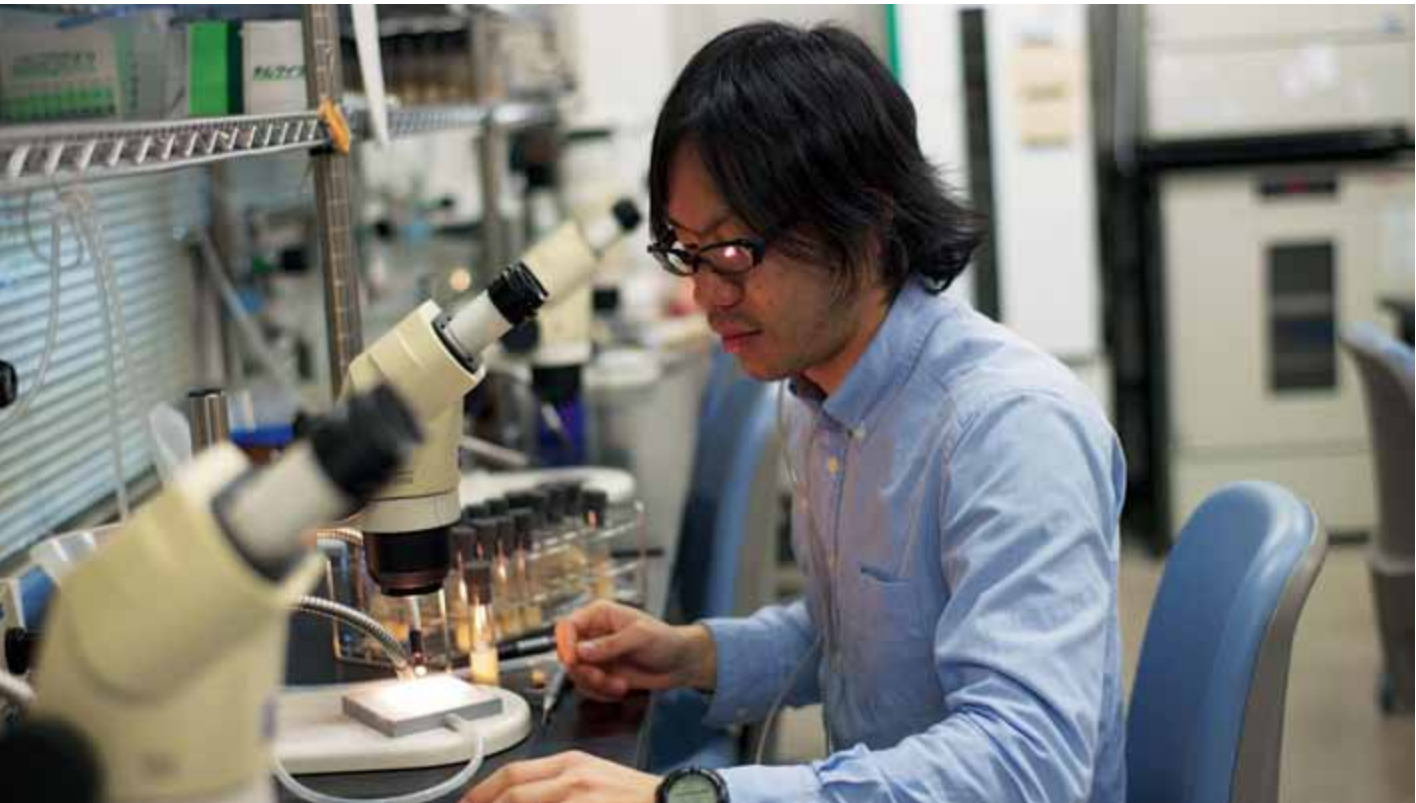
Other insulin-linked molecules are known to respond to changes in nutritional status. Looking at starved flies, in which the expression of some *Dilps* is down-regulated and that of Imp-L2 increased, a mechanism though to allow the body to sacrifice growth in favor of storing nutrients, they found that *SDR* expression and secretion remained unexpectedly stable under low-nutrition conditions. But when *SDR* mutants were bred under extremely adverse conditions where food availability was only one-tenth normal, the failure of growth inhibition led to poor survival, with over half of the flies dying during metamorphosis, suggesting that *SDR* is an essential safety mechanism for controlling insulin/IGF signaling under poor nutritional conditions.

“Although the *SDR* gene is found only in insects, similar decoy systems for Insulin/IGF signaling are likely present in other species. Recent observations have indicated that mammalian insulin receptor can potentially produce a soluble decoy,” says Nishimura. “Our initial focus was on the role of *SDR* in growth regulation, but from now we will be interested to learn more about its functions in other phenomena, such as metabolism and aging.”



Cartoon of *SDR* mode of action (left), shows binding to Dilps in hemolymph, preventing association with its membrane receptors. *SDR* mutant phenotypes (right) showing changes in body size; loss of function leads to larger sizes, overexpression to smaller.

Mitotic cell rounding as a driver of morphogenesis



Takefumi KONDO

The inward folding, or invagination, of epithelial tissues is an important morphogenetic phenomenon, converting flat sheets of cells into three-dimensional tissues. This process is often, but not always, associated with apical constriction of the actin cytoskeleton and produces indented hollows in epithelial surfaces, such as the placodes that give rise to sensory and other organs. Cells entering mitosis have generally been thought to be incapable of undergoing the intensive cell shape remodeling that underlies these processes, but as some tissues in which invagination takes place show signs of cell cycle activity, leaving open the question of how these two phenomena might interact.

Takefumi Kondo of the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) has now shown

that a process known as mitotic rounding drives invagination of the tracheal placode in the fruit fly, *Drosophila melanogaster*. This work, published in *Nature*, shows that mitosis plays an unsuspected role in this form of developmental tissue remodeling.

The apical constriction of epithelial cells, in which the apical regions of cells shrinks giving them a roughly conic structure, has long been thought to be the driving force behind invagination. But a number of tantalizing exceptions in which inward folding occurs without such constriction suggested that some other dynamic must also be involved. Using live imaging of the tracheal placode in *Drosophila* embryos, the group sought to answer this question. This placode begins as a flat sheet of about 40 cells, which



transforms into an L-shaped tubular structure. Watching the process with an eye to detail, Kondo observed that this invagination played out in two distinct phases. In the first, slow phase, cells in the center of the placode undergo apical constriction, and gradually move toward the embryo interior. A second, fast phase followed in which the tracheal pit grew and cells were more rapidly internalized.

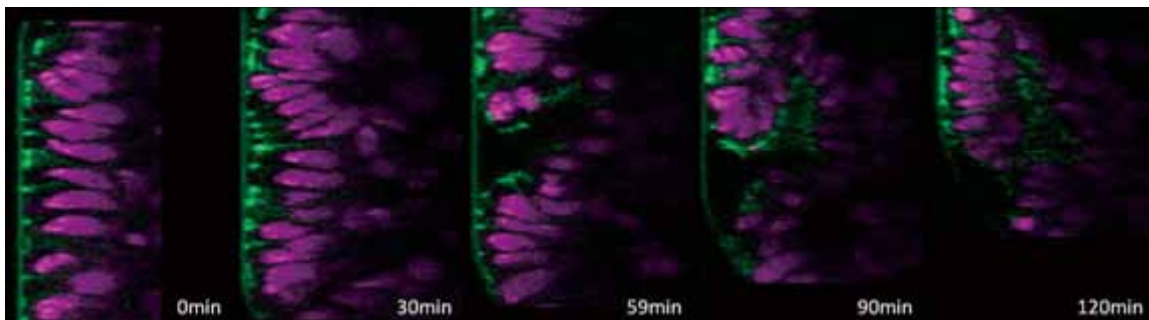
Interestingly, this fast phase was consistently associated with the mitotic entry of cells in the placode center. As cells enter the mitotic phase, they ball up. In orderly epithelial sheets, this 'rounding' behavior can destabilize the tissue, meaning that its site and timing need to be closely controlled, and indeed studies of mutants have shown that morphogenesis is disturbed when this program is dysregulated. Knowing this, Kondo used mutants of a cell cycle gene (*CycA*), which fail to enter the final stage of embryonic mitosis, and watched for effects on invagination. Although the slow phase proceeded as usual, the pace of the fast phase was appreciably decreased. Despite the delay, however, tracheal invagination did occur, suggesting that other forces might be at work as well.

The FGF signaling pathways is involved in inducing branching morphogenesis in the fly trachea, so Kondo tested next looked for a role for this molecule, but found that in single FGF mutants invagination was unaffected. In double mu-

tants for the cell cycle gene and FGF, however, invagination was slowed and incomplete, suggesting FGF might function as a backup mechanism in this process. In a separate experiment, chemical inhibition of microtubules showed that invagination can take place even when rounding occurs in the absence of mitotic spindles, indicating that it is the morphogenetic changes, not the cell division, that are required for the involution of the epithelial sheet.

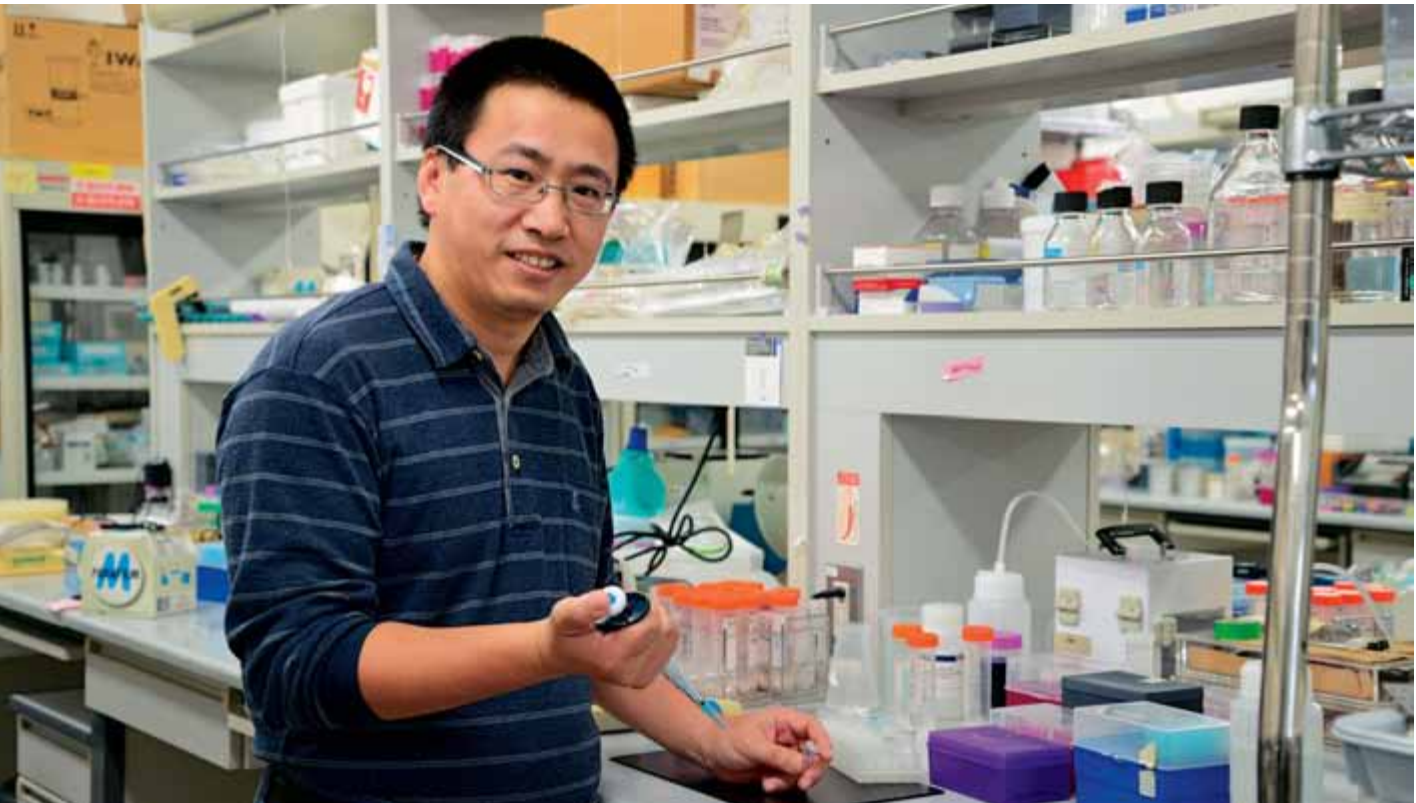
So how then do cells in the placodal center know when and where to begin invaginating? Kondo turned back to a previous study by the Hayashi lab, in which it was shown that the motor protein myosin confers constrictive force to actin fibers in cells peripheral to the tracheal placode center, forming a pressure front that may drive cells at the center inward. As this mechanism relies on the EGF receptor protein (EGFR), the group generated EGFR mutants and watched for effects. When this gene was ablated along with *CycA* and *FGF*, invagination failed completely.

"This work shows how multiple independent mechanisms can play overlapping and complementary roles in epithelial invagination," says Hayashi. "It is these kinds of elegant, robust programs that enable development to proceed in such as stable fashion. It seems that in order to give rise to the beauty of the animal form, embryos need to follow beautiful rules."



Tracheal invagination begins with a slow phase, later transitioning to a fast phase in which mitotic cells near the center of the internalizing epithelium undergo rounding and drive the formation of an L-shaped tube.

Retrograde recycling keeps tubules in shape



Bo DONG

Networks of tubular structures form the basis for many important organs and tissues in the animal body, such as the vasculature and airways. The need for such luminal structures developed as bodies grew larger over the course of evolution, necessitating conduits for the life-sustaining circulation of nutrients, gases and wastes throughout the body. Animals and their organs come in many different sizes and shapes, of course, so it is also necessary for lumens to fit the surrounding morphology. Width and length are the critical parameters in this equation, but how does the developing embryo make these measurements?

New work by Bo Dong and others in the Lab for Morphogenetic Signaling (Shigeo Hayashi, Group Director) now shows how an intracellular mechanism known as retro-

grade trafficking establishes the length of the developing trachea in fruit fly embryos. Published in *Nature Communications*, this study sheds light on the morphological regulation of an important class of tissue structures.

The formation of the *Drosophila* trachea is under the control of extracellular matrix proteins such as chitin secreted within its lumen. It has previously been shown that the diameter of this structure is regulated by an intracellular protein trafficking pathway mediated by actin complexes, while its length is controlled by a chitin deacetylase known as Serpentine (Serp). Loss of Serp results in an abnormally long trachea, but just how this mechanism is tied to the protein trafficking that regulates intraluminal secretion was poorly understood.

Dong B, et al. Rab9 and retromer regulate retrograde trafficking of luminal protein required for epithelial tube length control. *Nat Commun* 4.1358 (2013)

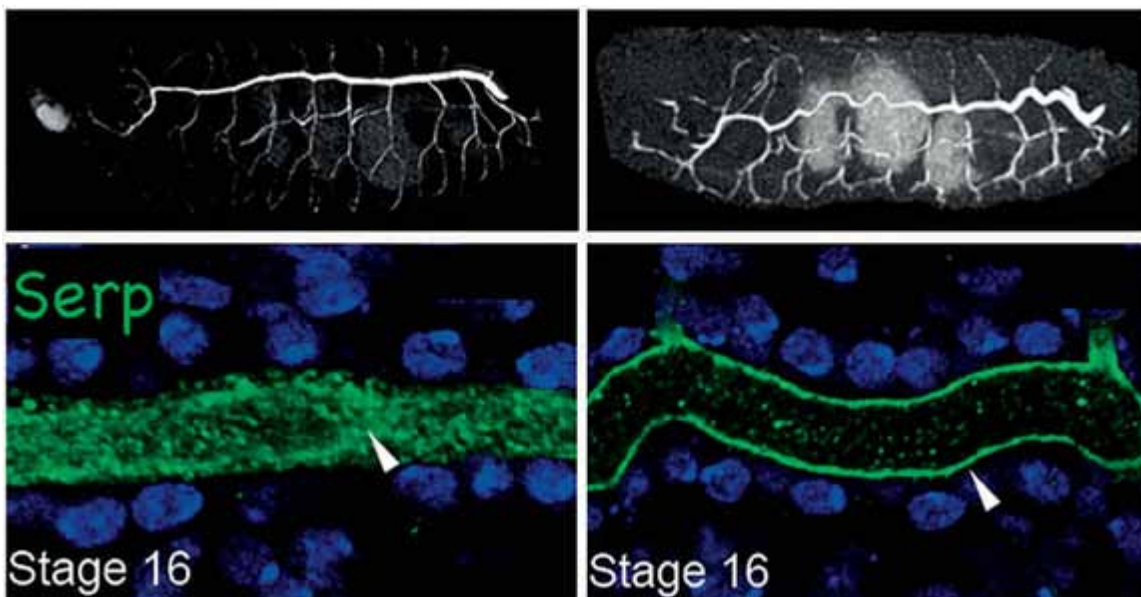


After searching for proteins showing specific localization patterns in the cells of the trachea, Dong et al. came to focus on Rab9, a protein known to be involved in retrograde trafficking, a kind of intracellular recycling process in which secreted molecules in the cell's environment are endocytosed and carried in endosomes to the *Trans-Golgi* network, where they are modified and readied to be secreted again.

Generating a *rab9* mutant, the group noted that while the width of the trachea and embryogenesis as a whole appeared normal, the trachea grew to unusual length, a phenotype resembling that seen in *Serp* mutants. Looking for co-localization of the two molecules, they found that after about 12 hours of development, the levels of *Serp* expression within the tracheal lumen dropped sharply, while chitin and other proteins were unaffected. In mutants of another known retrograde trafficking protein, *Vps35*, the over-long trachea phenotype was also observed, and biochemistry assays indicated that Rab9 and *Vps35* bind each other directly.

Using a live imaging system, Dong watched the dynamics of these three proteins and found that they co-localize as assemblies in an endosomal subtype known as late endosomes. Knowing that actin is crucial for the budding of vesicles from endosomes, they next looked for a spatial relationship between actin and the Rab9 endosomes. They found that actin and a newly identified actin-associated factor, WASH, localized with *Vsp35* at budding sites and are involved in the endosomal recycling of *Serp*.

“We think it is very interesting that loss of retrograde trafficking does not affect the tracheal diameter, which suggests that length and width are under the control of separate trafficking pathways,” says Hayashi. “Retrograde trafficking is widely conserved in animals, including humans, so we will be eager to discover whether it plays a more general role in morphogenesis.”



Formation of abnormally long trachea (top right), and reduced intraluminal expression of *Serp* (bottom right) in *Rab9* mutant.

Cutting the crosstalk: New confocal technique provides deeper look inside biological samples

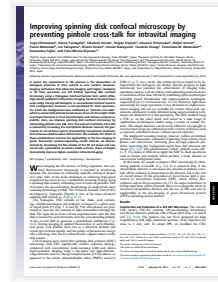


Togo (and Yuh) SHIMOZAWA

Confocal microscopy is a common tool for looking at the interiors of living samples in three dimensions. To increase the speed of image acquisition, multi-point scanning methods using a spinning disc embedded with thousands of pinholes that remove out-of-focus background light have been developed. But while spinning disk confocal microscopes cause less radiation damage to samples and achieve higher temporal resolution than conventional alternatives, the conventional model was limited in its ability to probe the interiors of large specimens due to a phenomenon known as ‘pinhole crosstalk,’ in which out-of-plane and scattered emissions bleed through inappropriate neighboring pinholes and increase the background haze that obscures images.

A new development by Togo Shimozawa and others in the Optical Image Analysis Unit (Yuko Mimori-Kiyosue, Unit Leader) shows a way out of the crosstalk by adjustments to pinhole placement and the use of two-photon excitation to reduce out-of-focus light. Published in the *Proceedings of the National Academy of Sciences*, these enhancements extend the range of applications of spinning disk confocal microscopy in the live imaging of larger specimens, including whole embryos. Shimozawa has since moved to Gakushuin University.

Expecting, based on theory, that the problematic pinhole crosstalk might be eliminated by increasing the space between these apertures, the lab modified a standard Confocal Scanner Unit (CSU), doubling the distance between pin-

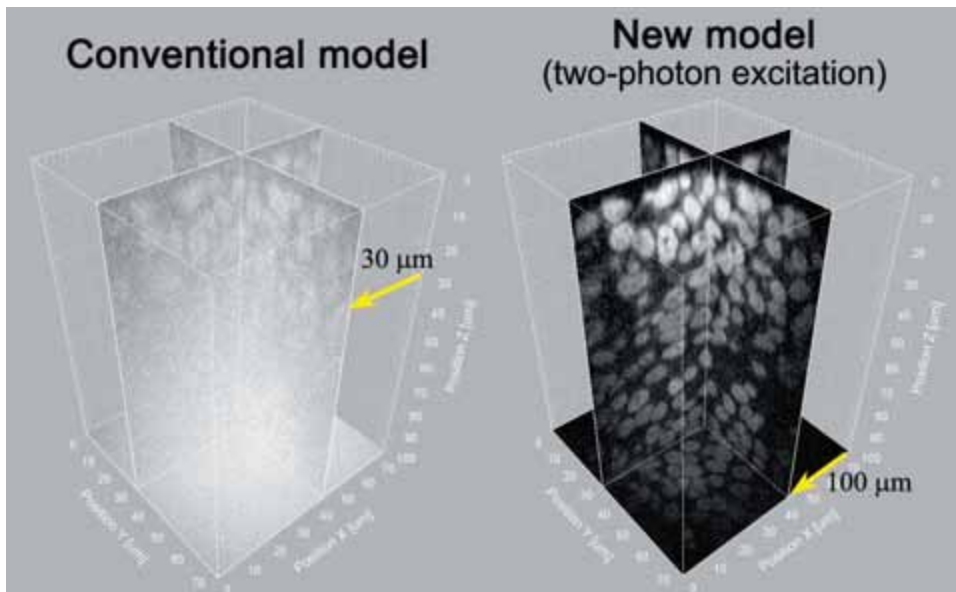


holes as well as the size of the microlenses that focus light onto them. The doubling of microlens size increases the concentration of light to the pinholes, boosting two-photon absorption efficiency, which is crucial to eliminate background light generated in out-of-focus planes, a key contributor to pinhole crosstalk.

A comparison of the two-photon excitation (2PE) setup with the standard CSU revealed the benefits of the new approach. While in the conventional technology background noise due to crosstalk can be as high as 60% of the total detected signals when imaging a thick specimen, with 2PE this dropped to as low as 0.5%. When they used the new apparatus to image a bulky (diameter 30 μm) pollen grain, not only was background light reduced, but 2PE also revealed rich new features of the particle's interior. And by assembling multiple images taken in this fashion, the lab was able to reconstruct its structure as a 3D image, pulling out details from even its far side that remained obscured in the background of single-photon images.

For their next demonstration, Shimozawa et al. used the 2PE system to examine GFP-expressing model organisms of the sort typically used in embryology studies. In mouse embryos, where traditional spinning disk confocal microscopes can only visualize to depths of around 30 μm , the new system could image three times as deep, to over 100 μm . The 2PE unit also showed the ability to visualize sub-micron structures in vivo. Looking at *Drosophila* embryos, they were able to follow the movements of a microtubule plus end-associated protein, EB1, by time-lapse imaging.

"We're very hopeful that this new technology will find applications across the life sciences and in other disciplines as well," says Mimori-Kiyosue. "But as the power of the lasers that generate the multiple beams needed for this approach is the limiting factor, we are looking to develop higher-power laser sources that will allow us to look even deeper and more clearly into microscopic specimens."



Imaging of sectioned mouse embryo expressing H2B-GFP which is distributing in nuclei. In the conventional image (left), pinhole crosstalk causes low contrast and prevents the visualization of nuclei outlines deeper than 30 μm , but in the 2PE image (right), contrast is high and nuclear outlines can be seen as deep as 100 μm .

Clones of clones can make clones



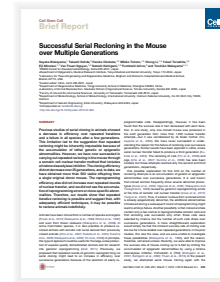
Sayaka WAKAYAMA

Since the first reports of successful cloning of mammals by somatic cell nuclear transfer, concerns have been raised about the efficiency and repeatability of cloning techniques, and the health of cloned offspring. Although it has been shown since the early 2000s that cloned animals can themselves be cloned, the efficiency of SCNT appeared to taper with each successive generation, leading some to speculate that there might be an inherent limit to cloning from clones due to the accumulation of genetic errors.

Now, however, a new report by the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) shows that mice can be cloned successively for at least 25 generations. Published in *Cell Stem Cell*, this work by Wakayama, now at the University of Yamanashi, suggests

that there is no inherent limit to SCNT repeatability, given sufficiently efficient methods.

The team used four genotyped female mice as its starting set of nuclear donors, removing the genetic material from cumulus cells for use in SCNT attempts, giving rise to a number of cloned offspring. Donor mice that showed the greatest ability to give rise to first generation clones was selected to serve as the donor for the subsequent serial cloning experiment, which began in late 2005. For each successive generation, cumulus cells were obtained from 3-month-old mice and used as donor cells for the next round. Wakayama found that the efficiencies varied by generation, ranging from 4–5% in early rounds to more than 20% in later generations, including the 25th (G25), the last



generation reported. Some of this variation may be attributable to improvements in cloning technology that emerged during the study period.

During this lengthy investigation, which gave rise to more than 500 viable cloned offspring, the team examined whether complete matching of donor nuclei with recipient oocytes would boost efficiency, but they found that even microinjection of donor somatic nuclei into oocytes from the same animal did not result in a higher success rate, suggesting that, in principle, heterogeneous donors are equally capable of giving rise to clones.

Cloned offspring in some species have shown a range of developmental and health defects. In mice, clones have been observed to have enlarged placentas (placentomegaly). In the 25-generation series, however, the body weights of the cloned offspring remained consistently within the normal range for wildtype animals, while placentas were two- to three-fold heavier. This effect did not increase with higher generations suggesting that the abnormality that underlies this phenomenon is not cumulative.

Fertility is another measure of the health status of cloned animals. Wakayama randomly selected four animals from

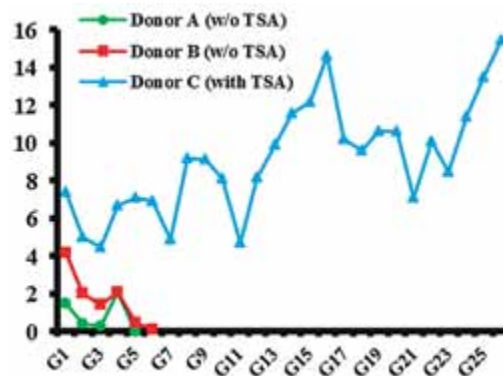
the 20th generation of the series and mated them with mice that had not been cloned, and found that all four were fertile and gave birth to healthy litters of pups. The lab also conducted tests of telomere lengths and gene expression patterns in the cloned mice. While no shortening of telomeres was observed even in later generation clones, there were differences in the expression of some genes. But again, these resembled differences observed in non-serially cloned control animals, and did not appear to change from generation to generation.

One key element of the cloning technique appears to be the addition of a reagent known as trichostatin A to the cell medium, which has previously been shown to raise SCNT efficiency by approximately double. In G20 cloning efforts, the team compared SCNT with and without trichostatin, and found that the effect on reprogramming remained the same.

“This series of experiments is exciting because it reveals how, with the right techniques, clones can continue to be made even after the death of the original donor animal,” says Wakayama.



Recloned mouse (black) and surrogate mother (white)



Cloning efficiencies over 25 generations

CandyFISH lights up gene expression



Satoko MORIWAKI, Lars Martin JAKT

Techniques for imaging the inner workings of cells are a backbone of modern biological investigations. In developmental biology studies, information on the behavior and properties, such as gene expression, of interacting cells is fundamentally important for gaining insight into their respective spatial arrangements and dynamics. Fluorescence in situ hybridization (FISH) is a well-known method for visualizing the localization of individual RNA molecules within cells, providing insight into the expression of genes of interest, but this technique is limited in the number of different genes it can be applied to simultaneously in a single cell.

In a new report, Lars Martin Jakt and colleagues in the Lab for Stem Cell Biology (Shin-Ichi Nishikawa, Group Director) describes a new variant of this technique that uses large

numbers of short probes making it possible to target transcripts by multiple fluorescent molecules, dramatically expanding the number of genes that can be visualized in situ. Published in *Development*, this new CandyFISH method may open the way to the simultaneous visualization of transcripts from 10 or more genes. In a proof of concept demonstration Jakt, now at Keio University, put the technique to use, revealing changes in gene expression in individual cells during the differentiation of mesoderm into endothelium.

The original FISH method involves labeling mRNA molecules with fluorescent RNA probes, enabling the visualization of the types and amounts of expressed genes in living cells. A recent improvement to this method have fused mul-

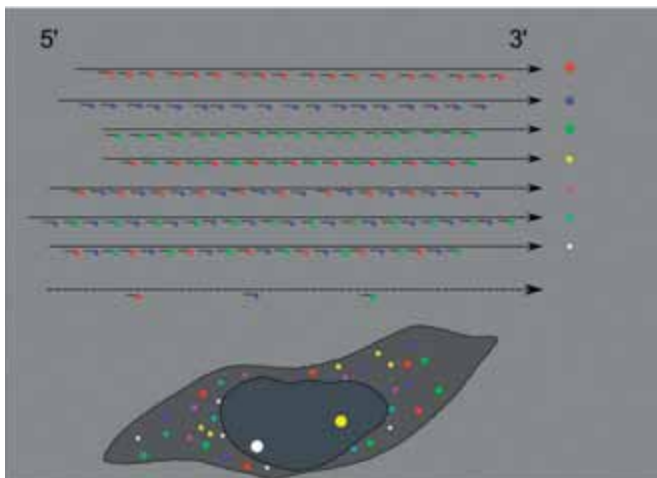


multiple short probes to single genes, yielding higher fluorescence and comparatively low cost, but with the limitation of being applicable to a maximum of only three genes. Jakt's refinement shows that using many short probes allows transcripts to be targeted by multiple fluorophores, allowing the quantification of gene expression from more than double that number of genes.

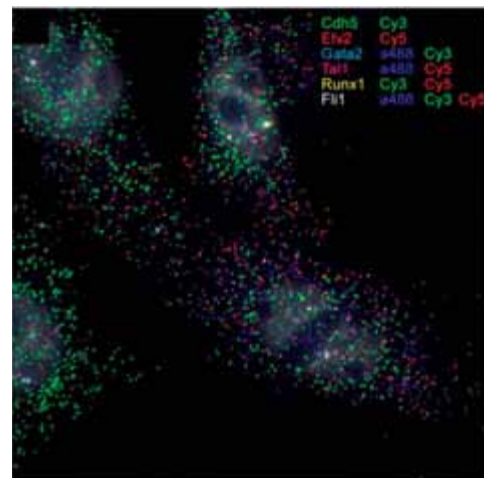
In a proof of principle experiment, the group next used the new method to examine changes in gene expression profile of cells during the differentiation of vascular endothelium from embryonic mesoderm. Previous in vitro studies had shown *Etv2* to be an important gene in this process, involved in the induction of endothelial genes, as well as a direct inducer of genes involved in stabilizing the hematopoietic state. This diversity of downstream targets made for a very fuzzy picture of the order in which *Etv2* activates other genes.

Jakt studied ES cells induced to differentiate into endothelium using six genes of interest marked using the CandyFISH technique, enabling him to visualize the intracellular localization of their RNA transcripts. By plotting changes in transcript density in individual cells over time, the group was able to work out a progression of the switching on and off of *Etv2* and its targets, yielding a much clearer image of how gene expression changes in single cells during endothelial differentiation.

CandyFISH represents an important new step in simultaneously measuring the abundances of multiple RNAs at the single-cell level, and consequently tracking changes in cellular identity. Additional refinements in microscopy and fluorescent labels may enable the tracking of the expression of as many as 10 genes in individual cells, and to analyze deeper tissue strata.



Combinations of three different-colored fluorescent tags enable labeling of six genes.



FISH analysis of six genes yields images resembling colored candy in a bowl.

2013 Events

The RIKEN CDB strives to engage with the public through a variety of media, including its website, media coverage and direct interactions, such as guided tours, visitors' day events, and other outreach activities. In addition to

this work in public engagement, the Center also organizes events for bringing scientists together outside of the laboratory environment.

CDB-IGDB-KAIST Joint Meeting

The CDB hosted a group of researchers from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (IGDB) and the Korea Advanced Institute of Science and Technology (KAIST) on February 19 and 20. This joint meeting provided a great opportunity to promote scientific exchanges between researchers in East Asia. Organized following the success of two prior meetings held between the CDB and IGDB in May 2009 in China, and between the CDB and KAIST in November 2011 in Korea, the meeting also led to the CDB and KAIST signing a Memorandum of Understanding in March 2013 to facilitate and encourage collaborations between the two institutions.



RIKEN signs MOU with Indian Institutions

On September 16, at the National Center for Biological Science (NCBS) in Bangalore, India, RIKEN signed a Memorandum of Understanding with three Indian institutions: the NCBS, the Institute for Stem Cell Biology and Regenerative Medicine (inStem) and the Center for Cellular and Molecular Platforms (C-CAMP). In attendance at the signing ceremony were RIKEN President, Ryoji Noyori, and Executive Director, Kenji Oeda, along with CDB Group Directors, Shigeo Hayashi and Shigeru Kuratani. The MOUs build on an already long-standing relationship of collaborative research exchanges established between the CDB and NCBS in 2004. A joint workshop was held after the signing ceremony as the first step toward firming relations between the institutions.



Open House 2013

The annual RIKEN Kobe Campus Open House was held Saturday, October 19. Despite the light drizzle outside, over 1500 people visited the CDB to learn about the research being done at the center, and enjoy the exhibits and activities organized to appeal to people of all ages. This year, the event featured a talk by Masayo Takahashi on using iPS cells in clinical applications, specifically for age-related macular degeneration (AMD), a science crafts corner for kids, hands-on experimental workshop extracting one's own DNA, and visits to some of the laboratories.

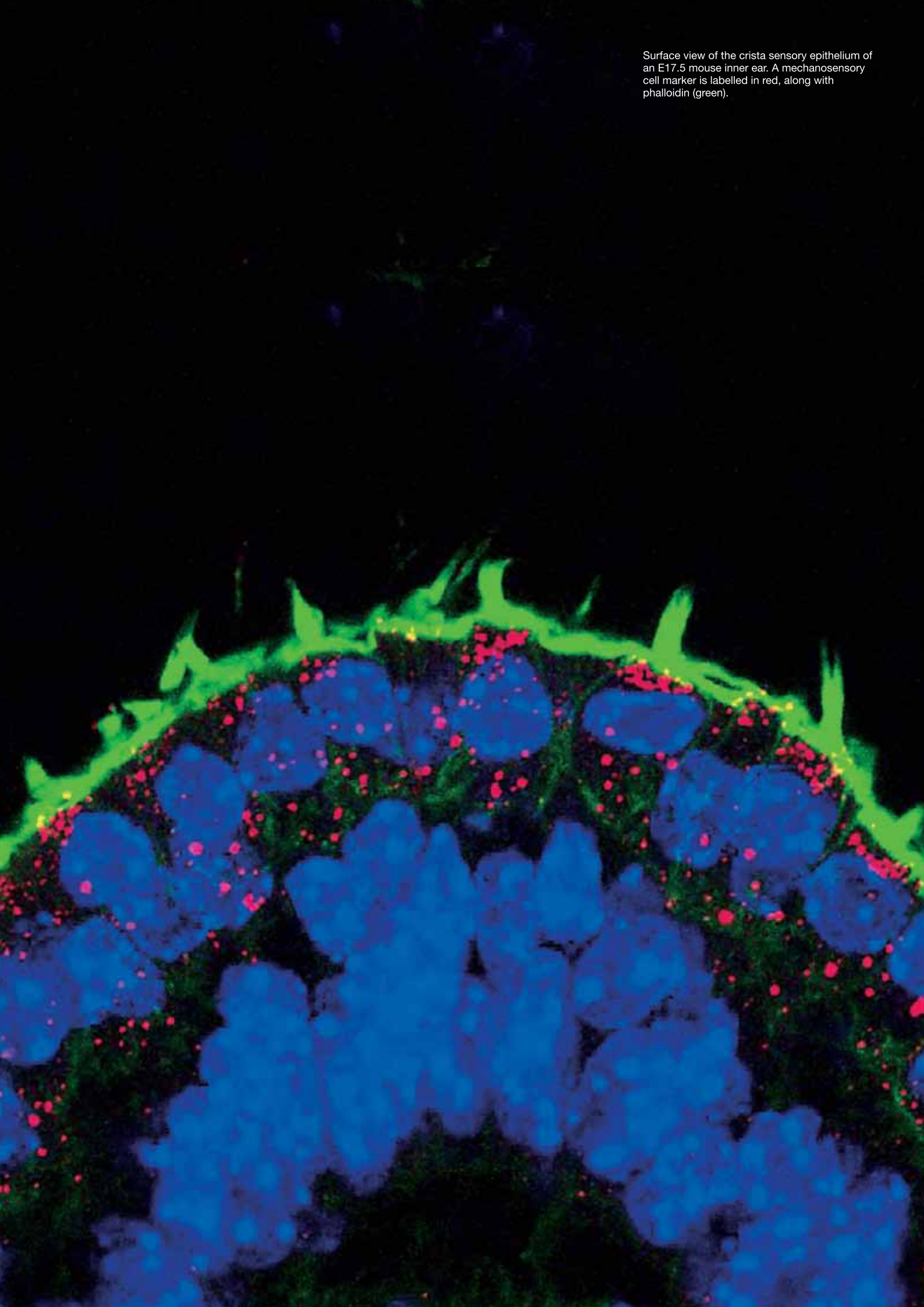


CDB Retreat in Awaji

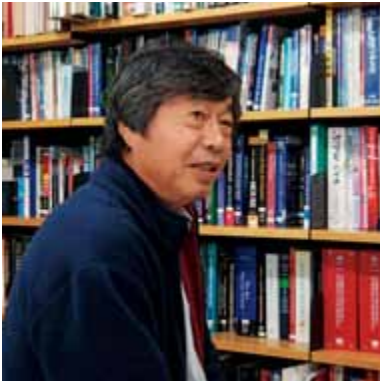
The CDB held its annual retreat for the research staff on October 7 to 8 on the nearby resort island of Awaji. CDB lab leaders, research scientists, student trainees, and technical staff took part in the closed meeting to share and discuss the latest developments of their research, and to also get better acquainted with each other through social activities and conversations away from the normal lab settings. The program featured talks given by recently arrived lab leaders, as well as short talks by young researchers and poster sessions with lively discussions.



Surface view of the crista sensory epithelium of an E17.5 mouse inner ear. A mechanosensory cell marker is labelled in red, along with phalloidin (green).



Vertebrate Body Plan



Shinichi AIZAWA Ph.D.

<http://www.cdb.riken.go.jp/en/aizawa>

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. He has left these positions at March 2013, and is now the Unit Leader of Laboratory for Vertebrate Body Plan.

Staff

Group Director

Shinichi AIZAWA

Research Specialist

Yoko SUDA

Michio YOSHIDA

Research Scientist

Hideharu HOSHINO

Visiting Scientist

Daisuke KUROKAWA

Masaki TAKEUCHI

Technical Staff

Eriko KAJIKAWA

Tomomi OHMURA

Daisuke YAMAMOTO

Part-Time Staff

Michiko INOUE

Recent Publications

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)

Kurokawa D, et al. A lineage specific enhancer drives Otx2 expression in teleost organizer tissues. *Mech Dev* 128.653-61 (2012)

Shibata M, et al. MicroRNA-9 regulates neurogenesis in mouse telencephalon by targeting multiple transcription factors. *J Neurosci*. 31.3407-22 (2011)

Kurokawa D, et al. Evolutionary origin of the Otx2 enhancer for its expression in visceral endoderm. *Dev. Biol.* 342.110-20 (2010)

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.



Sunc



Gray short-tailed opossum



Soft-shelled turtle

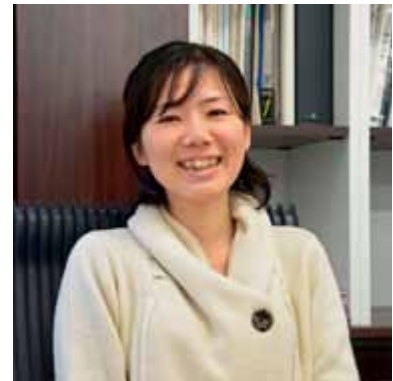


Gecko

Reconstitutive Developmental Biology

When examining a developmental phenomenon of interest, researchers often seek to identify the basic underlying elements (such as genes), and from them infer the mechanism. But after a certain degree of understanding has been achieved, approaches based on testing whether it is possible to reconstitute the phenomena from the inferred mechanisms become increasingly effective. In our lab, we seek to create artificial networks of around five genes in cultured cells to investigate whether it is possible to reconstitute the essence of developmental phenomena in vitro.

We are particularly focused on reconstituting mechanisms of intercellular communications. In metazoan development, such communications induce the spontaneous differentiation of groups of cells that underlies 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. This may allow us to identify minimal gene networks and sufficient conditions underlying such phenomena as well as discover unexplained or unexpected elements through observation. Such artificially reconstituted systems also have the advantage of facilitating measurement and the modification of parameters, which we hope will contribute to the quantitative understanding of communication between adjacent cells.

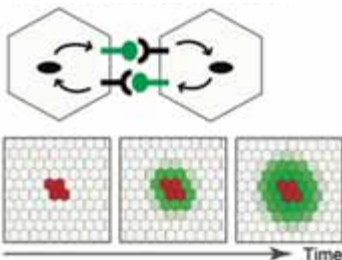


Miki EBISUYA Ph.D.

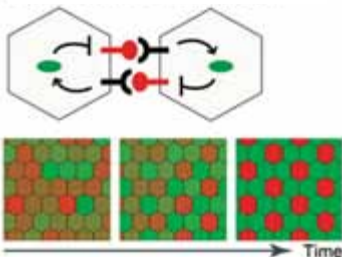
<http://www.cdb.riken.go.jp/en/ebisuya>

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.

Signal propagation



Pattern formation



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).

Synthetic signal propagation



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

Staff

Research Unit Leader

Miki EBISUYA

Research Scientist

Mitsuhiro MATSUDA
Ryoji SEKINE

Technical Staff

Yuko SAKAIDE

Student Trainee

Makiko KOGA

Recent Publications

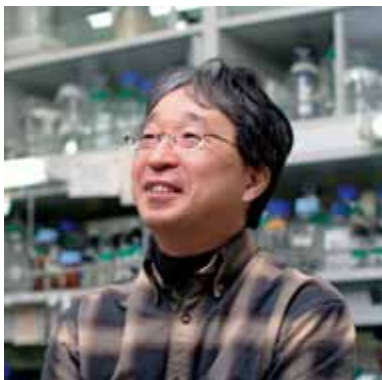
Koga M, et al. Foxd1 is a mediator and indicator of the cell reprogramming process. *Nat Commun* 5.3197 (2014)

Takashima S, et al. Regulation of pluripotency in male germline stem cells by Dmrt1. *Genes Dev* 27.1949-58 (2013)

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

Ebisuya M, et al. Ripples from neighbouring transcription. *Nat Cell Biol* 10.1106-13 (2008)

Neuronal Differentiation and Regeneration



Hideki ENOMOTO M.D., Ph.D.

<http://www.cdb.riken.go.jp/en/enomoto>

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 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, GRF α 1-4. 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.

Staff

Team Leader

Hideki ENOMOTO

Research Scientist

Pilaiwanwadee HUTAMEKALIN

Keisuke ITOH

Toshihiro UESAKA

Technical Staff

Chihiro NISHIYAMA

Junior Research Associate

Mitsuhiro IWASAKI

Yohei YONEKURA

Student Trainee

Sachie ONO

Part-Time Staff

Azusa FUKUDA

Toko KONDO

Akira YAMAOKA

Assistant

Yuka NODA

Recent Publications

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

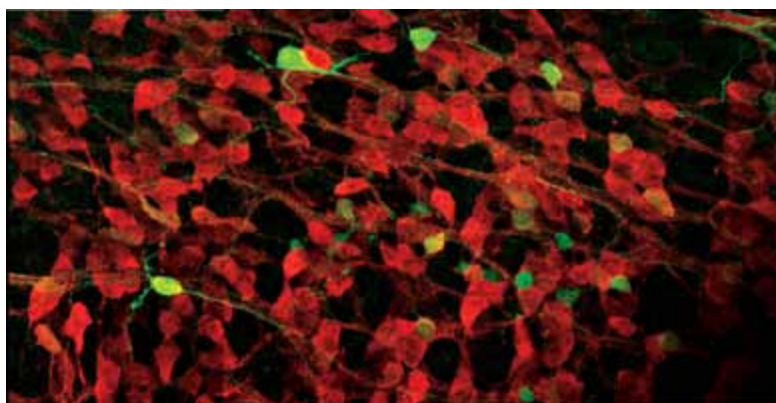
Nishiyama C, et al. Trans-mesenteric neural crest cells are the principal source for the colonic enteric nervous system. *Nat Neurosci* 15.1211-18 (2012)

Nagashimada M, et al. Autonomic neurocristopathy-associated mutations in PHOX2B dysregulate Sox10 expression. *J Clin Invest* 122.3145-58 (2012)

Uesaka T and Enomoto H. Neural precursor death is central to the pathogenesis of intestinal aganglionosis in Ret hypomorphic mice. *J Neurosci* 30.5211-8 (2010)

Uesaka T, et al. Diminished Ret expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice. *J Clin Invest* 118.1890-8 (2008)

Gould T W, et al. The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are restricted to fusimotor subtypes. *J Neurosci* 28.2131-46 (2008)



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

There is a wide diversity of environments on earth, from the North Pole to the Sahara, in which well-adapted organisms live. The traits and behaviors of all such organisms have been developed and modified through exposure to their specific environments.

In our own bodies, we also 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 (BM), a thin sheet-like ECM located at the borders of tissues. Stem cells in most tissues reside at this border, adhere to the BM and interact with neighboring niche cells. By virtue of its remarkable heterogeneity in composition, the BM 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 BM 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 BM. 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 BM specialization in the stem cell niche, 2) mechanisms by which the BM in the stem cell niche is regionally specialized, and 3) how the specialized BM controls stem cell niche formation, stem cell behavior and the conversation between stem cells and their neighboring cells.



Hironobu FUJIWARA Ph.D.

<http://www.cdb.riken.go.jp/en/fujiwara>

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.

Staff

Team Leader

Hironobu FUJIWARA

Research Scientist

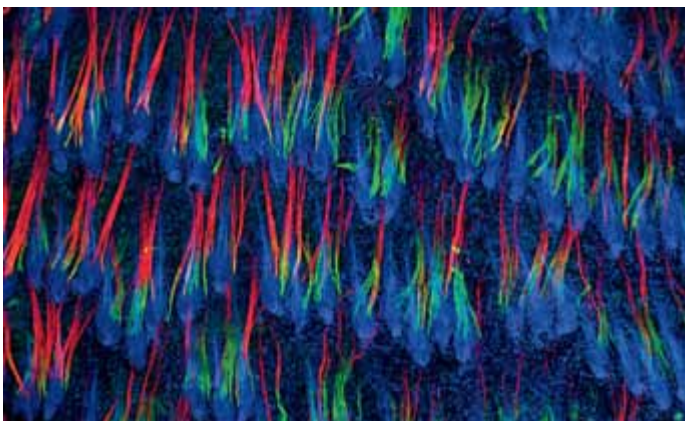
Chun-Chun CHENG
Ritsuko MORITA
Ko TSUTSUI

Technical Staff

Noriko BAN-SANZEN

Assistant

Asako NAKAGAWA



The image shows arrector pili muscles anchored to the bulge of hair follicles in a whole-mount preparation of dorsal skin in mouse, viewed from the dermal side. Arrector pili muscles are visualized by staining for α -smooth muscle actin (green) and SM22 α (red). The whole-mount is labelled with a nuclear counter-stain (blue).

Recent Publications

Fujiwara H, et al. The basement membrane of hair follicle stem cells is a muscle cell niche. *Cell* 144.577-89 (2011)

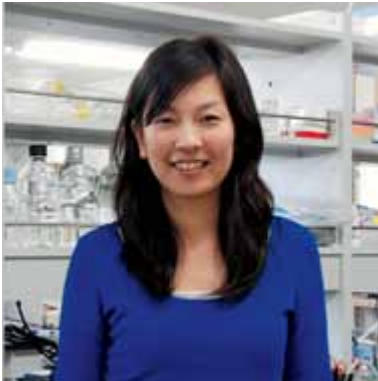
Watt F. M. and Fujiwara H. Cell-extracellular matrix interactions in normal and diseased skin. *Cold Spring Harb Perspect Biol* 3.(2011)

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

Fujiwara H, et al. Regulation of mesodermal differentiation of mouse embryonic stem cells by basement membranes. *J Biol Chem* 282.29701-11 (2007)

Fujiwara H, et al. Rac regulates integrin-mediated endothelial cell adhesion and migration on laminin-8. *Exp Cell Res* 292.67-77 (2004)

Neocortical Development



Carina HANASHIMA Ph.D.

<http://www.cdb.riken.go.jp/en/hanashima>

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.

Staff

Team Leader Carina HANASHIMA	Technical Staff Chihiro NISHIYAMA
Research Scientist Yuko GONDA Takuma KUMAMOTO	Part-Time Staff Chika KUMAMOTO Reiko ODA
Foreign Postdoctoral Researcher Torsten BULLMANN	Assistant Eri KOJIMA Miyuki MIURA
Visiting Researcher Tien-Cheng WANG	
Junior Research Associate Ken-ichi TOMA	

Recent Publications

Kumamoto T, et al. *Foxg1* Coordinates the Switch from Non-radially to Radially Migrating Glutamatergic Subtypes in the Neocortex through Spatiotemporal Repression. *Cell Rep* 3:931-45 (2013)

Gonda Y, et al. *Robo1* regulates the migration and laminar distribution of upper-layer pyramidal neurons of the cerebral cortex. *Cereb Cortex* 23:1495-508 (2013)

Kasukawa T, et al. Quantitative expression profile of distinct functional regions in the adult mouse brain. *PLoS One* 6: e23228 (2011)

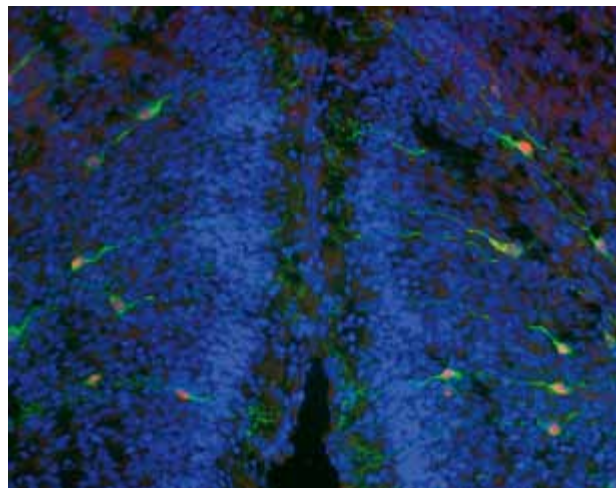
Fishell G and Hanashima C. Pyramidal neurons grow up and change their mind. *Neuron* 57: 333-8 (2008)

Hanashima C, et al. The role of *Foxg1* and dorsal midline signaling in the generation of Cajal-Retzius subtypes. *J Neurosci* 27: 11103-11 (2007)

Hanashima C, et al. *Foxg1* suppresses early cortical cell fate. *Science* 303: 56-9 (2004)

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 anterior-posterior (AP) and medial-lateral (ML) axes. We are exploring the extent to which intrinsic determinants control the specification of neuronal subtypes within discrete regions of the neocortex, as well as the extrinsic influences that refine the boundaries between functional areas of neocortex. To further these studies, we employ genetic manipulations in mice that will enable conditional loss of gene and cellular functions, recombination mediated cell-lineage tracing, and systematic approaches to identify novel molecules responsible for precise areal specification. Through these studies we wish to understand the mechanistic basis by which unique sensory perceptions and functional circuitries develop in the human neocortex.



Recruitment of late-born neocortical neurons expressing membrane-targeted GFP (green) and nuclear lacZ (red) to the motor cortex.

Morphogenetic Signaling

The main research interest in my lab focuses on the mechanisms by which cell-cell and tissue-tissue interactions are modulated during embryonic morphogenesis. A range of vital developmental processes, including the ability to build complex structures through selective cell adhesion, to move through the body, and to form epithelial tissues, rely on interactions between cells, and between cells and the extracellular matrix. The means by which cells are able to communicate, congregate, and work together to build a body is a central question in the study of morphogenesis. To tackle this problem, we use the model organism *Drosophila* which is suited for genetic dissection of biological processes and for high-resolution imaging, and study the problem at three levels of cellular organization: single-cell, multiple cells, and organ.

Our study is centered on the tracheal system, a respiratory organ that delivers air directly to tissues through a network of finely branched epithelial tubes. Tracheal development begins by invagination of ectodermal epithelium that subsequently forms branches with specific types of cells migrating in stereotyped patterns. Cellular forces produce mechanical strain in the epithelium, and alleviation of that strain is essential for smooth-tissue movement. As a consequence of epithelial cells behaving as elastic bodies, their shape changes and movement proceed with local fluctuations. We aim at elucidating (1) mechanisms that coordinate cell movement, (2) mechanisms for alleviating tissue strain, and (3) mechanisms of cross-talk between these two mechanisms. To accomplish these goals, the mechanical state of cells will be measured by combining techniques such as quantitative cell imaging, and various cell perturbation techniques are being used to assess the mechanical states of cells. The results of these analyses will be used to construct epithelial cell models and simulations.

In addition, we study intracellular mechanisms of cell polarization and elongation using model systems of mechanosensory bristles.



Shigeo HAYASHI Ph.D.

<http://www.cdb.riken.go.jp/en/hayashi>

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*.

Staff

Group Director

Shigeo HAYASHI

Research Scientist

Toshiya ANDO

Aki HAYASHI

Takuya MAEDA

Tetsuhisa OTANI

Foreign Postdoctoral

Researcher

Bo DONG

Special Postdoctoral

Researcher

Takefumi KONDO

Technical Staff

Akiyo KIMPARA

Housei WADA

International Program

Associate

Guangxia MIAO

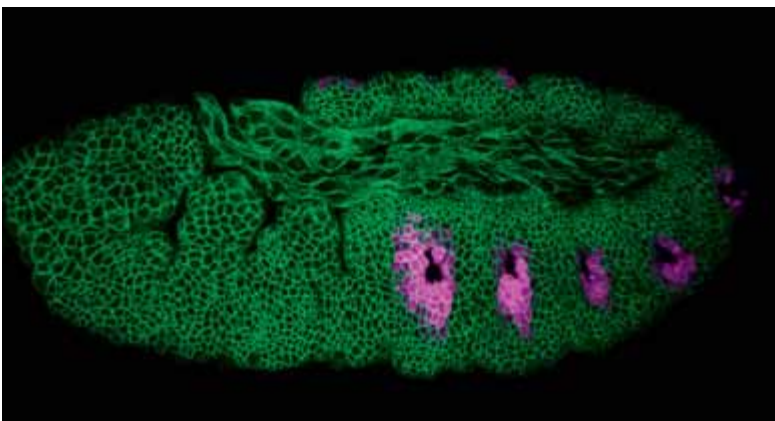
Part-Time Staff

Ikuko FUKUZYUO

Noriko MORIMITSU

Assistant

Ryoko ARAKI



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

Recent Publications

Niwa N, et al. Homeogenetic inductive mechanism of segmentation in polychaete tail regeneration. *Dev Biol* 381.460-70 (2013)

Dong B, et al. Rab9 and retromer regulate retrograde trafficking of luminal protein required for epithelial tube length control. *Nat Commun* 4.1358 (2013)

Kondo T. and Hayashi S. Mitotic cell rounding accelerates epithelial invagination. *Nature* 494.125-9 (2013)

Otani T, et al. IKKε regulates cell elongation through recycling endosome shuttling. *Dev Cell* 20.219-32 (2011)

Tajiri R, et al. Joint morphology in the insect leg: evolutionary history inferred from Notch loss-of-function phenotypes in *Drosophila*. *Development* 138.4621-6 (2011)

Noguchi T, et al. Sustained elongation of sperm tail promoted by local remodeling of giant mitochondria in *Drosophila*. *Curr Biol* 21.805-14 (2011)

Developmental Epigenetics



Ichiro HIRATANI Ph.D.

<http://www.cdb.riken.go.jp/en/hiratani>

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.

Staff

Team Leader
Ichiro HIRATANI

Technical Staff
Akie TANIGAWA

Recent 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)

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

Hiratani I, et al. Genome-wide dynamics of replication timing revealed by in vitro models of mouse embryogenesis. *Genome Res* 20:155-69 (2010)

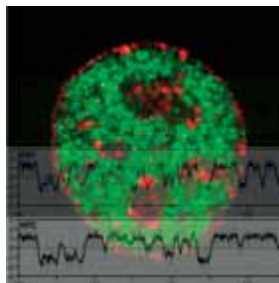
Hiratani I, et al. Replication timing and transcriptional control: beyond cause and effect--part II. *Curr Opin Genet Dev* 19:142-9 (2009)

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

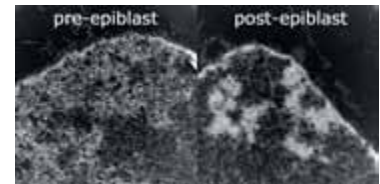
We wish to clarify the molecular mechanisms underlying global facultative heterochromatin formation during early mouse embryogenesis, with the belief that understanding the developmental regulation of higher-order chromosome organization will lead to a deeper understanding of cell differentiation.

The term facultative heterochromatin refers to chromosomal regions that condense, become inactivated, and are stably maintained in this manner after a certain developmental stage. A classic example is the inactive X chromosome in mammals, which becomes detectable immediately prior to the formation of germ layers and is stably maintained thereafter in all downstream lineages. Intriguingly, we recently discovered that many autosomal domains also undergo a similar process of facultative heterochromatin formation at the same developmental stage, which accounts for more than 6% of the genome. This suggests that facultative heterochromatin formation at this stage is not specific to the inactive X, but 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.

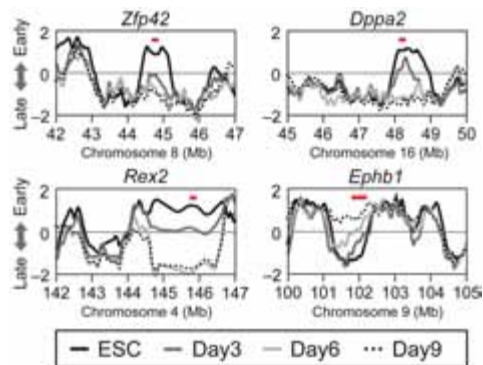
For these reasons, we combine genome-wide approaches with molecular and cell biology and imaging techniques to elucidate the molecular mechanisms underlying the facultative heterochromatin formation process. In the future, we will address the biological significance of this phenomenon and eventually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



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



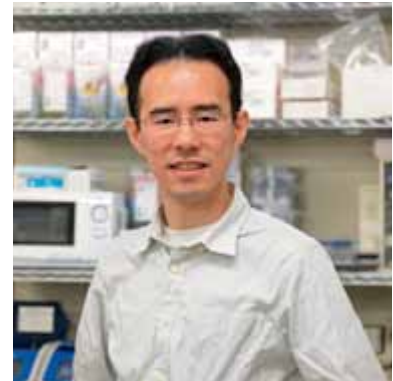
Genome-wide DNA replication profiling during ES cell differentiation can reveal domains that show large-scale changes in nuclear organization.

Sensory Circuit Formation

The mammalian nervous system is composed of enormous numbers of neurons, but how do these cells take on diverse fates and organize and array themselves during development? In recent years, it has become clear that the mouse olfactory system provides an excellent platform for addressing these questions experimentally. In this system, there are 1,000 types of odorant receptors that are capable of detecting and discriminating between odorant molecules. Each olfactory sensory neuron expresses a single type of odorant receptor, and the axons of neurons expressing the same receptor type converge on the same site in the olfactory bulb. Olfactory sensory neurons connect axons to the dendrites of mitral and tufted (M/T) cells in the bulb, where each receives inputs from a single specific type of olfactory sensory neurons.

It has generally been thought that neuronal identities are genetically programmed, and that neuronal connectivity is maintained by molecular “lock and key” mechanisms. The mouse olfactory system, however, is highly adaptive; olfactory neuronal identities are dependent on peripheral inputs, and form the basis for a self-organizing olfactory map. A better understanding of this flexibility may provide new insights into the diversification of function that took place during the evolution of the human brain.

Our lab will seek to develop a better understanding of odorant receptor-dependent axon projection of olfactory sensory neurons, and the formation of neuronal circuitry in the olfactory bulb dependent on inputs from these neurons. We are also seeking to develop next-generation genetic tools and imaging techniques to aid in our developmental and functional analyses of specific neuronal inputs in the brain. For example, we have recently developed a novel optical clearing agent named SeeDB, which is quick, efficient, and enables three-dimensional imaging of biological tissues at millimeter-scale levels.



Takeshi IMAI Ph.D.

<http://www.cdb.riken.go.jp/en/imai>

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.

Staff

Team Leader

Takeshi IMAI

Research Scientist

Satoshi FUJIMOTO

Ryo IWATA

Marcus LEIWE

Technical Staff

Miwako NOMURA

Student Trainee

Shuhei AIHARA

Meng-Tsen KE

Assistant

Eri YAMASHITA

Recent Publications

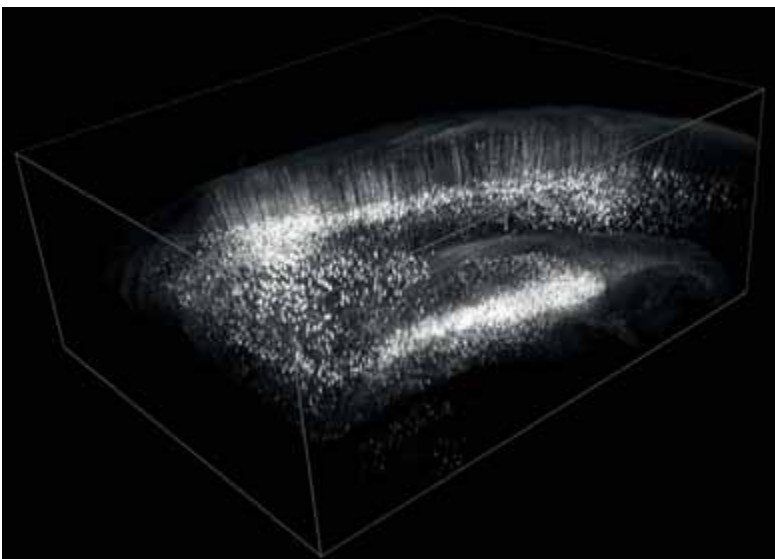
Nakashima A, et al. Agonist-Independent GPCR Activity Regulates Anterior-Posterior Targeting of Olfactory Sensory Neurons. *Cell* 154.1314-25 (2013)

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

Imai T, et al. Topographic Mapping - The Olfactory System. *Cold Spring Harb Perspect Biol* 2. A001776 (2010)

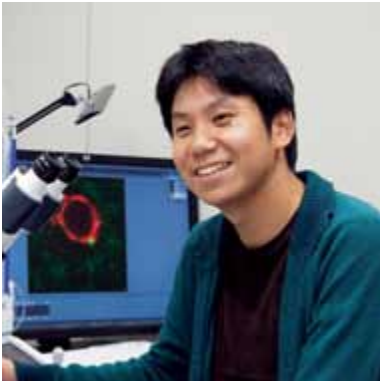
Imai T, et al. Pre-target axon sorting establishes the neural map topography. *Science* 325.585-90 (2009)

Imai T, et al. Odorant receptor-derived cAMP signals direct axonal targeting. *Science* 314. 657-61 (2006)



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 2mm, encompassing cerebral cortex and hippocampus, is shown.

Chromosome Segregation



Tomoya KITAJIMA Ph.D.

<http://www.cdb.riken.go.jp/en/kitajima>

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.

Staff

Team Leader Tomoya KITAJIMA	Technical Staff Masako KAIIDO
Research Scientist Yogo SAKAKIBARA Shuhei YOSHIDA	Student Trainee Yi DING
Visiting Scientist Aurélien COURTOIS Hirohisa KYOGOKU	Assistant Kaori HAMADA

Recent Publications

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

Lee J., et al. Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. *Nat Cell Biol* 10:42-52 (2008)

Kitajima T. S., et al. Shugoshin collaborates with protein phosphatase 2A to protect cohesin. *Nature* 441:46-52 (2006)

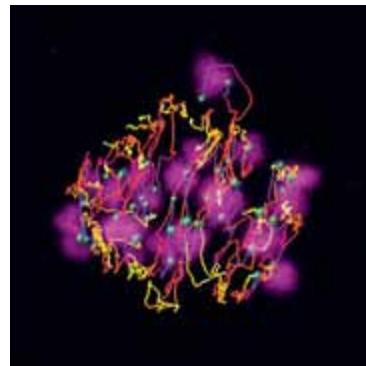
Kitajima T. S., et al. Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. *Curr Biol* 15:353-9 (2005)

Kitajima T. S., et al. The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. *Nature* 427:510-7 (2004)

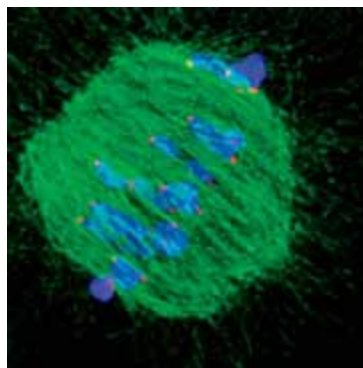
Kitajima T. S., et al. Distinct cohesin complexes organize meiotic chromosome domains. *Science* 300:1152-5 (2003)

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.

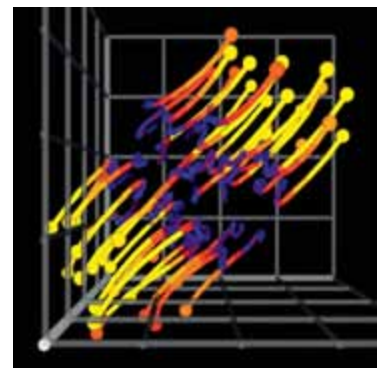
Using the mouse as a model, we will seek to conduct detailed and comprehensive analyses of the dynamics of chromosomes and the molecular machinery that underlies chromosome segregation during cell division. We plan to take advantage of the latest live imaging technologies to study the chromosome dynamics 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.



Tracking of kinetochores (green) and chromosomes



Prometaphase of meiosis I. Microtubules (green), kinetochores (red), chromosomes (blue).



Kinetochore tracking during anaphase of meiosis I.

Histogenetic Dynamics

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 an experimental approach to spatiotemporal information in living systems, such as can be obtained through the real-time live imaging of biological phenomena.

We have chosen the fruit fly *Drosophila melanogaster* as our primary research model, seeking to take advantage of its utility in developmental studies and wealth of genetic data in studying the coordination of histogenesis through live imaging and genetic screens. To elucidate the role of cell death in histogenetic processes, we will analyze caspase mutant phenotypes in which the exterior male genitalia (terminalia) develops abnormally. In normal *Drosophila* development, the terminalia rotates 360° as it forms, but in caspase mutants, this revolution is incomplete. Image analysis reveals that in wildtype, the speed of this rotation is variable, with distinct initiation, acceleration, deceleration, and termination stages; caspase inhibition results in loss of the acceleration phase, and failure in terminalia development. We will seek to identify how 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.



Erina KURANAGA Ph.D.

<http://www.cdb.riken.go.jp/en/kuranaga>

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.

Staff

Team Leader

Erina KURANAGA

Research Scientist

Kenichi KANAI

Emi MAEKAWA

Technical Staff

Ayako ISOMURA

Aimi TSUKIOKA

Student Trainee

Yuhei KAWAMOTO

Shuji SHIMAMURA

Part-Time Staff

Arata KURANAGA

Yoko UMEGAKI

Recent Publications

Takeishi A, et al. Homeostatic epithelial renewal in the gut is required for dampening a fatal systemic wound response in *Drosophila*. *Cell Rep* 3.919-930 (2013)

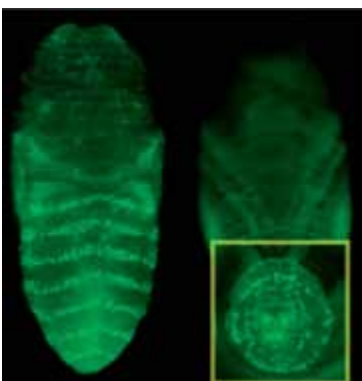
Sekine Y, et al. The Kelch repeat protein KLHDC10 regulates oxidative stress-induced ASK1 activation by suppressing PP5. *Mol Cell* 48.692-704 (2012)

Kuranaga E, et al. Apoptosis controls the speed of looping morphogenesis in *Drosophila* male terminalia. *Development* 138.1493-9 (2011)

Koto A, et al. Apoptosis ensures spacing pattern formation of *Drosophila* sensory organs. *Curr Biol* 21.278-87 (2011)

Nakajima Y, et al. Nonautonomous apoptosis is triggered by local cell cycle progression during epithelial replacement in *Drosophila*. *Mol Cell Biol* 31.2499-512 (2011)

Kuranaga E, et al. *Drosophila* IKK-related kinase regulates nonapoptotic function of caspases via degradation of IAPs. *Cell* 126.583-96 (2006)

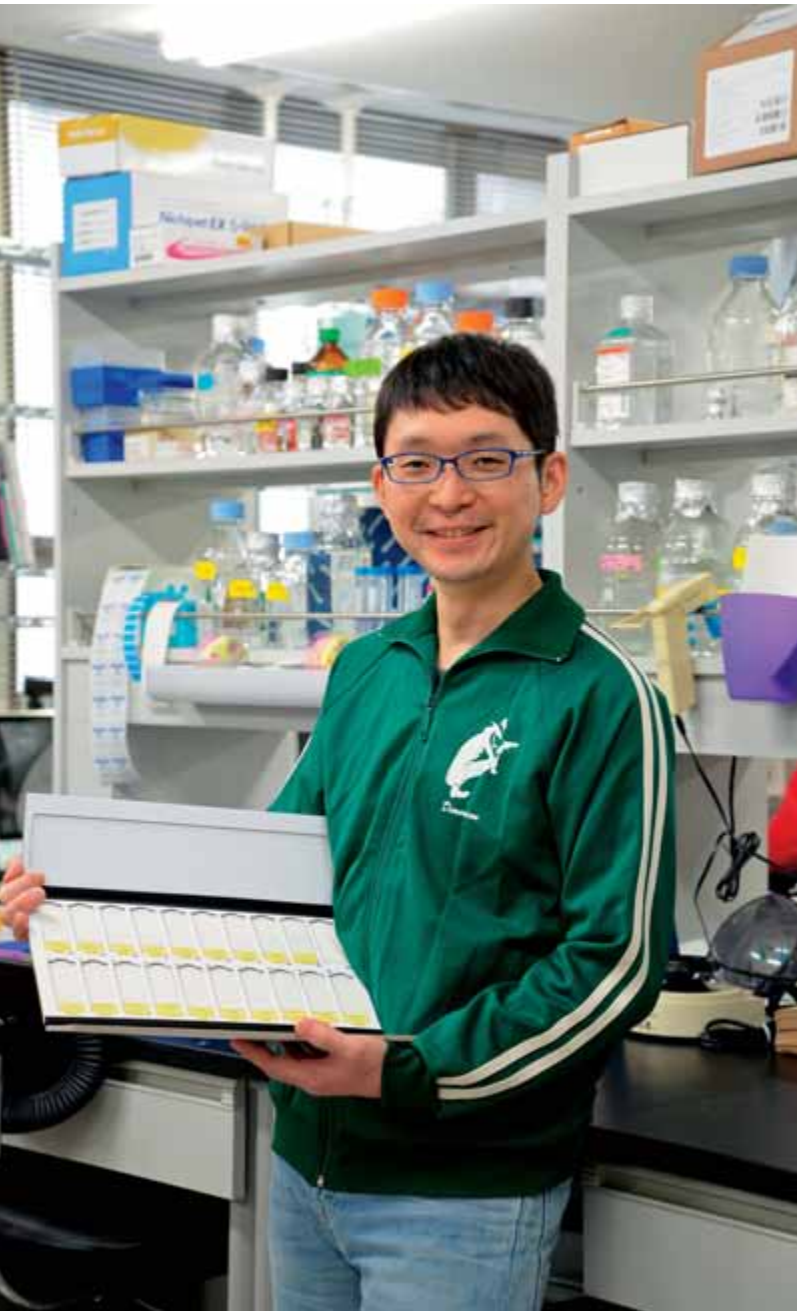


Dorsal (left) and ventral (right) views of *Drosophila* pupae that express fluorescent protein in cells located posterior component of each segment. Location of male genitalia is pointed in yellow square.



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

Transcriptional switch controls neuronal fate in neocortex



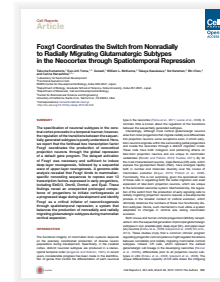
Takuma KUMAMOTO

There is something to the notion that it is our neocortex that makes us human. This sheet of neurons covering the brain is the seat of the senses, cognition, language and other cerebral faculties. Its development is thus a matter of intrinsic interest, but of elegant complexity as well. Cortical neurons of various types form in sequential order, with the older neurons inhabiting deeper layers and younger neurons forming the upper strata. In total, there are six such layers in the neocortex, but the genetic controls that direct the transitions from one neuronal subtype to another have remained elusive.

New work by Takuma Kumamoto and others in the Laboratory for Neocortical Development (Carina Hanashima, Team Leader) provides insight into how a transcription factor known as *Foxg1* functions as the earliest switch in regulating neuronal cell fate in the mouse neocortex. Published in *Cell Reports*, this study opens new inroads into our understanding of the formation of neocortical neuronal diversity.

Cajal–Retzius (CR) cells, the earliest glutamatergic neurons to emerge, play a central role in neocortical development. These cells secrete factors that direct the cortical stratification and the radial projection of later generations of neurons, but are gradually lost after birth, disappearing altogether by adulthood. CR cells are rare in birds and reptiles, leading to speculation that they are important to the formation of the comparatively massive mammalian cortex. It had earlier been thought that these cells arose from a different progenitor domain than the neocortex, but a previous study by Hanashima found that *Foxg1*, which is expressed in neocortical progenitors, suppresses CR cell differentiation, revealing that CR cells share a common progenitor with projecting neurons.

Kumamoto set out to build on that work, seeking the mechanism by which *Foxg1* regulates neural stem cell differentiation. The first step was to create a knockout mouse lacking *Foxg1*, and analyze its phenotype. In these mutants, he found that projection neurons of all layers failed to form, while CR cells developed in greater than usual numbers. Migration patterns of neurons that appear in a distinctive radial fashion in wildtype were also aberrant in the knockout embryos. The loss of *Foxg1* function thus appeared to



cause a failure in the transition to generation of projection neurons, resulting in overproduction of CR cells.

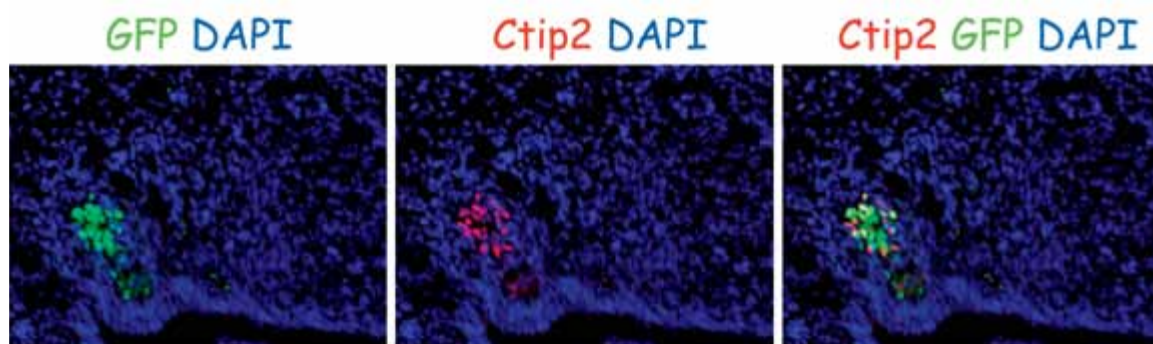
The team next made a conditional knockout (cKO) in which *Foxg1* expression could be switched off mid-development, to test whether upper-layer progenitor cells retain the ability to differentiate into CR cells following inactivation of the gene (something the lab had previously shown deep-layer progenitors capable of doing). Interestingly, upper-layer progenitor cells did not give rise to CR cells when *Foxg1* was inactivated at embryonic day (E) 15, suggesting that competence is progressively restricted during the transition from deep- to upper-layer neurons, implying that cortical progenitors use an intrinsic program to steer transitions between cortical subtypes.

To test that possibility, the team generated a conditional mutant in which *Foxg1* was induced at a later stage than normal, the time of the transition from deep to upper-layer progenitors in wildtype. When *Foxg1* was induced at E14.5, Kumamoto observed a nearly immediate generation of deep-layer projection neurons after the excess CR cell production, indicating that *Foxg1* works to direct cell identity decisions. Intriguingly, upper-layer projection neurons also subsequently appeared in these late *Foxg1*-induced em-

bryos, which ultimately developed a cortex with cells of the various types in numbers similar to those seen in wildtype. *Foxg1*, it seems, works as a transcriptional switch ensuring that the right types of cells emerge at the right timing during neocortical development.

In an effort to hunt for genes downstream of *Foxg1*, the team conducted microarray analyses of progenitor cells taken at different timepoints from cKO embryos in which its expression was switched on at E14.5. Their examination of gene expression patterns showed that *Foxg1* strongly inhibited genes associated with CR cells; chromatin immunoprecipitation suggested this was the result of direct binding between the factors. Interestingly, the majority of these binding domain sequences appears to have emerged after mammalian evolution.

“The robust and elegant spatiotemporal cell fate switching system mediated by *Foxg1* maintains the right balance between projection neurons and CR cells in mouse and human alike,” says Hanashima. “We are excited to continue in our efforts to understand just what goes on in the building of the neocortex that underlies many of humanity’s most fundamental traits.”



Induction of neocortical neurons by *Foxg1*. Images depicting *Foxg1* and EGFP electroporated cells (shown in green) within *Foxg1* knockout cells (blue), inducing *Ctip2* expression and neocortical projection neuron fate (red).

FASTER makes sleep staging a cinch



Genshiro SUNAGAWA

Sleep is a crucial daily activity for an extraordinarily wide range of animal species. Consciousness is typically divided into waking, REM (rapid eye movement), non-REM states, reflecting patterns of activity recorded in electroencephalography (EEG) and electromyography (EMG) data. Analyses of sets of such data are indispensable for studies of the mechanisms underlying sleep/wake states and sleep pathologies, but the need for inspection by individual human raters has imposed limits on the scale and speed of assessment and introduced problems of standardization and potential subjective biases.

A new automated approach to sleep staging developed by Genshiro A. Sunagawa and colleagues in the Lab for Sys-

tems Biology (Hiroki R. Ueda, Project Leader) now potentially makes the process more scalable and objective. Published in *Genes to Cells*, this new FASTER (Fully Automated Sleep sTaging method via EEG/EMG Recordings) method was developed in collaboration with researchers from Tokushima University, Osaka Bioscience Institute, and Nihon University College of Pharmacy.

The EEG and EMG waveforms observed during sleep differ between individuals, and between strains of laboratory mice. For this reason, sleep analyses have traditionally relied on the rating of data obtained from individual animals over a fixed period (such as 24 hours) to establish standards for the three sleep/wake stages. This process re-

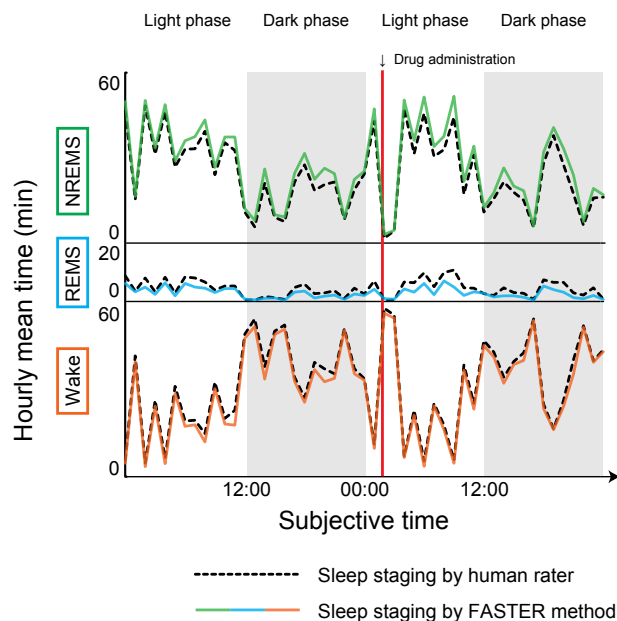


quires individual raters to take into consideration inter-individual differences and eliminate noise, but also introduces subjectivity and the risk of human error, as well as limiting the scale of data that can be evaluated. Semi-automated approaches have been developed, but these still require human supervision over the setting of scoring criteria.

The FASTER method developed by Sunagawa and colleagues now provides a fully automated alternative. The algorithm is composed of three main steps: character extraction, clustering, and annotation. The first of these involves breaking the observation period into epochs of fixed length (8 sec), converting the EEG/EMG outputs for each epoch into frequency domain data, and then subjecting them to principal component analysis to extract characters of their power spectra. After clustering regions showing similar features as determined by nonparametric density estimation, the annotation was performed without human supervision as determined by statistical characteristics for each cluster.

The team next tested the FASTER algorithm against results from human rater evaluations of mouse sleep/wake stages in wildtype animals in which these patterns were altered by drugs that induced wakefulness or sleep. When they compared the results of human raters' assessments with those generated by the FASTER algorithm, they found greater than 90% agreement between the two, indicating the high level of precision of this unsupervised, fully automated method. The FASTER approach was also...faster, taking only 10 minutes to complete an analysis that would require 1–2 hours using conventional methods.

“FASTER by itself won’t make it possible to automate the staging of huge sleep data sets,” cautions Sunagawa. “There is still quite a lot of manual work involved, such as attaching electrodes to mice, something we hope to tackle in future work. We are also looking forward to applying this new approach to sleep staging in humans, which will require handling much larger amounts of data.”



Sleep staging assessments made using visual inspection by human raters (dotted line) and FASTER methods (orange, blue, green)

Genomes shed light on turtle evolutionary history



Juan PASCUAL ANAYA, Naoki IRIE, Shigeru KURATANI

The turtle is something of a monster of evolutionary developmental biology, not by virtue of its stern physiognomy or powerful jaw, but for its utterly unique morphology. The turtle carapace, for example, is made of up ribs that have protruded through the animal's back and fused together in a bony shell, while its scapulae have moved to the body interior. Turtles also lack temporal fenestrae, small holes found in the skulls of other reptilians, further muddying the picture of their phylogeny.

A study of a pair of turtle genomes by Naoki Irie and others in the Laboratory for Morphological Evolution (Shigeru Kuratani, Group Director) now sheds new light on its evolutionary history, establishing turtles as a sister group of crocodylians and birds (collectively, Archosauria), both of

which diverged from the more ancient lizards and snakes (Lepidosauria). Published in *Nature Genetics*, this work reflects the collaborative efforts of an international turtle genome sequencing consortium with scientists from 11 institutions, including BGI and Wellcome Trust Sanger Institute.

A number of competing hypotheses have been proposed to find the turtles' true branch on the tree of life. These including placing turtles among early reptiles (anapsids), on account of their missing fenestrae; grouping them together with the snakes and lizards; and, categorizing them as archosaurs, along with crocodylians and birds. Using a massively parallel shotgun sequencing approach, the consortium generated and analyzed the genomes of the soft-shell turtle (*Pelodiscus sinensis*) and the green sea turtle (*Chelo-*

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)



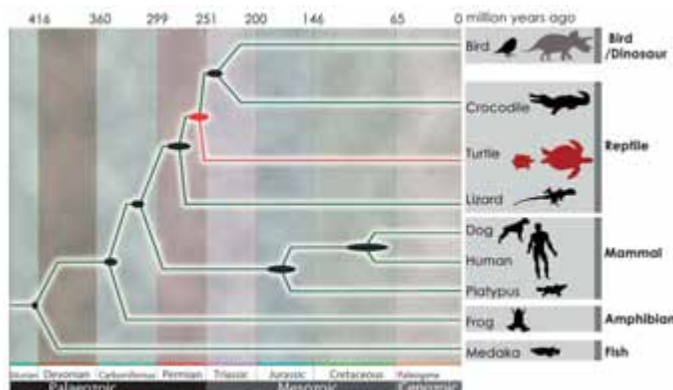
nia mydas). Both genomes weighed in at about 2.2 billion base pairs, encoding around 19,000 genes. Comparisons with 10 other vertebrate genomes (including those of the zebrafish, frog, alligator, chicken and human) identified 1,113 orthologous genes, phylogenetic analysis of which revealed that turtles are most closely allied with the archosaurian birds and crocs. Using this data supplemented, the authors place the turtle divergence to around 268–248 million years ago. This conclusion agrees nicely with the fossil record, as the oldest known turtle dates back 220 million years. The apparent timing of their emergence, which would have coincided with the transitional period known as the Permian–Triassic Transition, characterized by large-scale extinctions, raises intriguing questions about possible linkage between the two events.

Looking next to uncover details of the turtle genome itself, Irie et al. found an unexpectedly large number of olfactory receptor genes, as many as 1,137 in the soft-shell turtle, a number similar to that possessed by mammals. Of these, many coded for group a olfactory receptors, used to detect hydrophilic odorant molecules, which may indicate that turtles have an excellent ability to sniff out variety of smells. A number of genes involved in taste, appetite, and metabolic regulation, however, were missing, which may reflect these animals' low-metabolic strategy.

Previous work by Irie had focused on the developmental hourglass model that explains the evolutionary diversity of vertebrate embryos, and found support for the “hourglass”

model, that in which homology is highest at a midpoint in development (called the vertebrate phylotypic period, during which the basic body plan is being established), and differs more widely before and after. To confirm whether turtles would also fit this scheme, the group used RNA sequencing to compare turtle and chicken, and found hourglass-like divergence in the embryogenesis of these taxa, indicating that molecularly conserved turtle embryos exhibit a common vertebrate developmental plan, after which turtle-specific patterning is likely to begin in earnest. The group not only identified 233 genes that showed turtle-specific expression patterns after the phylotypic stage, but also found the *Wnt5a* gene expressed both in the limbs and the carapacial ridge, suggesting possible involvement of Wnt signaling in carapace evolution.

“Species make wide use of a shared toolbox of developmental processes and genetic information, but also generate new morphologies that could truly be called inventions. These new findings should be useful in guiding predictions about the course of vertebrate evolution,” says Kuratani. “But while these genomes are now sequenced, knowing the order of base pairs is not much more than being able to scan lines of text on a page. From now, we will need to explore how these genes are actually used during development to build body plans if we hope to decipher their true meaning.”



Phylogenetic history based on comparison of 12 vertebrate genomes and data from the fossil record. Turtles (red) diverged from crocodylian-avian archosaur lineage around the time of the massive Permian extinction event approximately 250 million years ago.

The remarkable tail of polychaete regeneration



Nao NIWA

Segmentation is a highly successful patterning strategy in a wide range of animals, including annelids, arthropods, and vertebrates, providing both flexibility and diversity in axial subdivisions of the body. While in many taxa, the number of body segments is strictly fixed, with anterior segments specified early, and more posterior segments added from a posterior segment addition zone (SAZ) as development progresses. This zone is lost in most post-embryonic animals, meaning that segments in general cannot regenerate. But certain annelids known as polychaetes have been shown to be able to regenerate amputated tail segments any number of times. However, previous studies have indicated that the SAZ in these species lack an identifiable population of stem cells, leaving the mechanisms behind this apparently limitless regenerative capacity an open question.

Nao Niwa and colleagues in the Lab for Morphogenetic Signaling (Shigeo Hayashi, Group Director) now report that posterior segment regeneration in the polychaete *Perinereis nuntia* relies on the provision of inductive signals from the pre-existing segment adjacent to the newly forming segment. Published in *Developmental Biology*, these new findings help to explain the mysterious ability of these animals to regrow body segments indefinitely.

The study began with observations of segment proliferation during the development of *P. nuntia*. Niwa and colleagues found that cells in the segment preceding the posterior-most region (pygidium) were in active cell cycle, while those in the terminal region remained at rest. The active cells expressed high levels of PCNA, and formed what the group



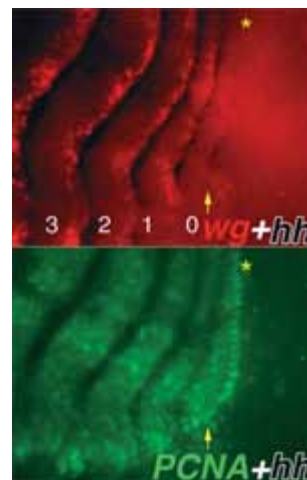
called the zone of cell cycle synchronization (ZCS). As the process of segment proliferation progressed slowly during development, the group decided to study it more closely in regenerating animals, which add segments more quickly. As in normal development, regenerative proliferation of segments involved the addition of rows of cells provided by the anterior segment, characterized by expression of PCNA, with one new segment forming for each five new rows of cells.

To determine the molecular mechanisms underlying this process, Niwa looked at signaling pathways common to segmentation in arthropods and vertebrates, suspecting that these might be conserved in polychaetes as well. The polychaete homolog of the *Drosophila* gene *wingless* (*wg*) is expressed in a stripe at the posterior edge of each segment during proliferation, and faces a similar band of the polychaete *hedgehog* (*hh*) homolog in the cell at the anterior margin of the newly forming segment. A closer observation of the expression of these genes suggested that *wg*, *hh*, and PCNA are sequentially expressed in an anterior to

posterior direction during segment formation, suggestive of a central instructive role for *wg* in the process. This sequence plays out repeatedly during segment proliferation, such that the program reinitiates for every fifth new cell, ensuring that individual segments are consistent in length.

The picture that emerges from these findings is that *P. nuntia* differs from other taxa in its approach to forming new segments—rather than generate signals from the edge of the newly forming segment, the signaling in polychaete appears to move from pre-existing cells at the border of the proliferation zone, a mechanism that bears resemblance to the theory of homeogenetic induction first proposed by Spemann and Mangold in 1927.

“We were surprised to find that segment formation in the polychaete differs so significantly from that in insects and vertebrates, specifically in its unique ability to regenerate segments,” says Hayashi. “I never cease to be amazed at the surprises the rich diversity of the animal world holds in store.”



Electron micrograph of *Perinereis nuntia*, showing tail and segments (left). Expression of *hedgehog* (*hh*) and *wingless* (*wg*) in a newly forming segment (top right); expression of PCNA in the last row of cells in the terminal segment shows active proliferation (bottom right).

Organizer signaling scales to embryo size



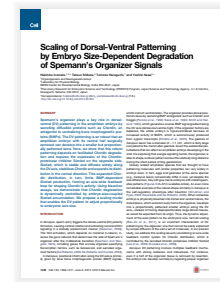
Tatsuo SHIBATA, Hidehiko INOMATA

While body sizes may differ significantly between individuals within the same species, body shape tends to remain constant. Even in closely related species, such as mouse and rat, while the overall size of the individual animals is quite different, the shape of their bodies are very similar. In a more extreme demonstration of this phenomenon, it has been shown that, after being cut in two, the dorsal half of a frog embryo can develop into a smaller, but morphologically ordinary tadpole, rather than one featuring only dorsal structures. The reason behind this conservation of pattern in differently sized embryos, however, has remained a mystery.

A new study by Hidehiko Inomata and colleagues in the Lab for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director), in collaboration with the Lab for Physical Biology (Tatsuo Shibata, Research Unit Leader), now points to a role for gradients of signaling factors from the embryonic organizer region of the African clawed frog, *Xenopus laevis*, in maintaining proportional patterning. Published in *Cell*, these new findings stand to answer a longstanding question of how embryonic shape can be scaled to embryo size.

In early amphibian development, BMP signals from the ventral side of the embryo are countered by BMP antagonists secreted from the organizer regions, first described by Hans Spemann in the 1920s. The interaction between these two streams generate a dorsal–ventral signaling gradient, which in turn sets the stage for further developmental routines: neural lineages form in dorsal regions of higher organizer signaling activity, while lower activity at the ventral side of the embryo triggers differentiation into blood and other lineages. What has remained an enigma is that that some mechanism must enable the maintenance of this gradient, even when the size of the embryo is halved, as suggested by the bisection studies described above.

To dig deeper into this question, Inomata first developed a ‘hyperventralized’ embryo to allow him to zero in on and analyze the functions of discretely expressed genes in reconstructing the dorsal–ventral (D–V) axis. When he misexpressed the organizer signal Chordin, he found that a gradient akin to that seen in wildtype embryos was established, leading to the formation of a normal D–V axis. Gradients are defined by distribution, so Inomata was curious about what



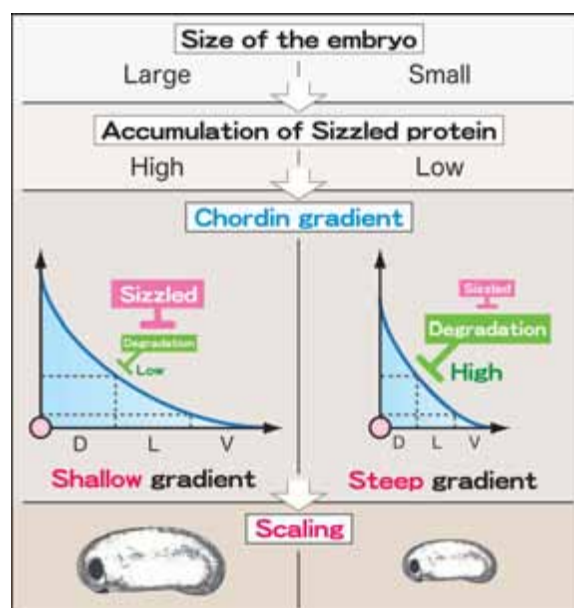
factors that control how Chordin is distributed in the embryo, and discovered that the action of Sizzled, which inhibits its degradation, plays a key role in stabilizing the Chordin molecule along a grade. Specifically, when he changed the level of Sizzled expression, he found that the Chordin gradient was altered dramatically, tilting from a gradual slope to a steep cliff. Inomata determined that the interaction between these two factors involved the following feedback loop: stabilization by Sizzled enables the broader diffusion of Chordin, which Chordin itself limits by inhibiting further Sizzled expression.

What then of dorsalized embryos? Knowing their ability to grow into small but proportional larvae, Inomata examined gene expression in bisected embryos, and found that the expression levels of dorsal markers in dorsal halves also dropped by around 50%, even as overall shape was maintained. But in loss-of-Sizzled-function embryos, bisection triggered no such reduction in dorsal gene expression, and

the embryos failed to develop normally, suggesting that Sizzled is responsible for maintaining the Chordin gradient in a manner scaled to embryo size.

The group's conclusion, that stabilization of Chordin by Sizzled and inhibition of Sizzled by Chordin maintain the Chordin gradient in a size-dependent manner, gained further support from the results of simulations of various starting values and perturbations for these factors and embryo size.

"It will be interesting to discover whether a similarly balanced feedback mechanism is at work in the embryos of other species," says Sasai, "as well as to determine whether the scaling mechanism changes over time as the embryo grows."



Sizzled concentration scales to embryo size, which proportionally affects Chordin stability (degradation), resulting in the formation of a Chordin gradient scaled to the size of the embryo and maintenance of homology.

Mesoderm induction: As in frog, so in bird?



Yuping WU, Cantas ALEV

British developmental biologist Lewis Wolpert famously remarked that the most important events in one's life take place during gastrulation. This crucial stage of embryogenesis involves dynamic morphological rearrangements that give rise to the three germ layers of the body: ectoderm, mesoderm, and endoderm. While gastrulation as a general phenomenon is highly conserved, the details differ between major phylogenetic groups. In fish and amphibians (anamniotes), for example, the induction of mesoderm is centered on a site known as the blastopore, while in amniotes such as birds and mammals, mesoderm forms at the primitive streak. Despite the central importance of this process, however, the evolutionary roots for this diversity in its developmental program have remained elusive.

Cantas Alev and colleagues in the Lab for Early Embryogenesis (Guojun Sheng, Team Leader) have now showed that, with a little manipulation, chicken embryos can be made to undergo an anamniote-like form of mesoderm induction. Published in *Development*, these results shed new light into the evolution of gastrulation strategies.

In frog and other anamniote embryos, gastrulation proceeds radially outward from the blastopore, inducing a ring of cells that migrate to the embryo interior, forming the mesodermal layer. In amniotes such as chicken, however, mesoderm cells are formed solely from a region known as the primitive streak, which evolved as an alternative to the 'circumblastoporal' routine. Despite these differences, both forms of gastrulation involve the expression of conserved



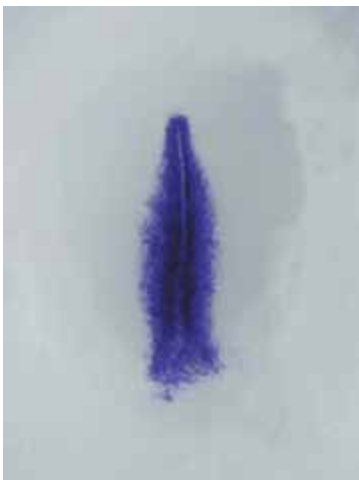
signaling factors, such as FGF, Wnt, and TGFβ. What Alev sought to determine in the present study was whether he could recapitulate the more ancient, anamniote approach to gastrulation in an amniote embryo by tweaking these signaling pathways.

He first developed a new method for injecting molecular signals into the subgerminal cavity of the developing embryo, and used it to test the effects of widespread ectopic presence of prominent signaling factors. He found that injection of FGF4 induced a ring of mesoderm (as labeled by *Brachyury*) in the marginal zone, irrespective of whether the primitive streak formed or not (the streak formed in fewer than half of the injected embryos). The mesoderm thus formed expressed epithelial–mesenchymal transition (EMT) markers, as in normal chick gastrulation, and dorsal–ventral axis formation was unperturbed, suggesting that these bird embryos could be led to undergo a very frog-like form of gastrulation, independent of primitive streak formation.

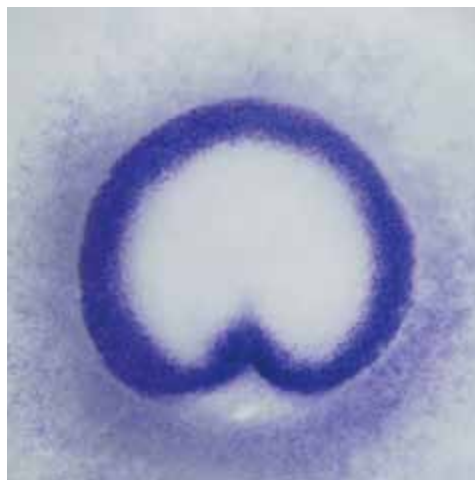
In these manipulated embryos, FGF was not able to induce mesoderm in the central epiblast, raising the likelihood that

other factors important for mesoderm formation were also involved. When Alev co-electroporated Wnt and FGF signaling factors into the medial epiblast of chick embryos, he found that the combined activity resulted in induction of mesoderm even in this central region. Additional experiments modulating a third signaling molecule, TGFβ, revealed that this pathway plays roles in the dorsalization of the embryo as well as the ingression of mesoderm cells. Suspecting that the effects of FGF activation might be conserved in amniotes other than chick, Alev repeated the experiment in quail, emu, and turtle embryos, and observed similar reversion to an anamniote-like circumblastoporal mode of gastrulation.

“Seeing dramatic differences in the morphogenetic processes taking place during gastrulation between anamniotes and amniotes, one would expect that this would also entail a significant change in the underlying molecular mechanisms” says Sheng. “But the results from these new experiments clearly show that, whether in frog or in bird, the essential process is fundamentally similar.”



In chicken embryos mesoderm is normally formed in the primitive streak. (blue: mesoderm marker *Brachyury*)



Subgerminal cavity injection of FGF, however, induces a ring of mesoderm not associated with the primitive streak. (blue: mesoderm marker *Brachyury*)

2013 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 teaching 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.

Out-of-classroom learning for high school students

The CDB organized its annual series of one-day summer school program for local high school students in August as part of its educational outreach activities. The program gives the students the opportunity to meet scientists and tour the research labs, and to also try out experimental techniques used in cell and developmental biology. This year, the students heard a short talk by Mitsuru Morimoto, team leader of the Laboratory for Lung Development, and also learned how to analyze proteins by running an SDS-PAGE.



Internship for undergraduate students

The CDB invited 32 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.



Lecture program for graduate students

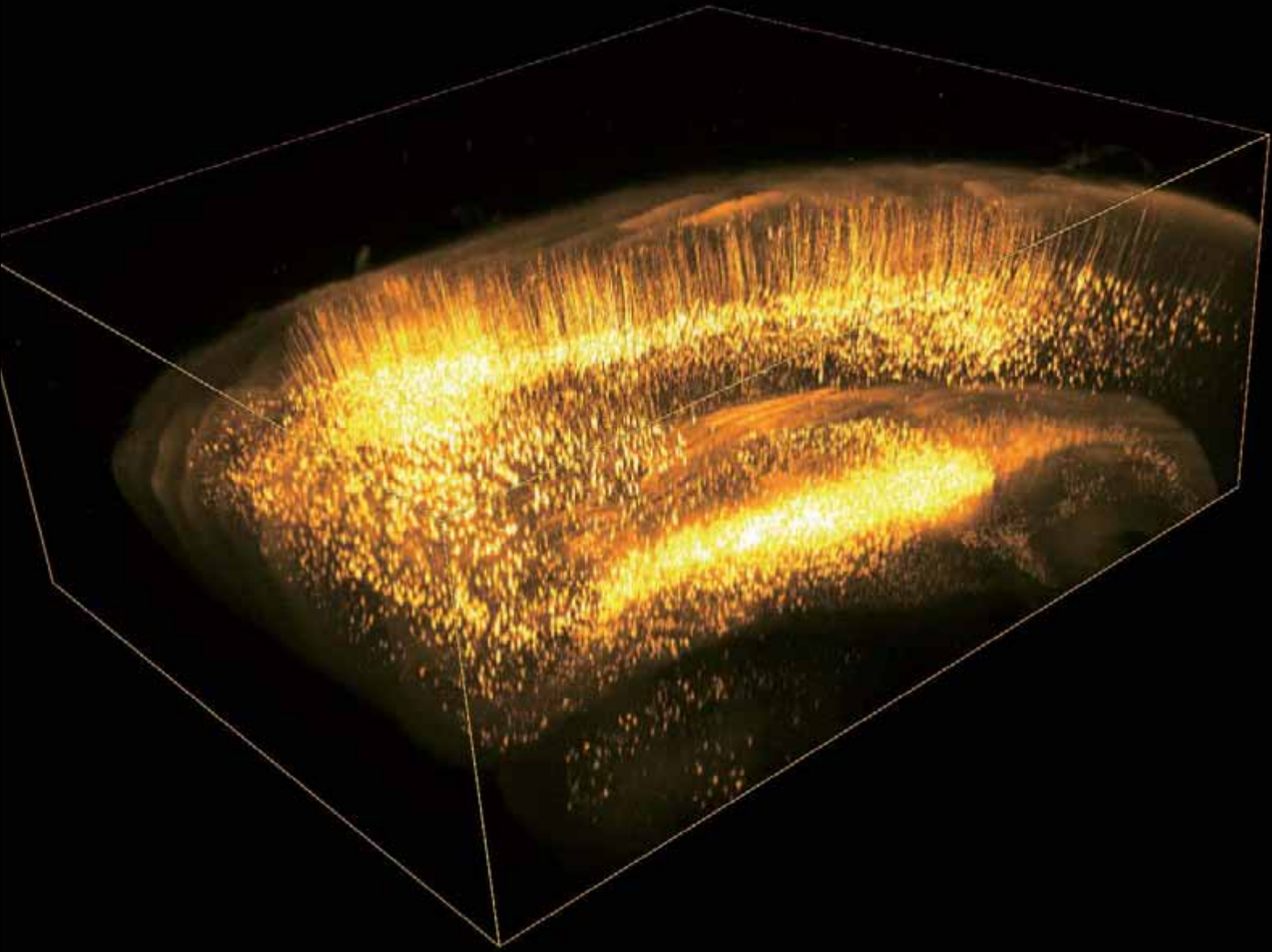
The RIKEN CDB maintains close ties with a number of major graduate and medical schools in the Kansai area, and hosts a two-day lecture program every year to introduce the students to the labs at the center. Over 150 students convened at the CDB this year to take part in this year's program, which was held on August 28 and 29. The event also included exhibitions of model organisms and tours of the lab facilities, such as the research aquarium and electron microscopy room.



Developmental biology training for teachers

The CDB, with support from the Japanese Society for Developmental Biologists (JSDB), organized a weekend workshop for high school teachers held October 5 and 6. This annually held 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.





3D rendering of two-photon images taken from a Thy1-YFP-H transgenic mouse brain cleared with SeeDB. A volume of 4 mm x 5 mm x 2 mm encompassing cerebral cortex and hippocampus is shown.

Evolutionary Morphology



Shigeru KURATANI Ph.D.

<http://www.cdb.riken.go.jp/en/kuratani>

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.

Staff

Group Director

Shigeru KURATANI

Research Scientist

Noritaka ADACHI
Tatsuya HIRASAWA
Naoki IRIE
Christian MITGUTSCH
Takayuki ONAI
Juan PASCUAL ANAYA
Fumiaki SUGAHARA

Technical Staff

Satoko FUJIMOTO
Tamami HIRAI
Yasuhiro OISI
Iori SATO

Student Trainee

Hiroki HIGASHIYAMA
Mana NAMETA

Intern

Jessica PLANADE

Part-Time Staff

Shigemitsu SHIBUYA
Kazuko YAMAMOTO

Assistant

Naeko MINAMI
Tomoko OYANAGI

Recent Publications

Hirasawa T, et al. The endoskeletal origin of the turtle carapace. *Nat Commun* 4.2107 (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)

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

Ota K G, et al. Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. *Nat Commun* 2.373 (2011)

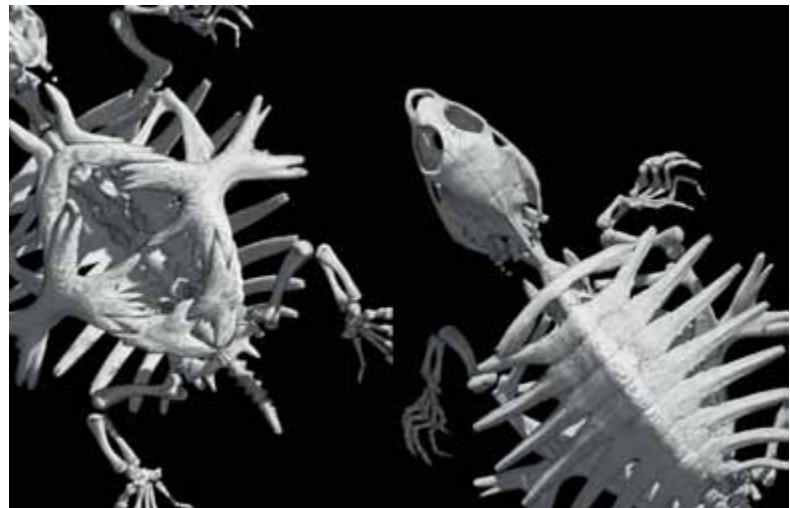
Nagashima H, et al. Evolution of the turtle body plan by the folding and creation of new muscle connections. *Science* 325.193-6 (2009)

Ota K G, et al. Hagfish embryology with reference to the evolution of the neural crest. *Nature* 446.672-5 (2007)

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.



CT-scanned skeleton of *Pelodiscus sinensis* juvenile

Sensory Development

Organogenesis is a monumental undertaking. From siting a given organ to its correct location, to confirming that it is correctly engineered and constructed, to ensuring the necessary functional connections with other organs, the embryo has a daunting task before it; indeed, perhaps we should be surprised that the result is so frequently successful. Taking sensory organogenesis as our model system, we hope to be able to follow the route an organ takes in its development, and seek to understand the steps that allow it to reach maturity. One aim is to be able to recapitulate these steps in vitro, with the ultimate goal of providing potentially clinically relevant applications.

The ear provides a particularly rich system for study. The inner ear, which converts mechanical stimulation to electrical impulses, is formed from a piece of tissue originally destined to become skin; a fate that is altered by instructive signals in a process known as induction. My lab has been focusing on the role that extrinsic signals play in its development. In particular, the roles that they play in cell fate, in balancing proliferation and differentiation and in directing morphogenesis. For this we have predominantly focused on the fibroblast growth factor (FGF) family. Our research aims is to bridge developmental events with the cellular mechanisms that drive development, either controlling quiescence, proliferation or imparting the distinctive phenotypic characteristics of particular cell types.

Our investigations have elucidated a number of mechanisms involved in the development of the inner ear. We have been instrumental in describing the earliest events of inner ear induction providing the clearest view of the developmental decisions that are made during induction. We have also discovered a role for extrinsic signals in regulating the cytoskeletal changes involved in tissue remodelling during the invagination of the inner ear. More recently, we have been investigating later signalling events in the generation of hair cells, the sensors of the inner ear, and how then their morphology is specified and maintained.

Environmental effects, genetic disorders or just the passage of time can damage hair cells, and the damage impairs our ability to hear. Our hope is that understanding inner ear formation allows us insights into potentially repairing or, at least mitigating the damage, elicited on hair cells.



Raj LADHER Ph.D.

<http://www.cdb.riken.go.jp/en/ladher>

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.

Staff

Team Leader

Rajesh LADHER

Research Scientist

Akira HONDA
Siu Shan MAK
Paul O'NEILL
Xiao Rei SAI

Visiting Researcher

Stephen FREEMAN
Tomoko KITA
Yuko MUTA

Research Associate

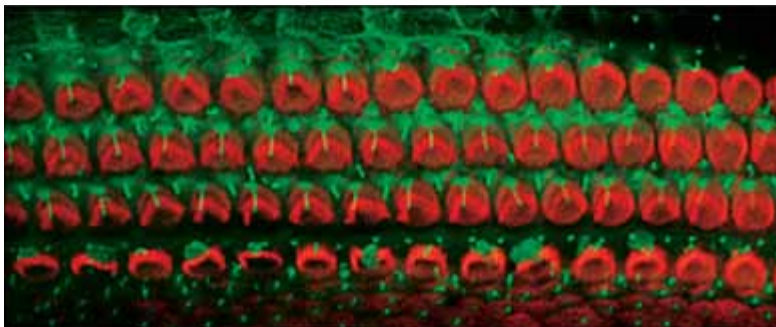
Kazuya ONO

Part-Time Staff

Yoshiko KONDO
Anna WRABEL

Assistant

Noriko HIROI



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).

Recent Publications

Ono K, et al. FGFR1-Frs2/3 signalling maintains sensory progenitors during inner ear hair cell formation. *PLoS Genet* 10(1): e1004118 (2014)

Paschaki M, et al. Retinoic Acid maintains olfactory progenitor cells during neurogenesis and regeneration. *Neural Development* 5: 13 (2013)

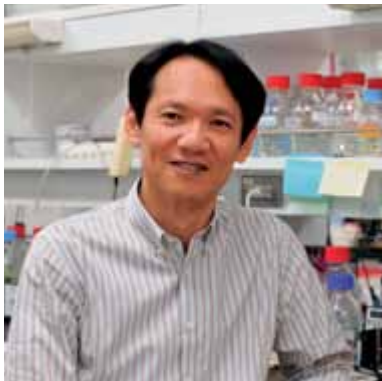
Honda A, et al. From placode to labyrinth: Culture of the chicken inner ear. *Methods* (2013)

Freter S, et al. Pax2 modulates proliferation during the specification of the otic and epibranchial placodes. *Dev Dyn* 241.1716-1728 (2012)

O'Neill P, et al. The amniote paratympanic organ develops from a previously undiscovered sensory placode. *Nat Commun* 3.1041 (2012)

Ladher R. K., et al. From shared lineage to distinct functions: the development of the inner ear and epibranchial placodes. *Development* 137.1777-85 (2010)

Cell Asymmetry



Fumio MATSUZAKI Ph.D.

<http://www.cdb.riken.go.jp/en/matsuzaki>

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.

Staff

Group Director	Chiaki KISHIDA
Fumio MATSUZAKI	Raymond Terhune-KUNIKANE
Research Scientist	Taeko SUETSUGU
Ikumi FUJITA	Nobuko TANIYAMA
Hiroshi ISHIMOTO	Student Trainee
Daijiro KONNO	Shun MASE
Keiko NAKANISHI	Kaga SAKAI
Atsunori SHITAMUKAI	Part-Time Staff
Shigeki YOSHIURA	Hiroimi ISHIMOTO
Research Associate	Erina MATSUMOTO
Noriko YUTSUDO	Yoko OTSUKA
Visiting Scientist	Megumi SHIBATA
Ayano KAWAGUCHI	Assistant
Yoichi KOSODO	Junko ISHIGAI
Technical Staff	
Tomoko AOKI	

Recent Publications

Pilz GA, Shitamukai A, Reillo I, Pacary E, Schwausch J, Stahl R, Ninkovic J, Snippert HJ, Clevers H, Godinho L, Guillemot F, Borrell V, Matsuzaki F, Götz M. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. *Nat Commun.* 4:2125. doi: 10.1038/ncomms3125 (2013)

Konno D, Iwashita M, Satoh Y, Momiyama A, Abe T, Kiyonari H, and Matsuzaki F. The mammalian DM domain transcription factor Dmrt2 is required for early embryonic development of the cerebral cortex. *PLoS One.* 7(10):e46577. doi: 10.1371 (2012).

Matsuzaki F, and Shitamukai A. Cell division modes and cleavage planes of neural progenitors during mammalian cortical development. *Cold Spring Harb Perspect Biol. Mammalian Develop.* doi 10.1101/cshperspect.a015719

Iwano T, et al. Prox1 postmitotically defines dentate gyrus cells by specifying granule cell identity over CA3 pyramidal cell fate in the hippocampus. *Development* 139:3051-62 (2012)

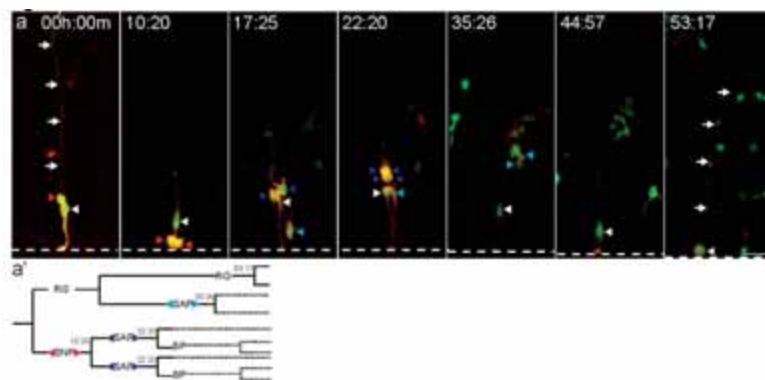
Yoshiura S, et al. Tre1 GPCR signaling orients stem cell divisions in the *Drosophila* central nervous system. *Dev Cell* 22:1-13 (2012)

Kosodo Y, et al. Regulation of interkinetic nuclear migration by cell cycle-coupled active and passive mechanisms in the developing brain. *EMBO J* 30:1690-704 (2011)

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. We focus our study on the programs by which behaviors of neural stem cells are controlled, using invertebrate (*Drosophila*) and vertebrate (mouse) model systems.

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.



During brain development, the ganglionic eminence in the ventral telencephalon generates a large number of diverse types of neurons including GABAergic interneurons that migrate tangentially into the cerebral cortex. Compared to the dorsal cortex, which has a well-organized pseudo-stratified structure, the ganglionic eminence is organized differently with a thicker germinal zone composed by a large number of intermediate progenitor cells. In collaboration with the Goetz laboratory in Germany, we have revealed that the ganglionic eminence produces novel types of self-renewing progenitor cells as well as intermediate progenitor cells that extensively proliferate. This results in rapid expansion of the population of descendant cells, and produces a range of different cell lineages. RG, radial glia; SAP, subapical progenitor; BP, basal progenitor.

Ref: Pilz GA, Shitamukai A, Reillo I, Pacary E, Schwausch J, Stahl R, Ninkovic J, Snippert HJ, Clevers H, Godinho L, Guillemot F, Borrell V, Matsuzaki F, Götz M. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. *Nat Commun.* 4:2125. doi: 10.1038/ncomms3125 (2013)

Lung Development

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.



Mitsuru MORIMOTO Ph.D.

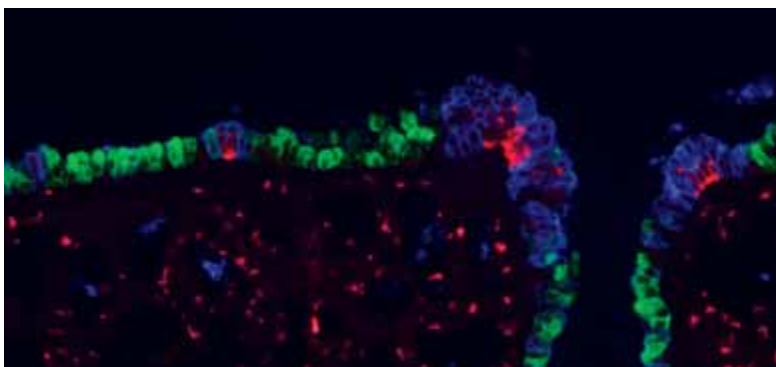
<http://www.cdb.riken.go.jp/en/morimoto>

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.

Staff

Team Leader
Mitsuru MORIMOTO
Research Scientist
Keishi KISHIMOTO
Research Scientist
Masafumi NOGUCHI

Technical Staff
Chisa MATSUOKA
Part-Time Staff
Junko SAKAI
Assistant
Yuka NODA



Airway epithelium is composed of various specialized cell types that benefit functional airways. Immunofluorescence staining of E18.5 lung with anti-CC10 (green), anti-CGRP (red) and anti-SSEA-1 (blue) shows localizations of Clara, neuroendocrine and SPNC cells.

Recent Publications

Morimoto M, et al. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. *Development* 139.4365-73 (2012)

Morimoto M, et al. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. *J Cell Sci* 123.213-24 (2010)

Demehri S, et al. Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. *PLoS Biol* 7. e1000067 (2009)

Morimoto M. and Kopan R. rTA toxicity limits the usefulness of the SP-C-rTA transgenic mouse. *Dev Biol* 325.171-8 (2009)

Morimoto M, et al. The negative regulation of *Mesp2* by mouse *Ripply2* is required to establish the rostro-caudal patterning within a somite. *Development* 134.1561-9 (2007)

Morimoto M, et al. The *Mesp2* transcription factor establishes segmental borders by suppressing Notch activity. *Nature* 435.354-9 (2005)

Developmental Morphogeometry



Yoshihiro MORISHITA, Ph.D.

<http://www.cdb.riken.go.jp/en/morishita>

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.

Staff

Research Unit Leader Yoshihiro MORISHITA	Technical Staff Kaori TSUJINO Yu WATAOKA
Research Scientist Yukitaka ISHIMOTO Daisuke OHTSUKA	Part-Time Staff Yuko FUJIYAMA Megumi MORIOKA
Student Trainee Ken-ichi HIRONAKA Sangwoo LEE	

Recent Publications

Hironaka K. and Morishita Y. Cellular sensory mechanisms for detecting specific fold-changes in extracellular cues. *Biophys J* (2014)

Hironaka K. I. and Morishita Y. Encoding and decoding of positional information in morphogen-dependent patterning. *Curr Opin Genet Dev* 22:553-61 (2012)

Morishita Y. and Iwasa Y. Coding design of positional information for robust morphogenesis. *Biophys J* 101:2324-35 (2011)

Morishita Y. and Iwasa Y. Estimating the spatiotemporal pattern of volumetric growth rate from fate maps in chick limb development. *Dev Dyn* 238:415-22 (2009)

Morishita Y. and Iwasa Y. Growth based morphogenesis of vertebrate limb bud. *Bull Math Biol* 70:1957-78 (2008)

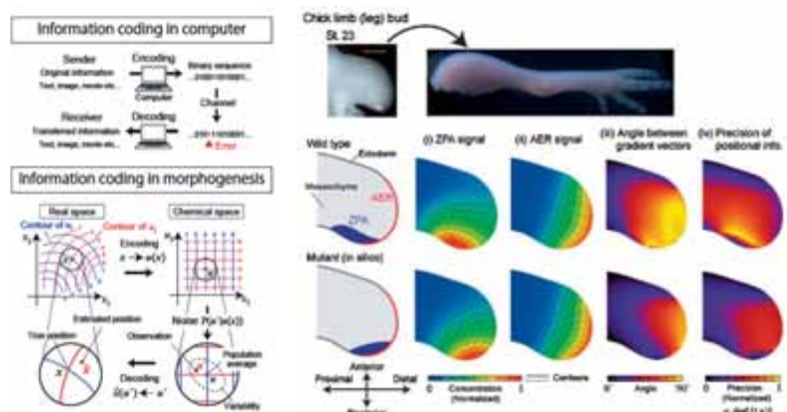
Morishita Y. and Iwasa Y. Optimal placement of multiple morphogen sources. *Phys Rev E Stat Nonlin Soft Matter Phys* 77:041909 (2008)

Developmental phenomena comprise a multiscale system extending across a range of spatial scales, from molecular to histological. Such phenomena are also multi-physical, 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 anisotropic 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.



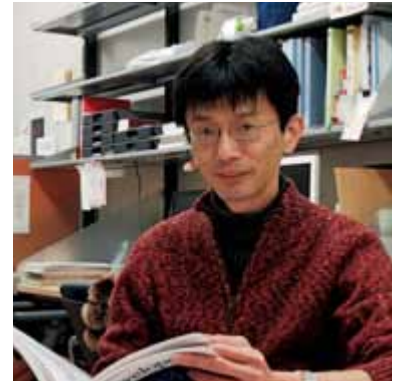
Optimal arrangement of morphogen source (Shh expression region) to maximize the precision of positional information in vertebrate limb bud.

Germline Development

Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the *Drosophila* fruit fly, the formation and differentiation of germ cells is controlled by mRNAs and proteins localized in a specific cytoplasmic region within eggs, called germ plasm. Germ plasm mRNAs are translated in a spatiotemporally regulated manner, but the means by which the germ plasm is formed and regulates germ cell development remain largely unknown. Our research team studies the establishment of the *Drosophila* germ line as a model of the processes of germ plasm assembly and germ cell differentiation. We expect that our study will also provide insights into the general mechanisms of mRNA localization and translation, which are important to a number of other developmental processes including asymmetric cell division, cell migration and axis formation, and postnatal synaptic plasticity as well.

One area of interest is the mechanism of translational repression in germline development. In one example of this critically important form of regulation, the translation of the RNA for the maternal gene *oskar*, which functions in embryonic patterning and the formation of germline cells in *Drosophila*, is repressed during its transport to the posterior pole of the oocyte. We are now studying the function of proteins, such as the recently identified factor, Cup, that regulates the repression of *oskar* translation during its localization to the oocyte posterior. In another concurrent project, we are focusing on the roles of *wunen2* and *polar granule component* (*pgc*), which are known to function in lipid signaling and global transcriptional regulation in germline cells during embryogenesis.

In addition to the study of fruit fly germline development, we are also beginning to undertake investigations using the ascidian, *Ciona intestinalis*. Our team will explore the genetic regulation of ascidian germline development by characterizing promoter regions of germline specific genes and trans-acting factors that regulate germline specific gene expression.



Akira NAKAMURA Ph.D.

<http://www.cdb.riken.go.jp/en/nakamura>

Akira Nakamura received both his baccalaureate and his Ph.D. from the University of Tsukuba. He spent a year as a post-doctoral fellow at the same institution before moving to the Department of Biology at McGill University in Montreal in 1995 to work as a postdoc under Paul Lasko. He returned to Japan in 1997 as a research associate at the University of Tsukuba. He was appointed assistant professor in the university's Gene Research Center and Institute of Biological Sciences in 2000, and began a three-year term as a PRESTO researcher in the Japan Science and Technology Corporation (JST) in December 2001. He was appointed CDB team leader in March 2002. From March 2013, Dr. Nakamura has been a professor in the Institute of Molecular Embryology and Genetics, Kumamoto University.

Staff

Team Leader

Akira NAKAMURA

Research Scientist

Kazuko HANYU-NAKAMURA

Yasuko KATO

Yuko SUKEGAWA

Assistant

Yoko UMEGAKI

Recent Publications

Shirae-Kurabayashi M, et al. Ci-Pem-1 localizes to the nucleus and represses somatic gene transcription in the germline of *Ciona intestinalis* embryos. *Development* 138:2871-81 (2011)

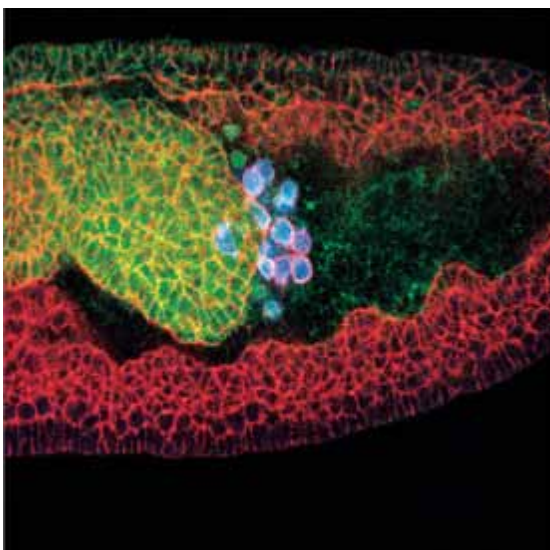
Tanaka T, et al. *Drosophila* Mon2 couples Oskar-induced endocytosis with actin remodeling for cortical anchorage of the germ plasm. *Development* 138:2523-32 (2011)

Tanaka T and Nakamura A. The endocytic pathway acts downstream of Oskar in *Drosophila* germ plasm assembly. *Development* 135:1107-17 (2008)

Hanyu-Nakamura K, et al. *Drosophila* Pgc protein inhibits P-TEFb recruitment to chromatin in primordial germ cells. *Nature* 451:730-3 (2008)

Shirae-Kurabayashi M, et al. Dynamic redistribution of vasa homolog and exclusion of somatic cell determinants during germ cell specification in *Ciona intestinalis*. *Development* 133:2683-93 (2006)

Nakamura A, et al. *Drosophila* Cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. *Dev Cell* 6:69-78 (2004).



Migrating germ cells (blue) in stage 10 *Drosophila* embryo

Chromatin Dynamics



Jun-ichi NAKAYAMA Ph.D.

<http://www.cdb.riken.go.jp/en/nakayama>

Jun-ichi Nakayama received his bachelor's, master's and Ph.D. degrees in bioscience from the Tokyo Institute of Technology, the last in 1999 for his work on the cloning and characterization of mammalian telomerase components. He spent the period from 1999 to 2001 as a postdoctoral researcher at Cold Spring Harbor Laboratory in Shiv Grewal's lab, before returning to Japan in December 2001 as a PRESTO researcher in the Japan Science and Technology Corporation (JST). He was appointed team leader of the Laboratory for Chromatin Dynamics in 2002.

Staff

Team Leader Jun-ichi NAKAYAMA	Technical Staff Yasuko OHTANI
Research Scientist Aki HAYASHI	Assistant Shinobu SHIMAHARA
Research Associate Mayumi ISHIDA	

Recent Publications

Mano Y, et al. Single cell visualization of yeast gene expression shows correlation of epigenetic switching between multiple heterochromatic regions through multiple generations. *PLoS Biol* 11. e1001601 (2013)

Kato H, et al. Spt6 prevents transcription-coupled loss of posttranslationally modified histone H3. *Sci Rep* 3. 2186 (2013)

Ishida M, et al. Intrinsic nucleic acid-binding activity of Chp1 chromodomain is required for heterochromatic gene silencing. *Mol Cell* 47.228-41 (2012)

Kawakami K, et al. A novel RNAi protein, Dsh1, assembles RNAi machinery on chromatin to amplify heterochromatic siRNA. *Genes Dev* 26.1811-24 (2012)

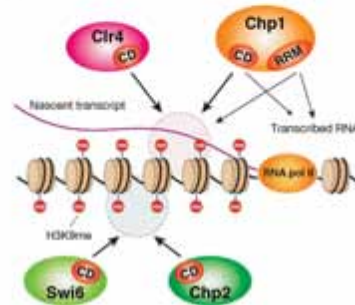
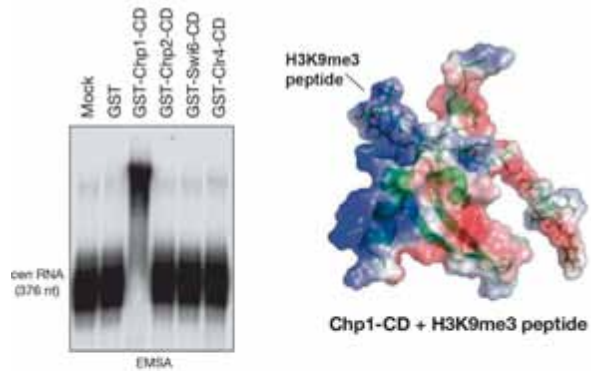
Hayashi A, et al. Heterochromatin protein 1 homologue Swi6 acts in concert with Ers1 to regulate RNAi-directed heterochromatin assembly. *Proc Natl Acad Sci USA* 109.6159-64 (2012)

Hiragami-Hamada K, et al. N-terminal phosphorylation of HP1 α promotes its chromatin binding. *Mol Cell Biol* 31.1186-200 (2011)

Multicellular organisms are made up of diverse populations of many different types of cells, each of which contains an identical set of genetic information coded in its DNA. Cell differentiation and the process of development itself depend on the ability of individual cells to maintain the expression of different genes, and for their progeny to do so through multiple cycles of cell division. In recent years, we have begun to understand that the maintenance of specific patterns of gene expression does not rely on direct modifications to the DNA sequence encoding the organism's genome, but rather takes place in a heritable, "epigenetic" manner. DNA methylation, chromatin modifications, and RNA silencing are some of the best known epigenetic phenomena. Recent studies have begun to show that these different mechanisms are closely inter-related, but a detailed understanding of these systems has yet to be developed.

Our team investigates how modifications to the structure and configuration of chromatin (complexes of nuclear DNA and proteins that provide the structural basis of chromosomes) contribute to epigenetic gene regulation and how such modifications are transmitted over generations of cellular division by studying events at the molecular scale in the excellent model organism, fission yeast (*Schizosaccharomyces pombe*), and in cultured mammalian cells.

Histones are a set of DNA packing proteins present in nucleosomes, the fundamental building blocks of chromatin. In our current studies, we are particularly interested in determining the specific histone modifications and the molecular recognition processes that enable modified histones to work together to establish and maintain higher-order chromatin structures. We also seek to clarify the picture of how dynamic rearrangements of chromatin structure are triggered by examining the structure and function of protein complexes that bind to and modify histones. Through these approaches we aim to understand the molecular mechanisms that underlie complex epigenetic phenomena in developmental processes.



Intrinsic nucleic acid-binding activity of Chp1 chromodomain (CD) is required for heterochromatin assembly. Electrophoretic-mobility shift assay (EMSA) of four CDs in fission yeast (upper, left). The 3D NMR structure of Chp1-CD in complex with H3K9me3 peptide (upper, right). Model for the role of CD-protein's intrinsic RNA binding in heterochromatin assembly (lower).

Stem Cell Biology

The stem cell system can be thought of as a means by which organisms maintain cell renewal. All forms of life require the endless recycling of both materials and energy, making it impossible for either organisms or individual cells to live independent of their environments. In bodies made up of many cells working in cooperation, such as our own, the need to replace and renew is not limited to simple materials and energy supply; the constant generation of new cells is also essential to the maintenance of the individual, a process that has developed in the evolution of multicellular life. In this sense, the process of cell renewal in maintaining the body's integrity and function provides a meaningful example of the relationship of the cell to the organism, the individual to the whole, which we hope to explore more fully using the biology of stem cells as our model.

We are especially interested in the question of how cells maintain the never-ending self-renewal that sustains the organism as a whole. This will require, at the very least, solving the riddles of two essential processes. The first of these is the need for old cells destined for replacement to be able to disengage or otherwise be dislocated from their environmental milieu. The second is the requirement for preparing new cells in replacement. To investigate the first of these mechanisms, our group uses a system for labeling cells with special dyes, which allows us to monitor their location and behavior. We have also developed a system for differentiating embryonic stem (ES) cells in culture to study the second question of new cell production. Currently, we are focusing on developing different stem cell systems, particularly hematopoietic stem cells. This ongoing research project in our lab will allow us to explore how the self-renewing stem cell system is formed and will lead to the development of methods to generate *bona fide* hematopoietic stem cells from ES cells.



Shin-Ichi NISHIKAWA M.D., Ph.D.

<http://www.cdb.riken.go.jp/en/nishikawa>

Shin-Ichi Nishikawa received his M.D. from the Kyoto University School of Medicine in 1973. He performed his internship and residency in the Department of Internal Medicine at the Kyoto University Chest Disease Research Institute, before taking an assistant professorship in the same department in 1979. He spent the period from 1980-82 at the University of Cologne Institute for Genetics before returning to the Chest Disease Research Institute, where he was appointed associate professor in the Department of Microbiology in 1983. He moved to the Kumamoto University Faculty of Medicine in 1987 to take a professorship in the Department of Immunopathology, and returned to Kyoto in 1993, as professor in the Department of Molecular Genetics at the Kyoto University Faculty of Medicine. He was appointed CDB group director in 2000.

Staff

Group Director
Shin-Ichi NISHIKAWA

Research Scientist
Guo DING
Yosuke TANAKA
Yan-Fung WONG

Research Specialist
Lars Martin JACT

Collaborative Scientist
Shin KAWAMATA

Visiting Scientist
Teruo AKUTA
Keitaro IMAIZUMI
Hiroshi KATAOKA
Herve Le MOUËLLIC
Taro MUTO

Visiting Researcher
Takao YAMADA

Technical Staff
Kumiko KOBAYASHI
Satoko MORIWAKI
Satomi NISHIKAWA
Iori SATO

Part-Time Staff
Miho HOSHIIJIMA
Yoko OTSUKA
Noriaki SATO
Kazumi YANAI

Assistant
Junko KOYA
Naeko MINAMI

Recent Publications

Jakt L. M, et al. A continuum of transcriptional identities visualized by combinatorial fluorescent in situ hybridization. *Development* 140.216-25 (2013)

Kataoka H, et al. Region-specific Etv2 ablation revealed the critical origin of hemogenic capacity from Hox6-positive caudal-lateral primitive mesoderm. *Exp Hematol* 41.567-81 e9 (2013)

Nakagawa R, et al. Peyer's patch inducer cells play a leading role in the formation of B and T cell zone architecture. *J Immunol* 190.3309-18 (2013)

Kobayashi K, et al. Role of Etv2-positive cells in the remodeling morphogenesis during vascular development. *Genes Cells* 18.704-21 (2013)

Wong Y. F, et al. Prolonged treatment with DNMT inhibitors induces distinct effects in promoters and gene-bodies. *PLoS One* 8.e71099 (2013)



Group Directors assemble for Dr. Nishikawa's farewell party

Growth Control Signaling



Takashi NISHIMURA Ph.D.

<http://www.cdb.riken.go.jp/en/nishimura>

Takashi Nishimura obtained his Ph.D. in Koza 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.

Staff

Team Leader

Takashi NISHIMURA

Special Postdoctoral Researcher

Naoki OKAMOTO

Research Associate

Hiroko MATSUDA

Eizo NAKAGAWA

Technical Staff

Tomoyuki TAKAHASHI

Takayuki YAMADA

Yuki YAMAUCHI

Student Trainee

Miki YOSHIDA

Part-Time Staff

Okiko HABARA

Kanako HIGUCHI

Noriko KAWASAKI

Tomomi OKJGOUCHI

Junko SHINNO

Recent Publications

Okamoto N, et al. A secreted decoy of InR antagonizes insulin/IGF signaling to restrict body growth in *Drosophila*. *Genes Dev* 27.87-97 (2013)

Okamoto N, et al. Conserved role for the Dachshund protein with *Drosophila* Pax6 homolog Eyeless in insulin expression. *PNAS* 109.2406-11 (2012)

Wirtz-Peitz F, et al. Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. *Cell* 135.161-73 (2008)

Nishimura T and Kaibuchi K. Numb controls integrin endocytosis for directional cell migration with aPKC and PAR-3. *Dev Cell* 13.15-28 (2007)

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.

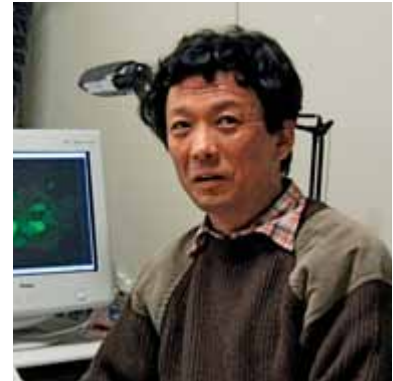


Wild-type female fly (right) and insulin-like receptor mutant female (left)

Pluripotent Stem Cell Studies

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.



Hitoshi NIWA M.D., Ph.D.

<http://www.cdb.riken.go.jp/en/niwa>

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.

Staff

Project Leader

Hitoshi NIWA

Research Scientist

Yoko NAKAI-FUTATSUGI
Satomie NISHIKAWA-TORIKAI
Satoshi OHTSUKA
Masaki SHIGETA
Robert Odell STEPHENSON
Toshimi SUGIMOTO
Tomoyuki TSUKIYAMA

Research Associate

Mariko YAMANE
Visiting Scientist
Yasuhide OHINATA
Hiroki URA

Technical Staff

Setsuko FUJII

Junior Research Associate

Shunsuke ITO

Student Trainee

Marius KLANGWART

Part-Time Staff

Sachiko HASHIMOTO
Yayoi NAKAI

Assistant

Miho SAKURAI

Recent Publications

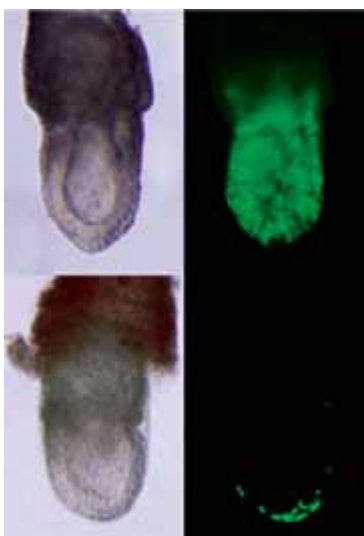
Adachi K, et al. Context-Dependent Wiring of Sox2 Regulatory Networks for Self-Renewal of Embryonic and Trophoblast Stem Cells. *Mol Cell* 52:380-92 (2013)

Ohtsuka S, et al. E-cadherin promotes incorporation of mouse epiblast stem cells into normal development. *PLoS One* 7:e45220 (2012)

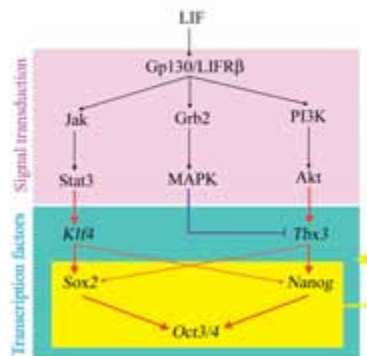
Martello G, et al. Esrrb is a pivotal target of the gsk3/tcf3 axis regulating embryonic stem cell self-renewal. *Cell Stem Cell* 11:491-504 (2012)

Niwa H, et al. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* 460:118-22 (2009)

Niwa H. How is pluripotency determined and maintained? *Development* 134:635-46 (2007)

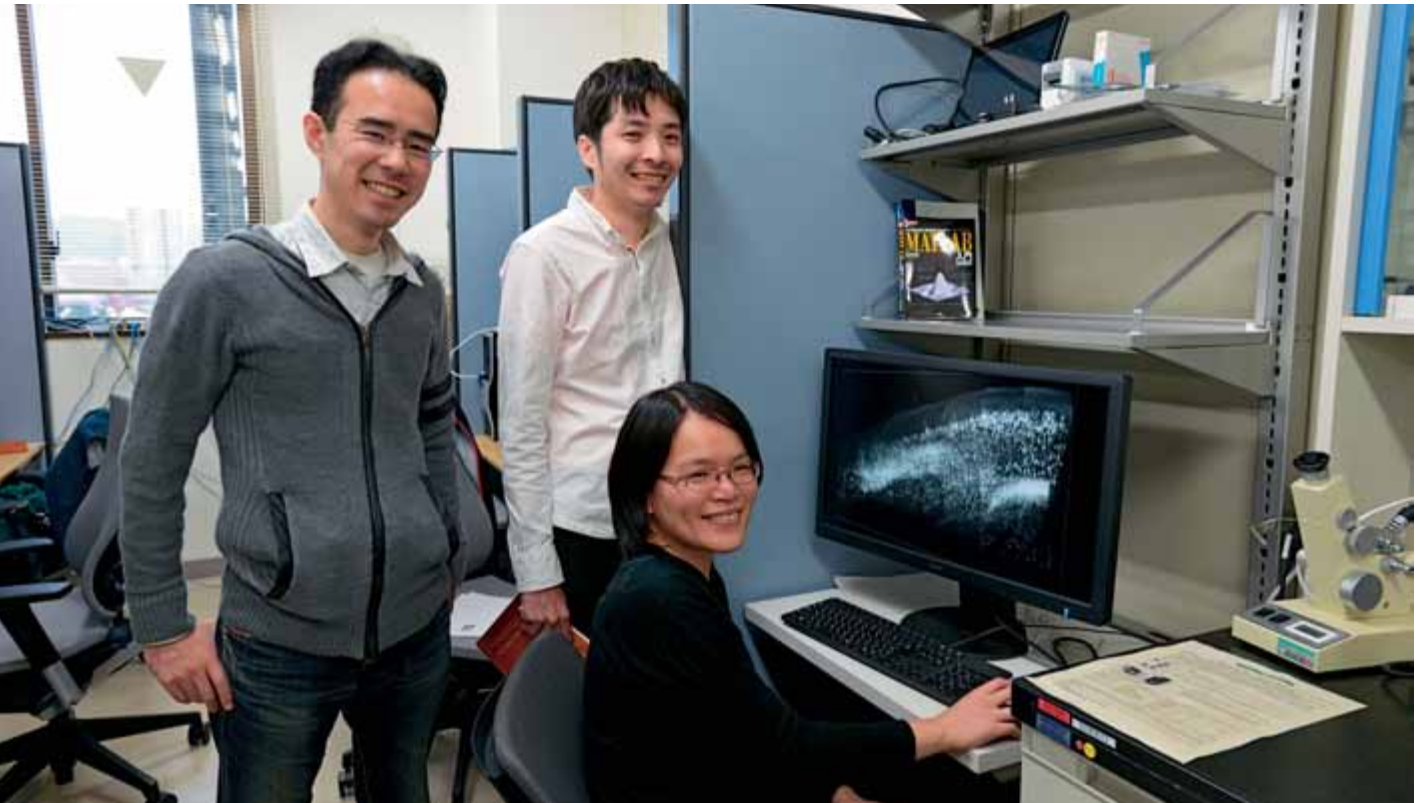


Chimeric embryos generated by injection of Gata6GR ES cells into blastocysts. These ES cells carry the constitutively-active Egfp transgene and contribute to primitive ectoderm (upper panels), whereas they contribute to parietal endoderm after induction of Gata6 activity with dexamethasone (lower panels).



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

Seeing neuronal circuitry with SeeDB



Takeshi IMAI, Satoshi FUJIMOTO, Meng-Tsen KE

Developmental biology is one of the most visually stunning fields in the life sciences, and the ability to 'see' within the embryo is a fundamentally important approach to its study. The advent of fluorescent marker proteins, such as GFP, has made it possible to visualize the localization of the expression of labeled proteins, but the scattering of fluorescent signals in opaque biological samples means that such imaging can only peer to within a few hundreds of micrometers of the surface of the tissue, even when using advanced technologies such as two-photon microscopy. This creates significant problems for the study of complex three-dimensional tissues, such as neuronal circuits; the traditional approach of slicing the tissue to obtain two-dimensional data to be used in attempts to reconstruct 3D

interconnected structures presents herculean difficulties. A number of alternative strategies involving the 'clearing' of tissue with solvents to render it more or less transparent have been developed, but these have also been complicated, time-consuming, and may affect fluorescent signals from labeled proteins.

Meng-Tsen Ke and colleagues in the Lab for Sensory Circuit formation (Takeshi Imai, Team Leader) now look to improve the toolkit with a report of a new technique for optically clearing embryonic tissues, which they have named SeeDB. This new method, published in *Nature Neuroscience*, uses a water-based agent that preserves fluorescence and tissue structure, and enables the deep-layer visualization of neuronal circuits.



In seeking a better optical clearing agent, Ke and colleagues resolved to identify aqueous solutions with refractive properties similar to those of the tissues they wished to study. Other groups had reported some success using sucrose solutions, but the Imai team decided to test fructose as an alternative. After long experimentation, they refined the recipe to what they named SeeDB (for See Deep Brain), a solution that made it possible to render an embryo optically transparent in just three days, a major advance over previous techniques requiring 2–3 weeks. SeeDB also excels in preserving the size and morphology of embryonic structures, and avoids the quenching of fluorescence from molecular labels such as GFP. Additional information for scientists interested in this new optical clearing solution are available at SeeDB Resources.

Ke et al. next sought to determine the tissue depths at which SeeDB imaging could be used, using samples from adult mouse brain. Working with a microscope manufacturer, they customized a two-photon microscope with a longer working distance lens, to overcome depth limitations of the commercially available version. Fluorescence imaging of 6 mm-thick samples cleared by SeeDB yielded beautiful, high-resolution images of even the deepest layers, demonstrating the utility of this technique at a scale suitable for whole-brain studies in mouse.

In a further test of the new clearing agent's utility, the team labeled axons of the corpus callosum, which link the left and right hemispheres of the brain, and traced their projections in a mouse brain made transparent with SeeDB. They were able to trace individual axon fibers over long distances from one side of the brain to counterparts projecting from the opposite hemisphere, and found that such callosal axon projection relies not only on positional guidance from target tissue, but also sorting at the pre-target site.

The team last moved to the lab's core interest of studying the circuits formed in the olfactory bulb of the brain. In the mouse, olfactory sensory neurons of like type converge in glomeruli within the olfactory bulb, which is the site of odor information processing. Odor inputs to a glomerulus are then relayed to mitral cells through dendritic connections, but the wiring of these mitral connections has remained unresolved. Using SeeDB, Ke et al. were able for the first time to get an unobstructed view of the circuit layout for the full set of mitral cells associated with a single glomerulus, revealing an unexpected architectural complexity.

"I am hopeful that this new technology will help usher in a new era of '3D biology' aimed at a better understanding of neural circuit formation and other complex developmental processes," says Imai. "However, we still need to fine-tune the microscopy and image data processing techniques to make this even more suitable for a wide range of studies."



Embryo prior to (left) and after (right) clearing with SeeDB agent



3D imaging of mouse brain treated with SeeDB

Role for RA in olfactory neurogenesis



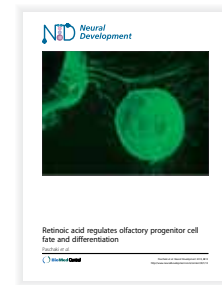
Marie PASCHAKI, Yuko MUTA

Uniquely among sensory tissues, olfactory neurons have the capacity to regenerate throughout life, a necessity given their direct exposure to the external environment. The olfactory neuroepithelium itself derives from a sensory placode includes a variety of supporting cell types in addition to neuronal cells, which are generated by the activity of progenitors also present in the tissue. Despite our increasingly detailed understanding of olfactory neural circuitry and functions, however, we still have much to learn about the ontogeny and regeneration of olfactory neurons.

Work by Marie Paschaki and colleagues in the Lab for Sensory Development (Raj Ladher, Team Leader) published in *Neural Development* has now begun to provide some insights into those processes. By studying the function of

retinoic acid in olfactory tissues from mouse and chicken, Paschaki et al. show that this signaling molecule plays a role in olfactory neurogenesis.

Retinoic acid (RA) is involved in a broad range of developmental processes, and had been implicated as a possible signal involved in olfactory epithelial patterning. But the results of this previous work had left open the question of whether it was involved only in induction of the tissue, or in prompting neuronal differentiation as well. Paschaki set out to pinpoint this role, as well as to determine the extent (if any) to which retinoic acid affects olfactory neurogenesis, by studying RA and an enzyme responsible for its synthesis, RALDH, in chicken and mouse.



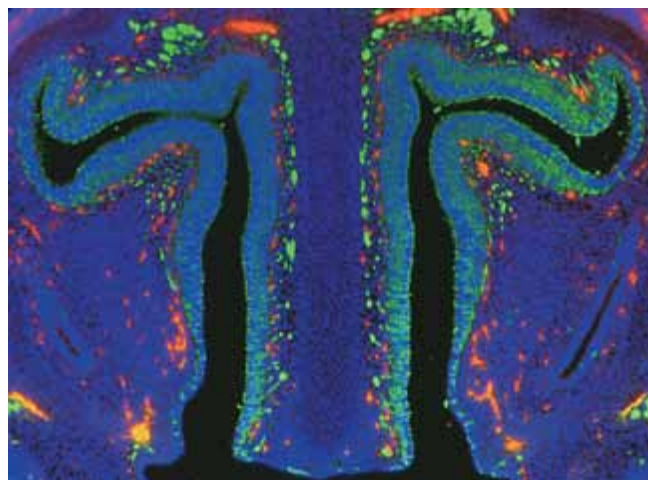
She first examined whether inhibition of RA signaling would perturb the induction of the olfactory placode in chicken embryos, but found that interference with RA synthesis had no perceptible effect. In mouse mutants lacking *Raldh* function as well, showed no abnormal placode phenotype, indicating that retinoic acid is dispensable in olfactory placode induction.

That picture changed dramatically however when she tested RA function later in development. When she implanted beads soaked in an inhibitor of RALDH activity into chicken embryos, she found that this led to a failure in the differentiation of olfactory stem cells, suggesting that RA is necessary for inducing or maintaining the olfactory progenitor population. In tissue explants, culture medium containing vitamin A, from which retinoic acid is metabolized, revealed an inhibitory effect on neurogenesis, pointing to the possibility that RA maintains stem cells by suppressing differentiation.

These observations raised the issue of where in the olfactory lineage RA plays its part. Loss of RA synthesis had no effect on apoptosis, but did increase the number of prolifer-

ating cells, prompting Paschaki to look at the olfactory progenitor phenotype in a mouse *Raldh* mutant. She found that a specific population of quiescent Pax6-expressing progenitors was depleted in the mutant embryos. Turning again to explanted tissue, she examined the effects of vitamin A and found that its addition to the culture medium supported greater numbers of progenitors than did medium without this RA precursor. More strikingly when she grew *Raldh* null mutant explants in the same conditions, the progenitor population collapsed irrespective of whether vitamin A was included in the medium, suggesting that massive differentiation had occurred at the expense of progenitor self-renewal. This hypothesis was borne out in the mutant embryos, which showed fewer Pax6-expressing progenitors than in wildtype, and consequent failure to maintain olfactory neurogenesis.

“The olfactory epithelium is the only neuronal population in mammals that can repair itself,” says Ladher. “If we can understand how it uses its developmental history to achieve this, it may give us some ideas on how we can help other neuronal populations repair themselves.”



Mouse olfactory epithelium at day 18.5 of development. Neurons are stained green, and can be seen through the epithelium, sending out axons, arranged as bundles, in the cephalic mesenchyme.

Endoskeletal origins of the turtle carapace



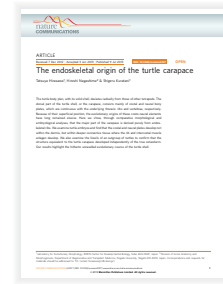
Shigeru KURATANI, Tatsuya HIRASAWA

Exoskeletons are widely associated with invertebrates, typically envisioned as the chitinous armor of a beetle or crustacean. But vertebrates have exoskeletal elements too. Our own cranium, which lacks an overlying layer of muscle, is the exception to our predominantly interior skeletal system. A number of mammals have also developed more extensive protective outer coats, but these vary dramatically in their composition – an armadillo’s scutes are completely different, for example, from a turtle’s carapace. While the armadillo’s scales are exoskeletal in origin, and the animal maintains internal ribs as in other vertebrates, only in the turtle does the endoskeleton make its way to the body exterior. Students of turtle evolution and development, however, have long debated whether there might not be some exoskeletal contribution to the formation of the carapace.

Tatsuya Hirasawa and colleagues from the Laboratory for Evolutionary Morphology (Shigeru Kuratani, Group Director) have now found new evidence that may help put that argument to rest, through analyses of anatomical and paleontological data that squarely trace the origins of the carapace to exteriorized ribs of endoskeletal origin. Published in *Nature Communications*, this work adds compelling new evidence to support the theory that the turtle’s shell grows out from the inside.

Our skeletons evolved through a process of territorial give and take, with some elements advancing while other gave ground. By studying how such movements differ in embryos of various species, biologists have gained insights into the mutability of fundamentally conserved body plans. For the past two centuries, however, the turtle has remained something of a conundrum. While some hold that the carapace represents a fusion of endo- and exoskeletal elements (as the result of signaling by endoskeletal elements, i.e., ribs, that have shifted into the dermis, triggering the formation of exoskeletal tissue), others view it as a purely endoskeletal structure that nonetheless finds its way outside.

To find an answer to this age-old question, Hirasawa analyzed the dorsal carapace of the Chinese soft-shelled turtle in close detail. The formation of this carapace begins with dorsal positioning of ribs and intercostal muscle primordia, after which the muscle element is gradually lost, leaving the



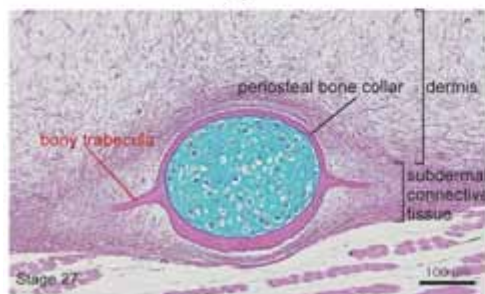
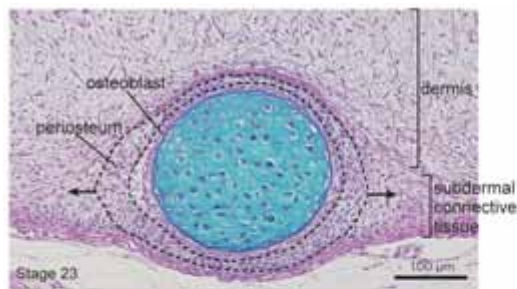
periosteum of rib to expand laterally and serve as a scaffold that is subsequently filled in by bony trabecula. These bony elements later invade the intercostal spaces to form a complete carapace entirely as the endoskeleton.

The group next compared the formation of the turtle carapace with that of ribs in a bird (chicken) and the bony dermal plates (osteoderm) of the alligator. While the carapace and avian ribs followed broadly comparable developmental sequences, the formation of the osteoderm differed dramatically, as it apparently involves epithelial-mesenchymal interactions within the dermis, further cementing the argument for an endoskeletal origin of the carapace.

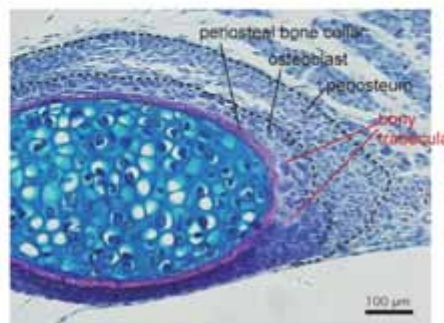
The paleontological record also holds clues to carapace evolution. *Odontochelys*, an ancient ancestral turtle, had bony plate-like outgrowth derived from ribs, although it lacked a fully-closed carapace. Most vertebrates have movable joints between ribs and vertebrae, which facilitate respiration, so the rigidity of these bones in both *Odonto-*

chelys and modern turtles is a remarkable feature. More recently unearthed intermediate fossils also underscore the evolution from axial endoskeleton to rigid shell. Examining the collection at the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP) in the Chinese Academy of Sciences (CAS), Hirasawa identified a marine reptile, *Sinosaurusphargis yunguiensis*, which shows anatomical similarities with both *Odontochelys* and turtles that suggest the growth of an immovable shell within the subdermal tissue otherwise typically occupied by intercostal muscle.

“We usually think of the turtle’s carapace as a form of protection, but it may have first emerged as an aid to swimming – a kind of surfboard if you like – that only latterly became hardened,” says Kuratani. “The study of unusual adaptations such as the turtle shell can teach us not only about the great diversity of the animal world, but the evolutionary programs that led to our own body plans as well.”



Turtle



Chicken

Development of turtle carapace (left) and chicken rib (right). Subdermal expansion of periosteum and bony trabeculae are seen in both processes.

Microtubules have a firm CLASP on the epiblast basement membrane



Yukiko NAKAYA, Erike Widyasari SUKOWATI

In higher animals, gastrulation lays the groundwork for subsequent development by establishing the three germ layers that give rise to all the tissue in the body. In chicken and other birds, cells of the epiblast at the body surface migrate to a site called the primitive streak, and subsequently move inward to the embryonic interior, forming the endoderm and mesoderm. Epiblast cells are epithelial in nature, form adhesive bonds and attach to a basement membrane. But during their ingress from the primitive streak, these cells shed their connections with other cells and the basement membrane, transforming into mobile mesenchymal cells in the process. Such epithelial–mesenchymal transitions (EMT) play roles in numerous developmental processes, but the molecular machinery that controls EMT is still poorly understood.

Yukiko Nakaya and colleagues in the Lab for Early Embryogenesis (Guojun Sheng, Team Leader) have now identified once such mechanism, finding that the microtubule-binding protein CLASP is required for maintaining cells in an epithelial state, and that downregulation of CLASP induces the degradation of basement membrane, a necessary event in gastrulation. Published in *Journal of Cell Biology*, this work was featured on the cover of the August issue.

Previous studies of CLASP had shown that it associates with microtubule plus-ends and maintain their density, but the physiological and developmental role of the protein has been a mystery. Nakaya had observed that EMT in gastrulating chick embryos appeared to involve microtubule-mediated anchoring of epiblast cells to the basement mem-

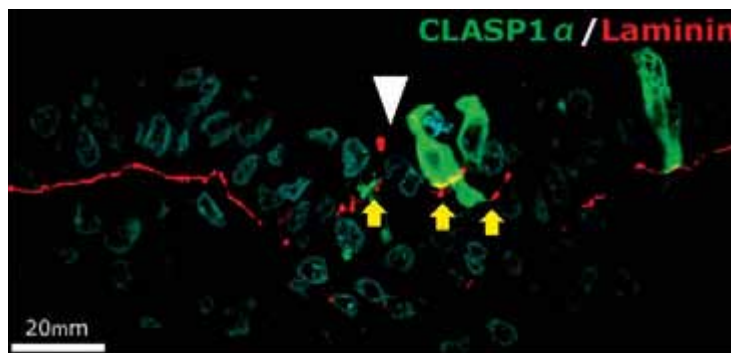
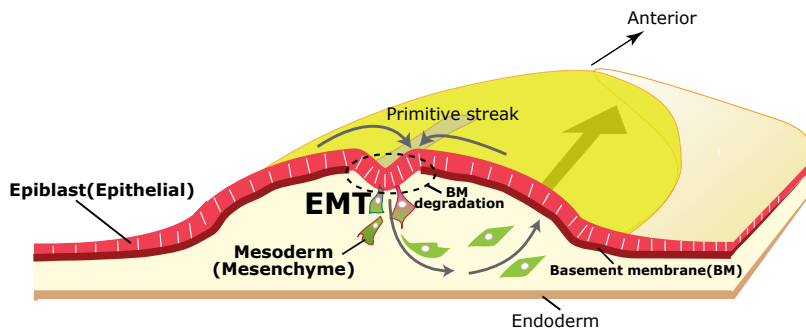


brane, presumably linked to its maintenance and degradation. Looking first at the expression of CLASP during this period, she observed that while it was expressed in the epiblast, it was downregulated in cells at the primitive streak. Overexpression of CLASP caused stabilization of the basement membrane, which is ordinarily destined to decay during EMT, while conversely, knocked down CLASP outside the streak led to abnormal degradation of basement membrane components.

Investigating CLASP function, Nakaya found that it works with its binding partner LL5 to tether microtubules to the basal cortex in cells, suggesting that its expression maintains epiblast cells in an epithelial state, while its downregulation is a necessary step in EMT. But how does a cytoplasmic molecule such as CLASP have this effect on the extracellular basement membrane? For a promising candidate, Nakaya next turned to Dystroglycan, a transmembrane protein known to interact both with the basement

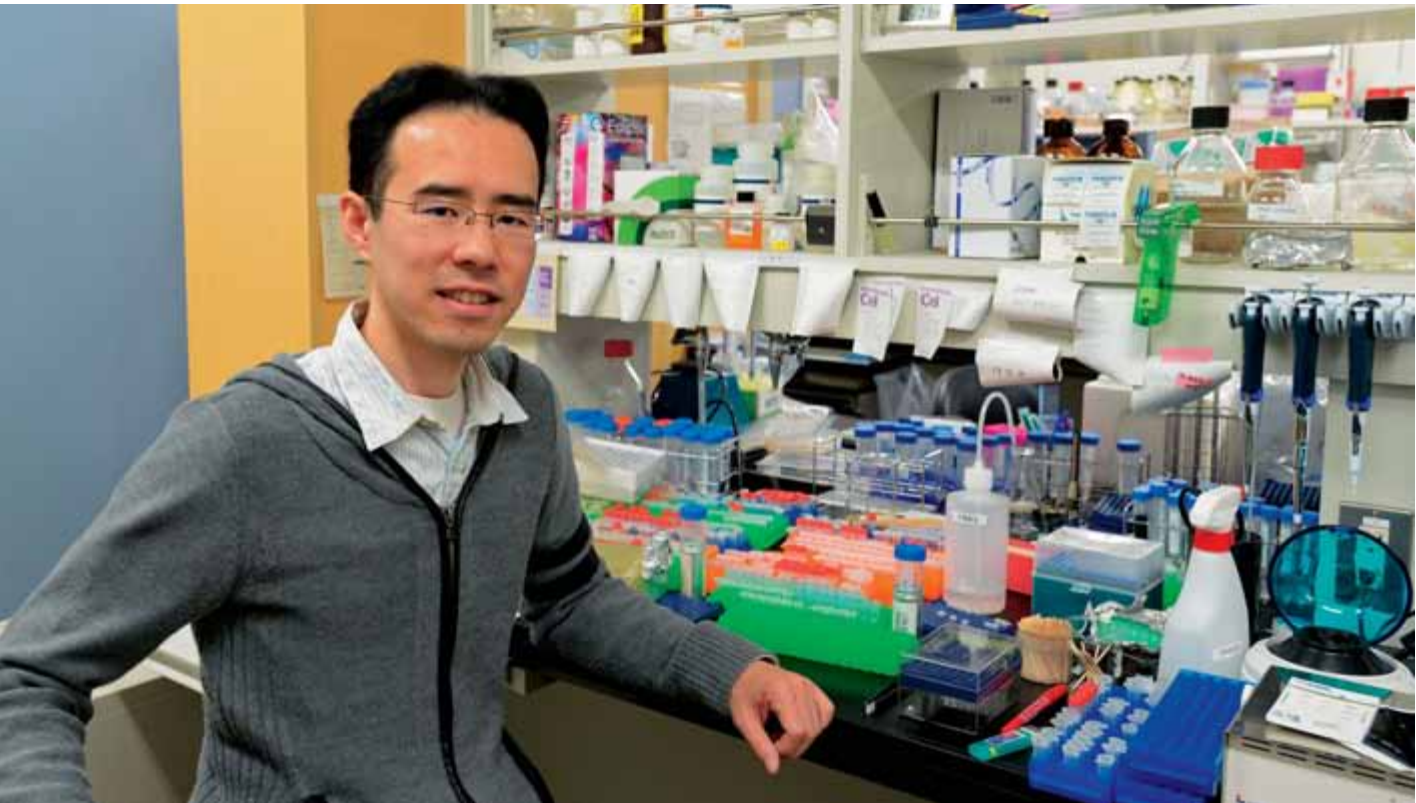
membrane and other intracellular factors. Overexpression or inhibition of Dystroglycan in the epiblast yielded similar results to those she observed in her experiments with CLASP, inappropriate stabilization or degradation of the basement membrane. Co-immunoprecipitation revealed colocalization of the two proteins in the basal cell cortex, indicating a physical interaction. And when she looked at Dystroglycan expression in the primitive streak, she found it decreased during EMT, just as with CLASP.

This series of experiments makes a solid case for the combined roles of CLASP and Dystroglycan in anchoring microtubules to the basal cell cortex and stabilization of the basement membrane in epiblast cells. “But we are still curious about why it is that loss of CLASP causes the basement membrane to decay,” says Sheng. “We suspect this may involve vesicle transport by microtubules, but we will have to study that possibility more closely in a future project.”



(Top) Cartoon scheme of epithelial-mesenchymal transition, showing degradation of basement membrane beneath the primitive streak and ingress of cells. (Bottom) Forced expression of CLASP (green) in the streak results in the stabilization of the basement membrane (yellow arrows).

Baseline receptor activity steers olfactory axon targeting along the A-P axis

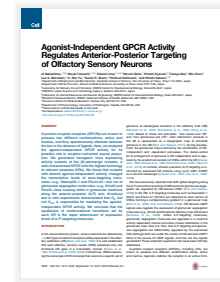


Takeshi IMAI

All the many odors we recognize and remember are first detected as molecular signals by G-protein coupled receptors (GPCRs) on specialized neurons in the olfactory system. The mouse has around 1000 such odorant receptors, which, when bound to an odorant molecule, convey signals to the olfactory bulb in the brain. An equal number of structures, known as glomeruli, serve as convergence points for neurons bearing the same odorant receptor, patterns of the collective activity of which are perceived as olfactory sensations by the brain. Establishing the right connections from receptor to glomerulus requires the precise wiring of olfactory neurons into elaborate circuits, by processes that remain imperfectly understood.

Takeshi Imai, Team Leader of the Lab for Sensory Circuit Formation, and colleagues have now discovered an essential role for the activation of olfactory receptors unprovoked by ligand binding in the control of axon targeting from olfactory neuron to glomerulus. Reporting in *Cell*, they also describe how the co-action of the protein G_s supports the efficient transduction of such weak, unstable signals, making guided axon projection in the fetal mouse possible even in the absence of odor stimuli.

Odorant receptors are expressed on neurons lining the olfactory epithelium, each of which typically expresses only a single receptor type. Axons from these neurons of the same type converge to glomeruli specific to that odorant receptor profile, following a general targeting gradient along the an-



terior-posterior (A-P) axis, followed by more precise local sorting. Both of these have been thought to rely on signals arising from odorant receptor activation, but the details of these mechanisms have eluded explication.

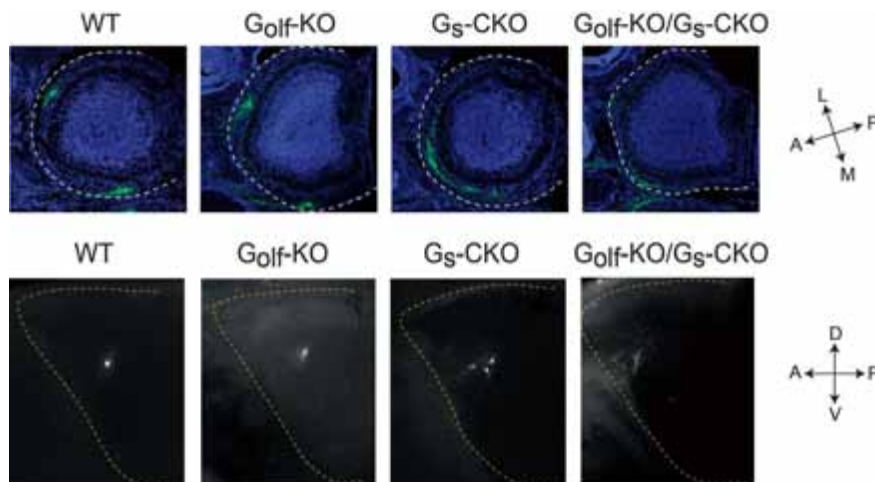
Binding of a GPCR by an odorant molecule triggers a shift from inactive to active state that entails structural changes in the conformation of the receptor molecule. Past studies have shown, however, that activation by such an agonist is not necessary; GPCRs show a certain baseline level of activity even in the absence of ligand binding, although this has often been considered little more than noise.

Imai and colleagues set out to determine whether this agonist-independent receptor activation plays a role in olfactory neuron axon guidance along the A-P axis by developing mutant mice with altered baseline activity. A-P axon guidance is governed by factors during mid-development, while those responsible for local sorting are expressed later. In an extensive screening study, Imai was able to identify G_s as the A-P factor and G_{olf} as the glomerular segregation molecule. Both proteins are structurally similar, and mediate odorant receptor cAMP signals; G_{olf} is known to cooperate with odorant receptors in detection of odor stimuli but the

role of G_s and how it works with G_{olf} had never been determined. Undertaking a detailed biochemical analysis, Imai found that while G_s effectively responded to baseline OR activity, G_{olf} did not.

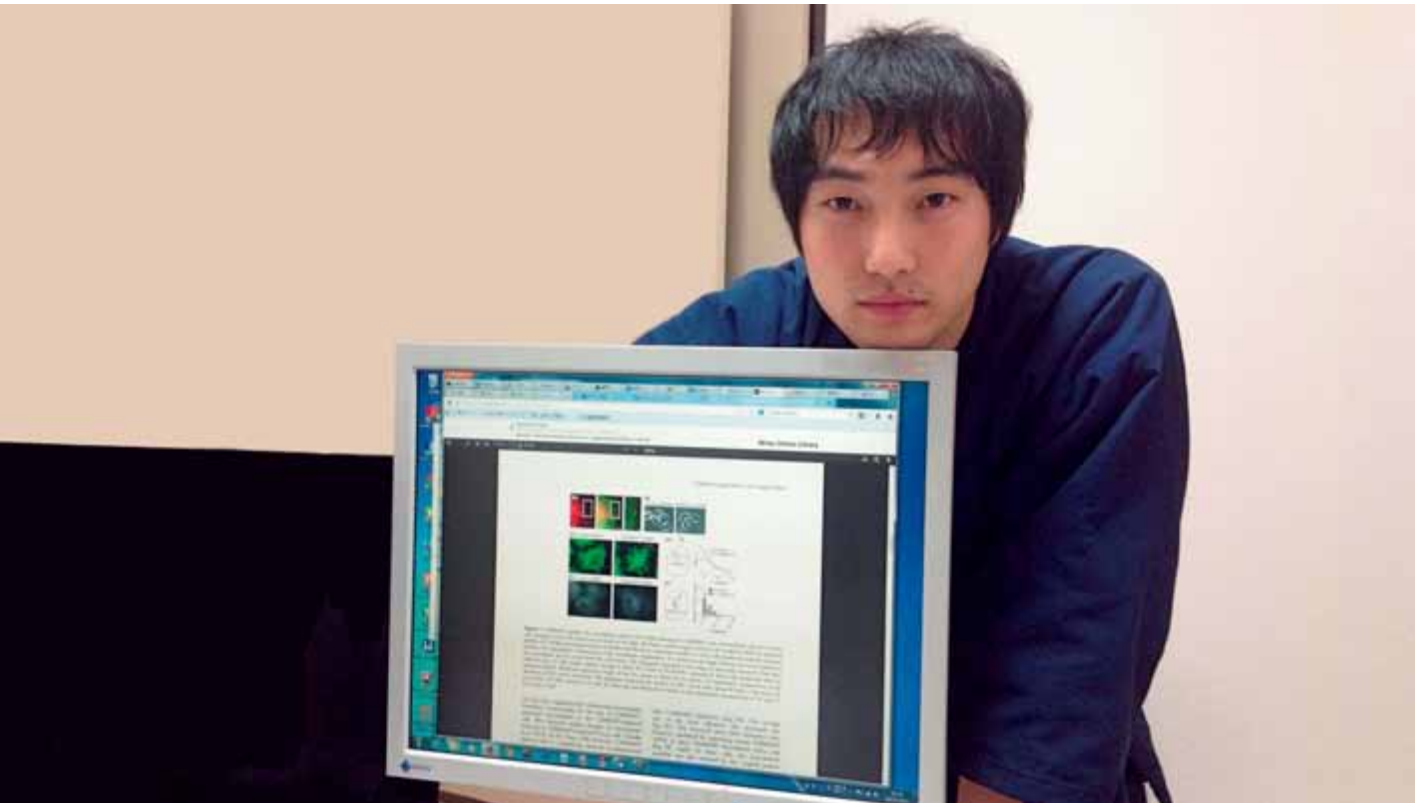
The team next generated knockout G_{olf} and G_s mice; the G_s KO was necessarily conditional, as the mutant is embryonic lethal. Imai found that A-P axon guidance was dramatically affected in the G_s mutant, and glomerular sorting in the G_{olf} KO, and that these defects were specific to the respective mutations. G_s mutants additionally showed a high frequency of axonal wiring errors, indicating that its role is to regulate A-P axon targeting in response to baseline signaling by still-developing olfactory neurons.

“The ability odorant receptors to achieve two highly distinct functions simply by the use of differential responses by G_s and G_{olf} to baseline signals is truly amazing,” says Imai. “Many have suggested that stimulus-independent signaling might play a role in neural circuit formation, and it appears that is just what we are observing here. We next want to study the mechanism behind how connection specificity during embryonic neural circuit formation depends on neuronal activity.”



Axon projection from olfactory neurons expressing a specific receptor (OR-17). While the G_{olf} knockout mouse shows no abnormalities, the G_s conditional knockout shows disturbance of axonal targeting. Top row shows stained sections of olfactory bulb (OR-17 in green), bottom row shows wholemount olfactory bulb preparations.

New roles for microtubules in actin regulation



Shigenori NAGAE

Microtubules are components of the cytoskeleton known for their roles in maintaining cellular structure and cytoplasmic transport. These elongated proteins form two distinct populations, one anchored to the centrosome by its minus-end and a second that grows from other sites within the cell, through interactions with other molecules. It has previously been shown that the cytoplasmic protein CAMSAP3 (also known as Nezha) stabilizes non-centrosomal microtubules, but it remains unclear whether the roles of non-centrosomal in cellular architecture and other functions differ from those of the centrosomal type.

Now, a study by Shigenori Nagae of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) and the Kyoto University Graduate School

of Biostudies, working in collaboration with the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (Beijing, China), reveals that non-centrosomal microtubules mediated by CAMSAP3 regulate the organization of a second cytoskeletal protein, F-actin, which plays roles in cellular morphology and behavior. Published in *Genes to Cells*, these findings shed new light on the molecular machinery underlying microtubule function.

CAMSAP, which serves as the nucleus of non-centrosomal microtubule formation, was recently identified by the Takeichi group, who showed that CAMSAP stabilizes microtubules through binding their minus-ends. In this latest study, Nagae et al. used the HeLa line of immortalized human cells to study the function of microtubules bound to CAMSAP.



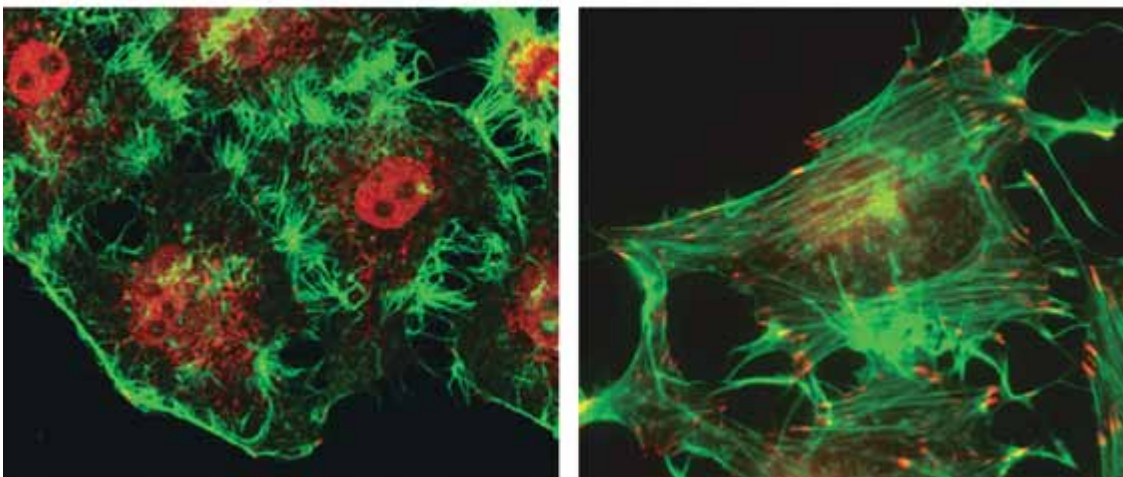
The group focused specifically on CAMSAP3, which they found to be expressed in a punctate fashion throughout the cells, binding with microtubule minus ends, with the plus ends growing outward from these CAMSAP loci.

Using RNAi to inhibit CAMSAP expression, the group found that the ordinarily smooth-edged cells became spiky and distorted, taking on a distinct 'thorny' morphology, with a concomitant increase in the number of centrosomal microtubules. Curious about the cause of the morphological changes, they next examined the activity of a second cytoskeletal protein, actin, in CAMSAP-inhibited cells, and found that actin stress fiber was upregulated on loss of CAMSAP function.

But how then do changes in non-centrosomal microtubule formation affect actin fibers? In looking for the underlying mechanism, they next focused on RhoA, a protein known to induce actin stress fiber formation. Intriguingly, they found that depletion of CAMSAP3 led to an increase in

detyrosinated microtubules (which were found to be at higher levels in centrosomal microtubules than in non-centrosomal microtubules). Due to this, the Rho activator GEF-H1 became unbound from microtubules, leaving it free to activate RhoA, resulting in the promotion of actin stress fiber formation and, ultimately, changes in cell shape. Thus, it appears that under normal conditions binding of microtubules by CAMSAP3 inhibits RhoA activity maintaining actin fiber formation at a fixed level. These new insights help to clarify the mechanistic and functional differences between centrosomal and non-centrosomal microtubules.

"We are now finding that a balance between these two types of microtubules serves to regulate actin fibers, which may contribute to cell type-dependent morphological processes," says Takeichi. "By clearly distinguishing between these different microtubule populations, we hope to contribute even more to our understanding of how these molecules work in the cell."



In HeLa cells, inhibition of CAMSAP3 (right panel) results in an increase in actin fibers (green) and changes in cell morphology. Control cells shown to left.

2013 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

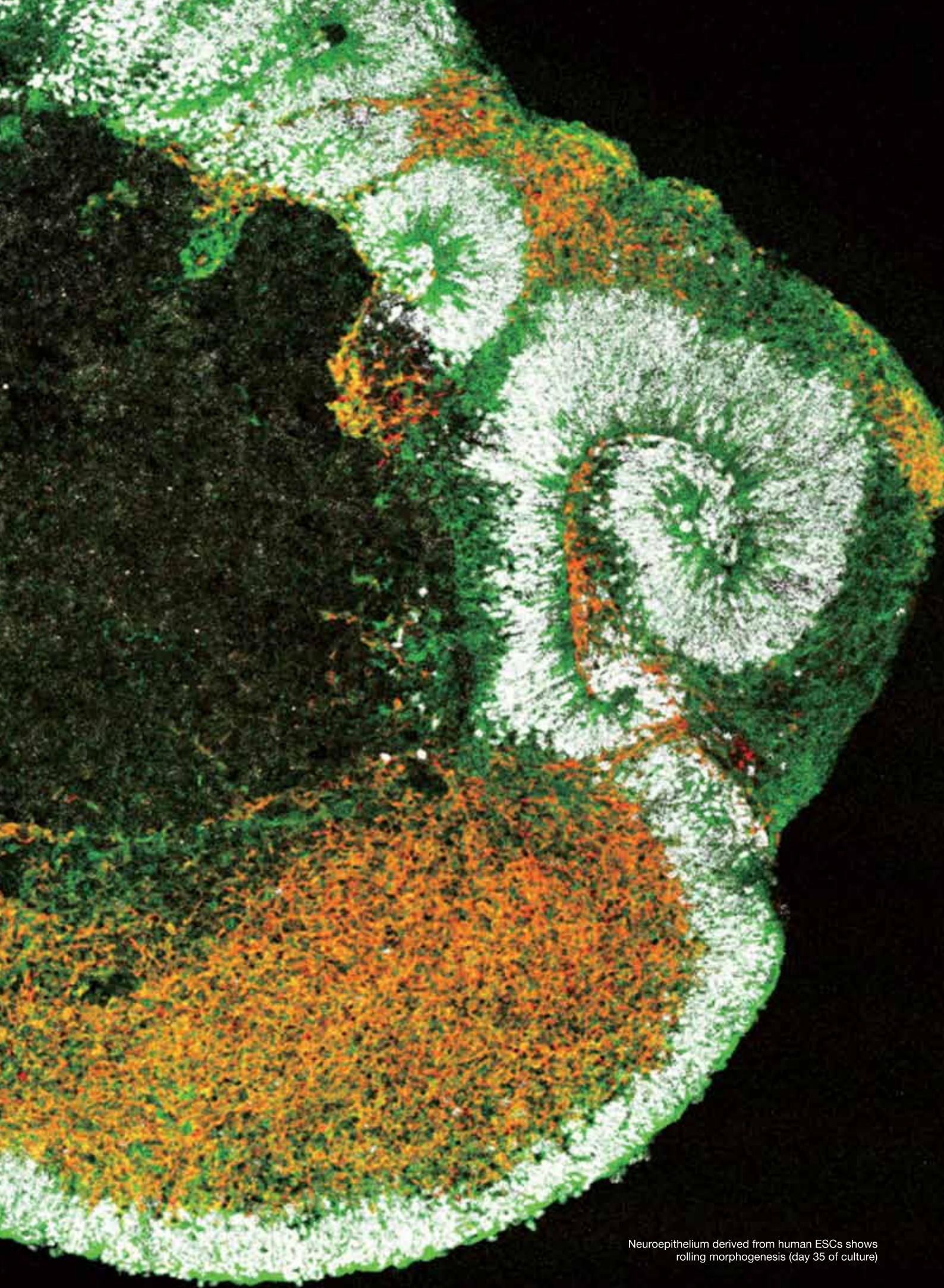
organizations, including government ministries and agencies, private foundations and academic societies.

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
Miki Ebisuya	Unit Leader	Reconstitutive Developmental Biology	The Young Scientists' Prize, The Commendation for Science and Technology	The Minister of Education, Culture, Sports, Science and Technology
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Systems of Biology Award	The Society of Photo Optical Instrumentation Engineers
Tatsuya Hirasawa	Research Scientist	Evolutionary Morphology	Best Presentation Award for Young Researchers (1st place winner)	The Society of Evolutionary Studies
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Hans Sigrist Prize	Hans Sigrist Foundation of University Bern
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Uehara Award	The Uehara Memorial Foundation



Photo : Eriko Nakatsuta



Neuroepithelium derived from human ESCs shows rolling morphogenesis (day 35 of culture)

Cellular Reprogramming



Haruko OBOKATA Ph.D.

<http://www.cdb.riken.go.jp/en/obokata>

Haruko Obokata graduated from Waseda University with a B.Sc. in applied chemistry (2006), and a M.Sc. (2008) and Ph.D. (2011) in engineering. Her foray into regenerative medicine and tissue engineering began during her master's, doing her bench work as a student trainee at the Tokyo Women's Medical University. During her doctoral program, she studied for almost two years under Charles Vacanti at the Brigham and Women's Hospital, Harvard University Medical School looking at the relationship between stem cells and regeneration. She took a postdoctoral fellowship in Vacanti's lab after completing her Ph.D. in 2011 to continue the research she began in his lab, and also began collaborations with Teruhiko Wakayama (now at Yamanashi University) at the CDB as a visiting scientist. In March 2013, she was appointed to her current position as a unit leader at the CDB.

Staff

Research Unit Leader
Haruko OBOKATA

Technical Staff
Kumi MATSUURA
Azusa NOMA
Rieko YAKURA-NISHIZAWA

Student Trainee
Yukari TERASHITA

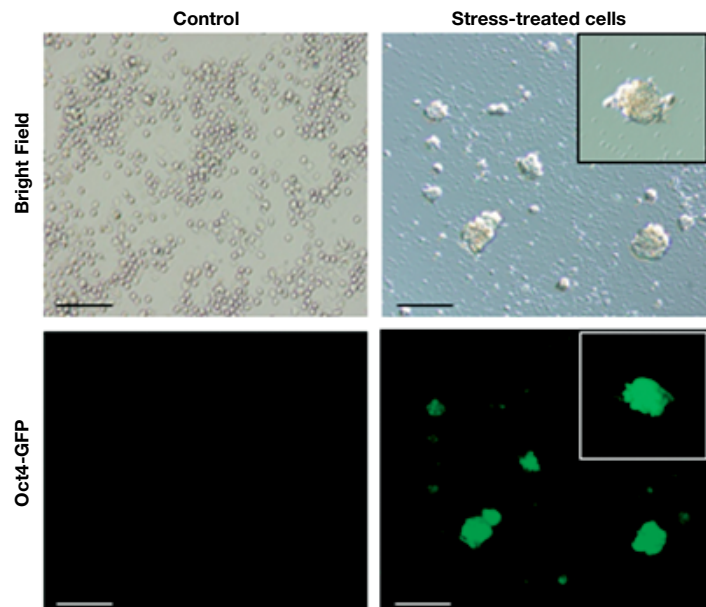
Recent Publications

Obokata H, et al. Stimulus-triggered fate conversion of somatic cells into pluripotency. *Nature* 505.641-647 (2014)

Obokata H, et al. Bidirectional developmental potential in reprogrammed cells with acquired pluripotency. *Nature* 505. 676-680 (2014)

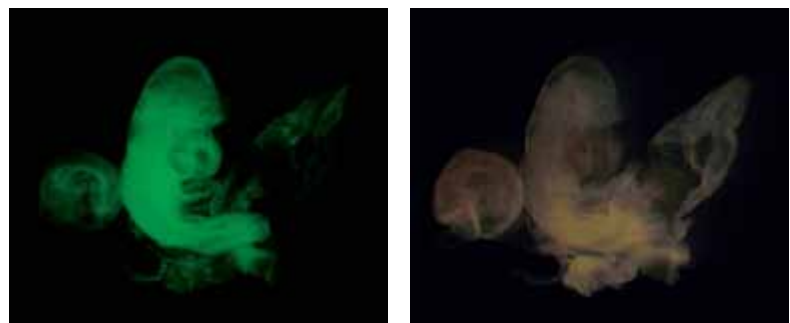
Obokata H, et al. Reproducible subcutaneous transplantation of cell sheets into recipient mice. *Nat Protoc* 6.1053-9 (2011)

All organisms possess instincts to survive exposure to external stresses by adapting to their environment and, to some degree, regenerating injured tissues or organs. It is thus unsurprising to observe dramatic cellular plasticity after exposure to significant external stresses, such as an injury. In both vertebrates and plants, cellular reprogramming can be induced by exposure to external stimuli, which suggests that organisms that possess significant capacity for regeneration show great plasticity when exposed to external stresses and generate "stem cells" from somatic cells. Given these observations, true 'stemness' appears to be linked to cellular plasticity. The main goal of our research is to elucidate the relationship between stemness and cellular plasticity.



Oct4 expressing cell generation from CD45 positive somatic cells.

Oct4-GFP expression of stress treated cells. Stress-treated cells express Oct4-GFP, while untreated controls did not. Magnification of Oct4-expressing colony is shown in the upper right in the stress-treated group. Scale bar indicates 100 μ m.



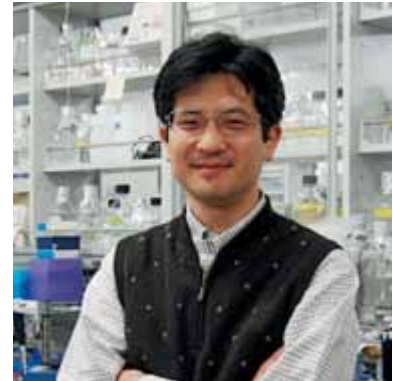
4N embryos at E9.5 generated with stress altered somatic cells.

Stress altered somatic cells contributed not only to fetus but also to placenta and amniotic membrane.

Mammalian Epigenetic Studies

The stable inheritance of cellular traits and gene regulatory programs through multiple rounds of cell division is a fundamental process in animal development and homeostasis. Chemical modifications and remodeling of chromatin play critical roles in such epigenetic processes. Research in our laboratory has focused on cytosine DNA methylation, a heritable genome-marking mechanism. In mammals, DNA methylation is catalyzed by three DNA methyltransferases, Dnmt1, Dnmt3a and Dnmt3b, and plays important roles in the regulation of gene expression and development. During early embryogenesis, genome-wide reprogramming of DNA methylation establishes new DNA methylation profiles of the embryos. Once established, these cell-specific DNA methylation profiles are stably maintained in a lineage-dependent manner. Using mouse embryos and embryonic stem (ES) cells as model systems, we investigate the regulation and functions of DNA methylation in mammalian development.

During embryogenesis, many key transcription factors are used repeatedly, triggering different outcomes depending on cell type and developmental stage. Using a drug-inducible Gata4 system and an ES cell model of mesoderm differentiation, we found that DNA methylation modulates transcription factor output in the context of cell differentiation. The activation of inducible Gata4 in mouse ES cells is known to drive their differentiation into endoderm. We found that the differentiation of wild-type ES cells into mesoderm blocks their Gata4-induced endoderm differentiation, while mesoderm cells derived from *Dnmt3a*^{-/-}*Dnmt3b*^{-/-} ES cells can retain their response to Gata4, allowing lineage conversion from mesoderm to endoderm. Our study revealed groups of endoderm and mesoderm developmental genes whose expression were induced by Gata4 only when DNA methylation was lost, suggesting that DNA methylation restricts the ability of these genes to respond to Gata4, rather than controlling their transcription *per se*. Our data indicate that epigenetic regulation by DNA methylation functions as a heritable safeguard to prevent transcription factors from activating inappropriate downstream genes, thereby restricting the differentiation potential of somatic cells.



Masaki OKANO Ph.D.

<http://www.cdb.riken.go.jp/en/okano>

Masaki Okano received his baccalaureate and master's degrees from Kyoto University, and his doctorate from the Research Division in Agriculture at the same institution in 1994. He spent the period from 1994 to 1995 as a research fellow in cell biology at the Nagoya University BioScience Center before moving to Massachusetts, where he worked as a research fellow in medicine at Harvard Medical School and Massachusetts General Hospital from 1995 to 2002. He was appointed team leader at the RIKEN Center for Developmental Biology in 2001, and returned to work in Japan full-time in 2002. In 2012, he served as a senior investigator at the CDB.

Staff

Senior Investigator

Masaki OKANO

Research Scientist

Takashi TAMURA

Technical Staff

Chisa MATSUOKA

Recent Publications

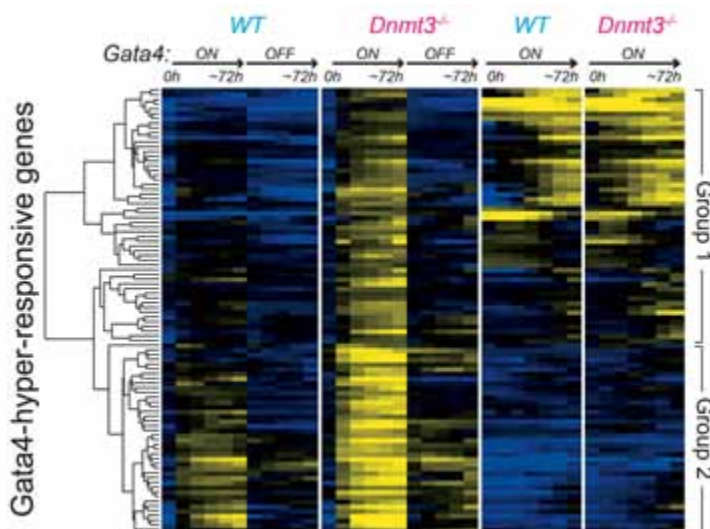
Oda M, et al. DNA methylation restricts lineage-specific functions of transcription factor Gata4 during embryonic stem cell differentiation. *PLoS Genet* 9:e1003574 (2013)

Kubiura M, et al. Chromosome-wide regulation of euchromatin-specific 5mC to 5hmC conversion in mouse ES cells and female human somatic cells. *Chromosome Res* 20.837-48 (2012)

Ehara T, et al. Role of DNA methylation in the regulation of lipogenic glycerol-3-phosphate acyltransferase 1 gene expression in the mouse neonatal liver. *Diabetes* 61.2442-50 (2012)

Sakaue M, et al. DNA methylation is dispensable for the growth and survival of the extraembryonic lineages. *Curr Biol* 20.1452-7 (2010)

Mesoderm ES



Gene expression changes (blue, low; yellow, high) in wild-type or *Dnmt3a*^{-/-}*Dnmt3b*^{-/-} cells in response to stimulation with transcription factor Gata4. The mesoderm cells deficient for DNA methylation are hypersensitive to the transcription factor-stimulation.

Organogenesis and Neurogenesis



Yoshiki SASAI M.D., Ph.D.

<http://www.cdb.riken.jp/en/sasai>

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*.

Staff

Group Director

Yoshiki SASAI

Research Specialist

Keiko MUGURUMA

Senior Scientist

Hidehiko INOMATA

Research Scientist

Satoru OKUDA

Nozomu TAKATA

Momoko WATANABE

Foreign Postdoctoral

Researcher
Nicholas Robert LOVE

Visiting Scientist

Satoshi ANDO

Atsushi KUWAHARA

Makoto NASU

Morio UENO

Technical Staff

Masayo FUJIIWARA

Tomoko HARAGUCHI

Masako KAWADA

Ayaka NISHIYAMA

Mika SOEN

Rieko YAKURA-NISHIZAWA

Junior Research Associate

Hideya SAKAGUCHI

Student Trainee

Taisuke KADOSHIMA

Atsushi SHIRAIISHI

Kazuhiro TAKASHIMA

Part-Time Staff

Masako SUZUKI

Assistant

Mako MIYAGI-INOMATA

Masayo OKUGAWA

Ayumi TANAKA-IKEYA

Fumi WAGAI

Recent Publications

Inomata H, et al. Scaling of Dorsal-Ventral Patterning by Embryo Size-Dependent Degradation of Spemann's Organizer Signals. *Cell* 153.1296-1311 (2013)

Suga H, et al. Self-formation of functional adeno-hypophysis in three-dimensional culture *Nature* 480.57-62 (2011)

Eiraku M, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 472.51-56 (2011)

Kamiya D, et al. Intrinsic transition of ES cell differentiation into neural progenitors. *Nature* 470.503-509 (2011)

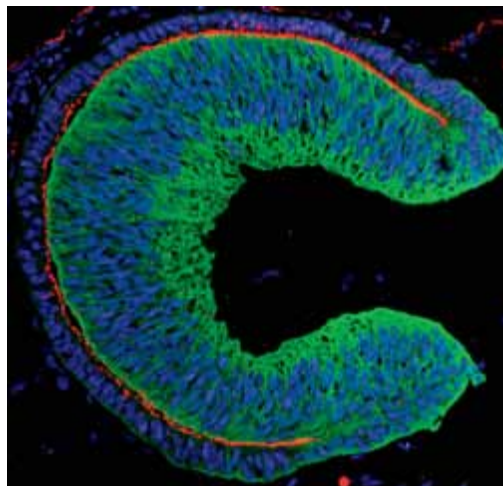
Muguruma K, et al. Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. *Nat Neurosci* 13.1171-80 (2010)

The complexity of the fully formed brain defies description, yet this organ arises from a non-descript clump of cells in the embryo. The specification of the dorsal-ventral 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.

Our 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 application in the treatment of neurodegenerative disorders, such as Parkinson's disease. Using a system developed in our lab, we have succeeded in inducing mouse and human embryonic stem (ES) cells to differentiate into a range of specialized neuronal types, including those of the cerebral cortex, cerebellar cortex and retina.

By studying very early neurogenesis, and the mechanisms of neuronal differentiation, our lab aims to understand the molecular basis underpinning the formation of so intricate a system as the mammalian neural network. In addition, we seek to understand the principles underlying the shapes of organs, and are currently studying self-organization of cortex, retina, and adenohypophysis using three-dimensional ES cell culture.



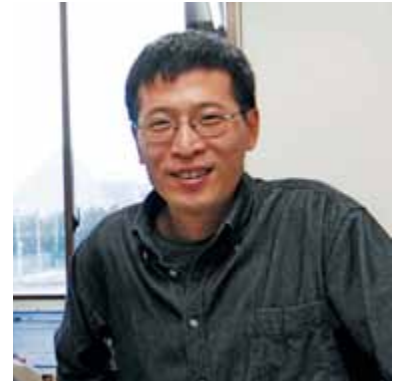
Self-organized formation of optic cup from human ES cells

Early Embryogenesis

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.



Guojun SHENG Ph.D.

<http://www.cdb.riken.go.jp/en/sheng>

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.

Staff

Team Leader

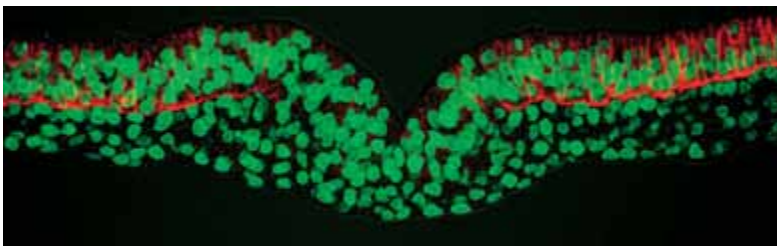
Guojun SHENG
Research Scientist
Cantas ALEV
Yukiko NAKAYA
Wei WENG

Technical Staff

Hiroki NAGAI
Kanao OTA
Eriko Widyasari SUKOWATI
YuPing WU



An amniote-like, circumblastoporal ring of mesoderm precursors (Blue: *Brachyury*) is induced by subgerminal cavity injection of FGFs, suggesting that the primitive streak is not an essential component of mesoderm formation in amniotes.



This is a cross-section of the primitive streak. Epiblast cells in the middle of the streak are undergoing EMT. Red: beta-Dystroglycan (basolaterally localized in lateral epiblast cells and downregulated in medial epiblast cells); Green: nuclei.

Recent Publications

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

Nakaya Y, et al. Epiblast integrity requires CLASP and Dystroglycan-mediated microtubule anchoring to the basal cortex. *J Cell Biol* 202.637-51 (2013).

Alev C, et al. Decoupling of amniote gastrulation and streak formation reveals a morphogenetic unity in vertebrate mesoderm induction. *Development* 140.2691-6 (2013)

Alev C, et al. Transcriptomic landscape of the primitive streak. *Development* 137.2863-74 (2010)

Shin M, et al. Notch mediates Wnt and BMP signals in the early separation of smooth muscle progenitors and blood/endothelial common progenitors. *Development* 136.595-603 (2009)

Nakaya Y, et al. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nature Cell Biol* 10.765-775 (2008)

Physical Biology



Tatsuo SHIBATA Ph.D.

<http://www.cdb.riken.go.jp/en/shibata>

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.

Staff

Research Unit Leader Tatsuo SHIBATA	Technical Staff Satoru IWATANI Yuko WADA
Research Scientist Akinori BABA Naotoshi NAKAMURA Katsuhiko SATO	Junior Research Associate Prabhat SHANKAR
Foreign Postdoctoral Researcher Marcel HOERNING	Part-Time Staff Toshinori NAMBA
Visiting Researcher Seirin LEE	Assistant Satoko KINOSHITA

Recent Publications

Shibata T, et al. Intracellular encoding of spatiotemporal guidance cues in a self-organizing signaling system for chemotaxis in dictyostelium cells. *Biophys J* 105.2199-209 (2013)

Hiraiwa T, et al. Theoretical model for cell migration with gradient sensing and shape deformation. *Eur Phys J E Soft Matter* 36.9846 (2013)

Baba A, et al. Directional sensing of deformed cells under faint gradients. *Phys. Rev. E* 86.060901(F) (2012)

Shibata T, et al. Modeling the self-organized phosphatidylinositol lipids signaling system in chemotactic cells using quantitative image analysis. *J Cell Sci* 125.5138-50 (2012)

Namba T, et al. The relation of signal transduction to the sensitivity and dynamic range of bacterial chemotaxis. *Biophys J* 103.1390-9 (2012)

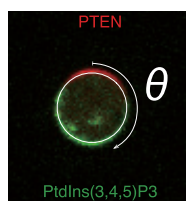
Arai Y, et al. Self-organization of the phosphatidylinositol lipid signaling system for random cell migration. *Proc Natl Acad Sci U S A* 107.12399-404 (2010)

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

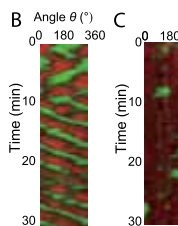


$$\frac{\partial [PIP3]}{\partial t} = -V_{PTEN}[PTEN] \frac{[PIP3]}{K_{PIP3} + [PIP3]} + V_{PI3K} \frac{[PIP2]}{K_{PI3K} + [PIP2]} - \lambda_{PIP3}[PIP3] + DV^2[PIP3]$$

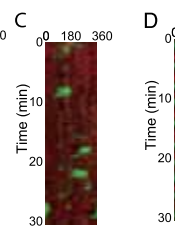
$$\frac{\partial [PIP2]}{\partial t} = V_{PTEN}[PTEN] \frac{[PIP3]}{K_{PIP3} + [PIP3]} - V_{PI3K} \frac{[PIP2]}{K_{PI3K} + [PIP2]} + k - \lambda_{PIP2}[PIP2] + DV^2[PIP2]$$

$$\frac{\partial [PTEN]}{\partial t} = V_{in} \left([PTEN]_{total} - \chi \frac{1}{L} f([PTEN]) \right) \frac{K_{PIP2}}{K_{PIP2} + [PIP2]} - \lambda_{PTEN}[PTEN] + D_{PTEN} \nabla^2 [PTEN]$$

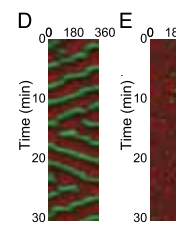
B



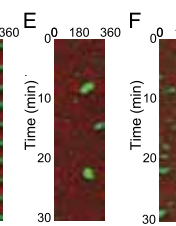
C



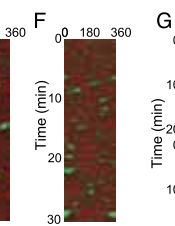
D



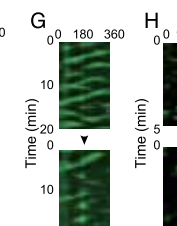
E



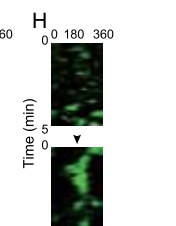
F



G



H



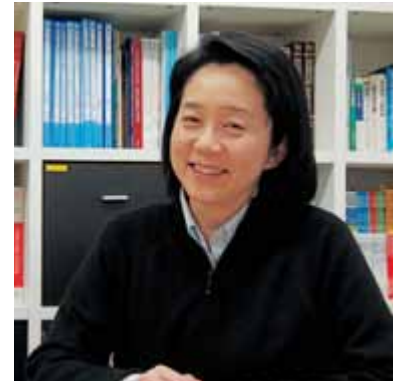
(A) Spontaneous asymmetry formation of phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3, green) and PTEN (red), and an explanatory theoretical model. (B, C) Spatiotemporal diagrams of the membrane distributions. (D-F) Stochastic numerical simulations of the theoretical model. (G-H) Experiment of gradient response in PtdIns(3,4,5)P3 (green) (Shibata, et al. 2012, 2013).

Retinal Regeneration

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.



Masayo TAKAHASHI M.D., Ph.D.

<http://www.cdb.riken.go.jp/en/takahashi>

Masayo Takahashi received her M.D. from Kyoto University in 1986, and her Ph.D. from the same institution in 1992. After serving as assistant professor in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1995, where she discovered the potential of stem cells as a tool for retinal therapy. She returned to Kyoto University Hospital in 1997, and since 2001 served as an associate professor at the Translational Research Center in the Kyoto University 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.

Staff

Project Leader

Masayo TAKAHASHI

Deputy Project Leader

Michiko MANDAI

Sunao SUGITA

Research Scientist

Hiroshi AKIMARU

Jun KANEKO

Naoshi KOIDE

Chikako MORINAGA

Akishi ONISHI

Akiko SUGA

Research Associate

Chie ISHIGAMI

Akihiro TACHIBANA

Technical Staff

Momo FUJII

Kanako KAWAI

Tomoyo HASHIGUCHI

Naoko HAYASHI

Shino HIRAHARA

Yukako HIRAO

Ayumi HONO

Kyoko ISEKI

Hiromi ITO

Miwa OKADA

Noriko SAKAI

Yumiko SHIBATA

Hitomi TAKAKUBO

Motoki TERADA

Chikako YAMADA

Ryo YOSHIDA

Part-Time Staff

Tomoko KATAYAMA

Ryoko TAKEDA

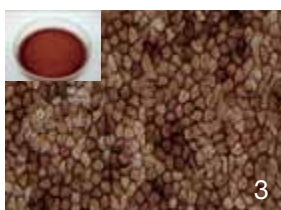
Tomoko YOKOTA



Human iPS cells



Retinal pigment epithelial cells differentiated from iPS cells



Purified retinal pigment epithelial cells



Photoreceptor cells differentiated from iPS cells

Recent Publications

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)

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

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

Osakada F, et al. Wnt signaling promotes regeneration in the retina of adult mammals. *J Neurosci* 27. 4210-9 (2007)

Ooto S, et al. Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. *Proc Natl Acad Sci USA* 101. 13654-9 (2004)

Haruta M, et al. In vitro and in vivo characterization of pigment epithelial cells differentiated from primate embryonic stem cells. *Invest Ophthalmol Vis Sci* 45. 1020-5 (2004)

Cell Adhesion and Tissue Patterning



Masatoshi TAKEICHI Ph.D.

<http://www.cdb.riken.go.jp/en/takeichi>

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. He assumed the directorship of the CDB in 2000.

Staff

Group Director

Masatoshi TAKEICHI

Research Scientist

Shuichi HAYASHI

Shoko ITO

Shigenori NAGAE

Anna PLATEK

Nobutoshi TANAKA

Mika TOYA

Vassil VASSILEV

Collaborative Scientist

Wenxiang MENG

Shoko NAKAMURA

Tamako NISHIMURA

Technical Staff

Sylvain HIVER

Yoko INOUE

Saeko KOBAYASHI

Miwako NOMURA

Hiroko SAITO

Part-Time Staff

Haruka MORISHITA

Assistant

Mutsuko AISO-WATANABE

Recent Publications

Tanaka N, et al. Nezha/CAMSAP3 and CAMSAP2 cooperate in epithelial-specific organization of non-centrosomal microtubules. *Proc. Natl. Acad. Sci. USA* 109.20029-34 (2012)

Nishimura T, et al. Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell* 149.1084-97 (2012)

Ishiyoshi T. and Takeichi M. Willin and Par3 cooperatively regulate epithelial apical constriction through aPKC-mediated ROCK phosphorylation. *Nat Cell Biol* 13.860-6 (2011)

Taguchi K, et al. Mechanosensitive EPLIN-dependent remodeling of adherens junctions regulates epithelial reshaping. *J Cell Biol* 194.643-56 (2011)

Ishiyoshi T, et al. Mammalian Fat and Dachshous cadherins regulate apical membrane organization in the embryonic cerebral cortex. *J Cell Biol* 185.959-67 (2009)

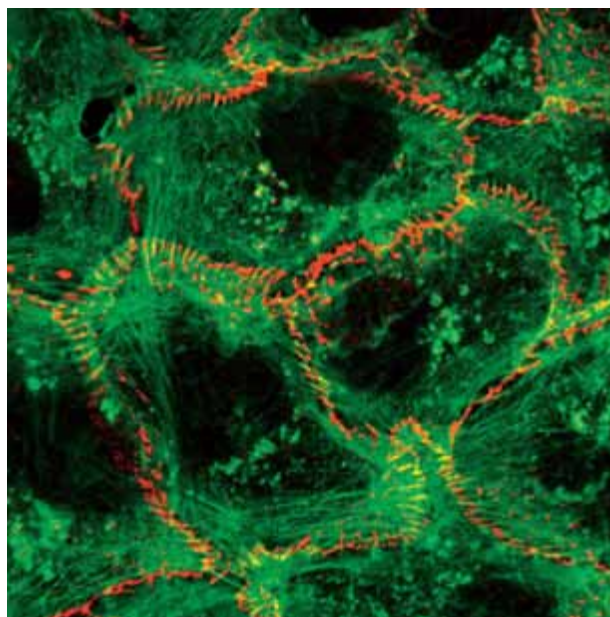
Meng W, et al. Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. *Cell* 135.948-59 (2008)

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.



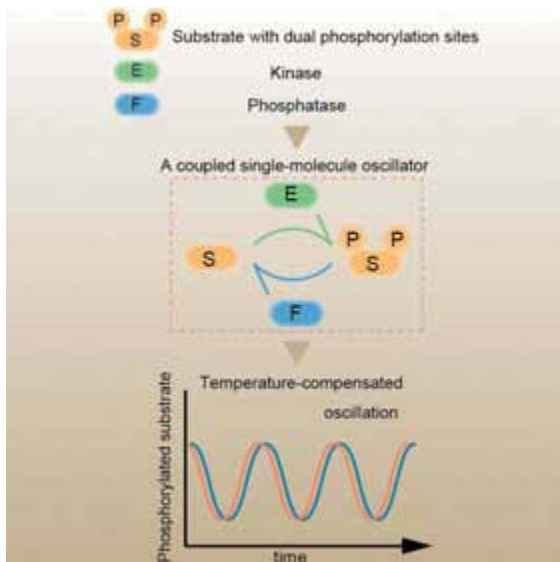
Double-immunostaining for F-actin (green) and Kusabira Orange-tagged E-cadherin (red) introduced into A431D cells. In these cells, E-cadherin dynamically moves along cortical actin filaments, resulting in the unique distributions shown here.

Systems Biology

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.



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

<http://www.cdb.riken.go.jp/en/ueda>

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.

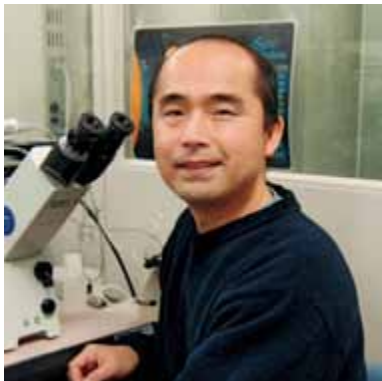
Staff

Project Leader Hiroki R. UEDA	Visiting Researcher Arthur MILLIUS
Research Scientist Yohei M. KOYAMA Yasutaka NIWA Genshiro SUNAGAWA Hideki UKAI Rikuhiko G. YAMADA	Technical Staff Hiroshi FUJISHIMA Junko HARA Naoshi KOIDE Kaori TSUJINO Maki UKAI-TADENUMA Junko YOSHIDA
Special Postdoctoral Researcher Hiroki DANNO Etsuo A. SUSAKI	Student Trainee Genki KANDA Hiroyuki TAMIYA
Foreign Postdoctoral Researcher Craig C. JOLLEY Dimitri PERRIN	Visiting Scientist Kohei MASUMOTO Atsushi WADA Assistant Noriko OKAMOTO

Recent Publications

- Tsujino K, et al. Establishment of TSH β real-time monitoring system in mammalian photoperiodism. *Genes Cells* 18:575-88 (2013)
- Sunagawa G. A, et al. FASTER: an unsupervised fully automated sleep staging method for mice. *Genes Cells* 18: 502-18 (2013)
- Jolley C. C, et al. A design principle for a posttranslational biochemical oscillator. *Cell Rep* 2: 938-50 (2012)
- Ukai-Tadenuma M, et al. Delay in feedback repression by cryptochrome 1 is required for circadian clock function. *Cell* 144: 268-81 (2011)
- Masumoto K. H, et al. Acute induction of Eya3 by late-night light stimulation triggers TSH β expression in photoperiodism. *Curr Biol* 20: 2199-206 (2010)
- Isajima Y, et al. CKIepsilon/delta-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc Natl Acad Sci USA* 106: 15744-9 (2009)

Genomic Reprogramming



Teruhiko WAKAYAMA Ph.D.

<http://www.cdb.riken.go.jp/en/wakayama>

Teruhiko Wakayama received his B.Sc. and M.Sc. from Ibaraki University, and was awarded a Ph.D. in reproductive biology from the University of Tokyo Department of Veterinary Anatomy in 1996. He received a postdoctoral fellowship from the Japanese Society for the Promotion of Science in 1996 and spent the next two years at the Yanagimachi lab in the University of Hawaii Medical School, where he succeeded in creating the world's first cloned mouse. He was appointed to an assistant professorship at the same institution in 1998, and moved to the Rockefeller University as a research assistant professor in 1999. He spent a year as a researcher at Advanced Cell Technology before returning to Japan to take his current position at the RIKEN CDB.

Staff

Team Leader

Teruhiko WAKAYAMA

Research Scientist

Sayaka WAKAYAMA

Visiting Scientist

Satoshi KISHIGAMI

Koji KOJIMA

Haruko OBOKATA

Mikiko TOKORO

Kazuo YAMAGATA

Student Trainee

Hirohisa KYOGOKU

Yukari TERASHITA

Technical Staff

Yuko SAKAIDE

Kaori YAMANAKA

Assistant

Sakura HIRAUCHI

Recent Publications

Wakayama S, et al. Successful Serial Recloning in the Mouse over Multiple Generations. *Cell Stem Cell* 12:293-297 (2013) (Cover article of issue)

Terashita Y, et al. Latrunculin A treatment prevents abnormal chromosome segregation for successful development of cloned embryos. *PLoS One* 8. e78380 (2013)

Terashita Y, et al. Latrunculin A Can Improve the Birth Rate of Cloned Mice and Simplify the Nuclear Transfer Protocol by Gently Inhibiting Actin Polymerization. *Biol Reprod*.86:180 1-6 (2012)

Yamagata K, et al. Fluorescence cell imaging and manipulation using conventional halogen lamp microscopy. *PLoS One* 7.e31638 (2012)

Mizutani E, et al. Abnormal chromosome segregation at early cleavage is a major cause of the full-term developmental failure of mouse clones. *Dev Biol* 364.56-65 (2012)

A theoretically limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual cells and their nuclei, that fiction has become reality. The efficiency of the cloning procedure (measured as the ratio of live offspring to cloning attempts), however, remains quite low, at only a few percent, and even in those attempts that do lead to live births, the cloned animals consistently exhibit a range of severe developmental abnormalities and pathologies.

The cause of these defects is thought to be due to imperfections in the nuclear transfer technique used to remove the nucleus from a host cell and replace it with another from a donor; imperfections which presumably lead to total or partial failure of reprogramming, the process in which a cell's developmental potential is reset to a totipotent state (such as is exhibited by a naturally fertilized egg). However, the specific details of the technical flaws, the ways in which they lead to reprogramming failure, and possible solutions to the problem all remain completely unknown. Despite these challenges, cloning continues to be a biotechnology of great promise, given its potential applications in the field of regenerative medicine, such as the generation of embryonic stem (ES) cells by nuclear transfer, which may one day allow us to grow replacement cells that perfectly match a patient's own genetic and immune profile, potentially eliminating the risk of rejection.

We use the mouse as a model system to study cloning under a range of experimental conditions with the goals of achieving improvements in the efficiency of the cloning procedure, gaining a better understanding of reprogramming mechanisms and analyzing the properties of ES cells derived via somatic nuclear transfer. We also use nuclear transfer technology to develop methods for preserving embryonic lethal and infertile strains of laboratory mice, and continue to explore the development of new and better techniques for sperm and oocyte preservation and other reproductive biological research technologies.



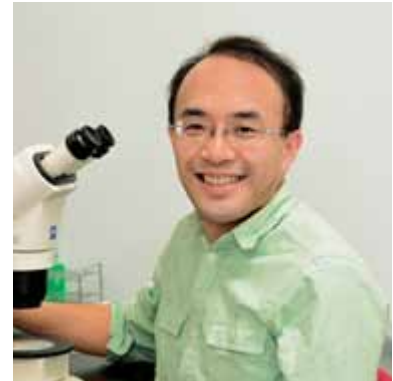
Transfer of a somatic nucleus into an enucleated egg

Epithelial Morphogenesis

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.



Yu-Chiun WANG Ph.D.

<http://www.cdb.riken.go.jp/en/wang>

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

Staff

Team Leader	Assistant
Yu-Chiun WANG	Yuko FUJUYAMA
Research Scientist	
Niraj K. NIRALA	
Technical Staff	
Mustafa SAMI	
Michiko TAKEDA	

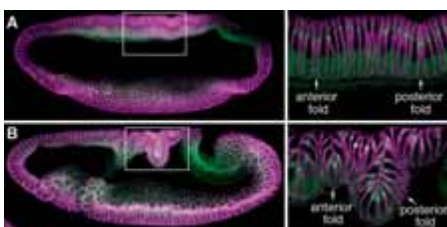
Recent Publications

Wang Y. C, et al. Distinct Rap1 activity states control the extent of epithelial invagination via alpha-catenin. *Dev Cell* 25.299-309 (2013)

Wang Y. C, et al. Differential positioning of adherens junctions is associated with initiation of epithelial folding. *Nature* 484.390-3 (2012)

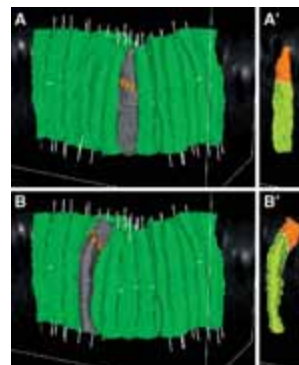
Wang Y. C, and Ferguson E. L. Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. *Nature* 434.229-34 (2005)

Podos S. D, et al. The DSmurf ubiquitin-protein ligase restricts BMP signaling spatially and temporally during *Drosophila* embryogenesis. *Dev Cell* 1.567-78 (2001)



The formation of dorsal folds during *Drosophila* gastrulation.

The dorsal epithelium of the gastrulating *Drosophila* embryo forms two epithelial (anterior and posterior) folds. These pictures show mid-sagittal optical sections of an early (A) and a late (B) embryos that display the initiation (A) and final morphology (B) of these dorsal folds. The highlighted areas are shown on the right panels. 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) to perform 3D reconstruction of cell shape based on cell membrane labeling. Immunolabeling of adherens junction (orange in A and B) defines the boundaries between the apical and basal-lateral compartments (A' and B').

Sox2 sits atop different transcriptional networks in ESC and TSC self-renewal



Kenjiro ADACHI

The first unmistakable differentiation events in mammalian development take place just as the blastocyst is forming, with the outer layer of cells committing to form the extra-embryonic trophoblast, and the inner cell mass giving rise to the earliest embryonic lineages. When grown in culture, these inner cell mass cells are known as embryonic stem cells (ESCs), and show the ability, known as pluripotency, to give rise to every embryonic lineage, as well as the ability to proliferate indefinitely by self-renewal in vitro. Trophoblast likewise arises from trophoblast stem cells (TSCs), which form the embryonic component of the placenta. The genetic regulation of such 'stemness' in ESC, TSC and neural stem cells relies in part on the function of the transcription factor Sox2, but how the function of this protein functions differs across these cell types has never been resolved.

A new in-depth study by Kenjiro Adachi and colleagues in the Lab for Pluripotent Stem Cell Studies (Hitoshi Niwa, Project Leader) reveals that Sox2 sits atop two different networks of stemness genes in mouse ESCs and TSCs. Conducted in collaboration with the CDB labs for Genomic Reprogramming, Functional Genomics, Systems Biology and Genome Resources and Analysis, this work was published in *Molecular Cell*.

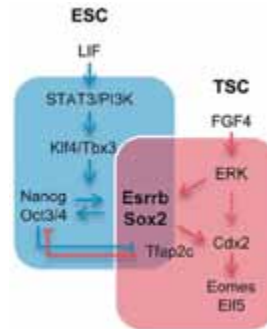
Previous work by the Niwa lab had shown that deletion of Oct4, a known pluripotency gene, caused ESCs to transform into TSCs, which are known to rely on the activity of FGF4. In the present study, Adachi and colleagues examined changes in gene expression during this transition, and determined that in TSCs, FGF4 activates Sox2 and a second gene, Esrrb. They found that by misexpressing these genes in TSCs, they could maintain their stemness even in the absence of FGF4. In contrast, in mouse ESCs, Sox2 is under the control of LIF, indicating to the team that Sox2 might interact with different sets of genes to maintain stemness and inhibit differentiation in trophoblast and embryonic stem cells.

What then is responsible for the switch in Sox2 targets? Adachi et al. next looked at Sox2 DNA and protein binding, and discovered something interesting: Sox2 does not act alone, but functions by binding with other transcription fac-

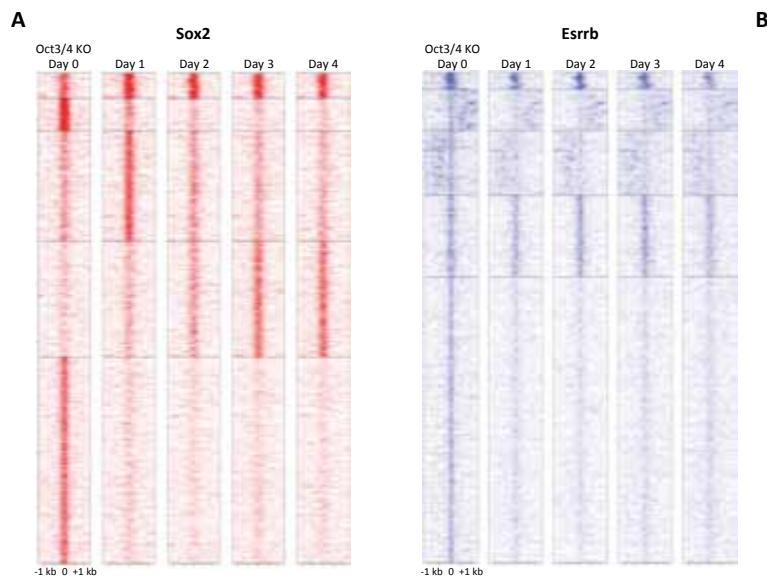


tors in a cell context-dependent fashion. While it binds Oct4 in ESCs, in TSCs it partners with Tfap2c; these changes in binding partner trigger conformational changes that enable interactions with independent networks of target genes in these stem cell types.

“The idea that combinations of the activity of various signaling pathways helps to give rise to cellular diversity is widely accepted, but it is generally thought that the factors involved and the order of activation within a pathway are fairly uniform,” says Niwa. “But what we have shown here is that changes in upstream and downstream factors can play significant roles in driving cell diversity, and we suspect perhaps evolutionary processes as well.”

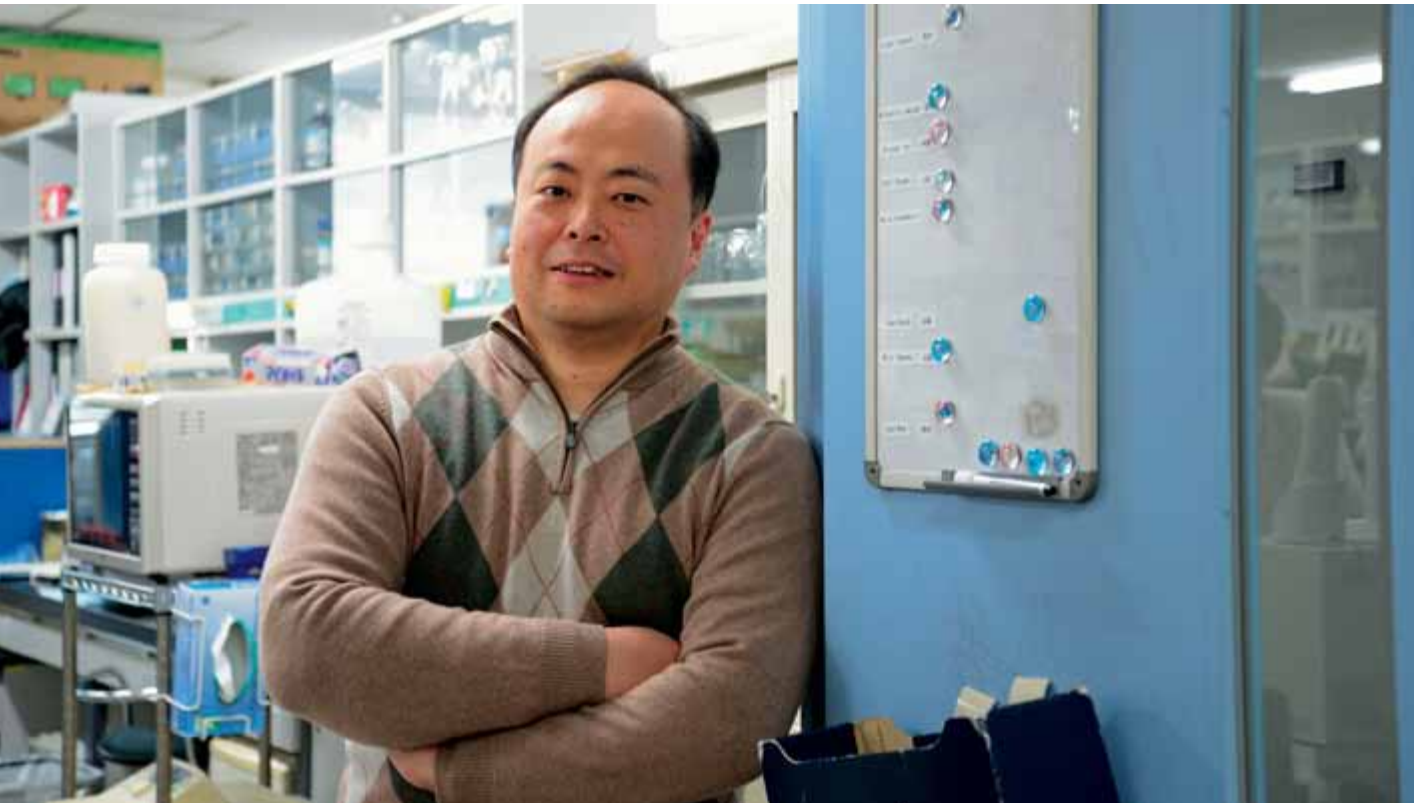


A schematic of genetic networks regulating stemness in early embryonic cells. In mouse ESCs, Sox2 is controlled by LIF, and in TSCs by FGF4. The switch of Sox2’s binding partner from Oct4 to Tfap2c changes the set of downstream genetic interactions, inducing a transition from ESC to TSC state. In this scheme, Oct4 and Tfap2c are mutually inhibitory.



Heatmaps of Sox2 and Esrrb ChIP-seq signals around peak regions. Sox2 binding sites changed dynamically after elimination of *Oct3/4*; most of the peaks were found specifically in ESCs or TSCs. In contrast, Esrrb binding showed less dynamic behavior, with only persistent or ESC-specific peaks apparent.

GDNF levels control neuronal migration and differentiation in the ENS



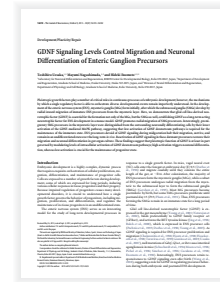
Toshihiro UESAKA

The neurons of the enteric nervous system undergo among one of the longest journeys in development, as precursors migrate downward and proliferate to innervate the digestive tract from end to end. This process involves two main stages: primary caudal migration by neural crest-derived precursors, and a second round of radial migration of a subset of precursors that radiate to the submucosal layer. While precursors in the myenteric ganglia (MG) typically leave mitosis by the time of birth, many submucosal ganglia (SMG) precursors remain active for two weeks thereafter, suggesting that these cells at least in part remain in an incompletely differentiated state. The molecular controls over this exquisitely coordinated activity, however, have remained elusive.

Toshihiro Uesaka and colleagues in the Lab for Neuronal Differentiation and Regeneration (Hideki Enomoto, Team

Leader) now reveal that GDNF signaling is an essential regulator of both the primary and secondary migration of ENS precursors in mouse. Published in *The Journal of Neuroscience*, these new findings also show that intracellular signaling levels controlled by GDNF regulate differentiation states in these precursors as well.

At embryonic day 9.5 in mouse embryonic development, neural crest-derived cells begin to enter the foregut and migrate down the enteric tract toward the colon, completing this primary migration by embryonic day 14. Most of this first wave of migrants differentiates to form myenteric ganglia, but a subpopulation secondarily migrates radially into the submucosal layer, giving rise to submucosal ganglia. GDNF is expressed in the gut mesenchyme, and activates the RET tyrosine kinase through binding with the receptor



GFR α 1. All of these factors are essential for proper ENS development, as evidenced by the loss of enteric neurons in mice lacking any of these genes. Previous work by the Enomoto lab has shown that enteric precursors are receptive to GDNF signaling into the postnatal period, suggesting that this factor plays a long-term central role in controlling multiple events during ENS development.

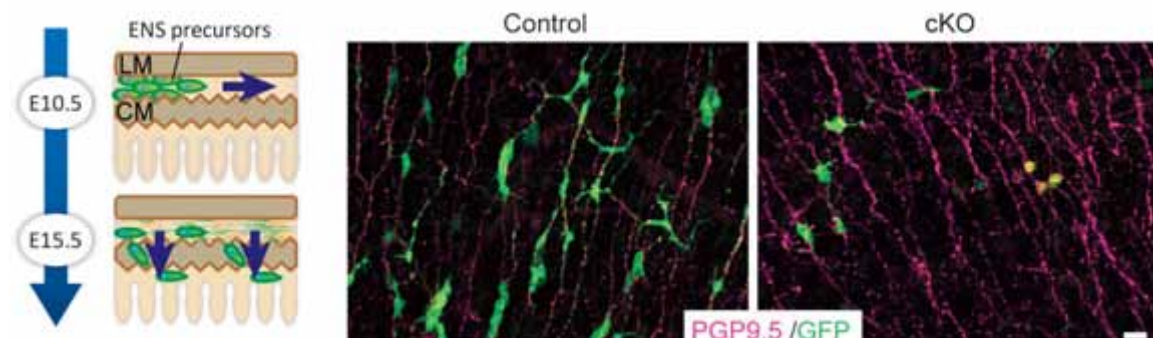
Uesaka used a number of conditional knockout mouse lines to gain a more detailed understanding of GDNF's function. When he examined GFR α 1 expression, he found that in both myenteric and submucosal ganglia, GFR α 1⁺ cells (i.e., those carrying receptors for GDNF) also expressed Sox10, a marker of undifferentiated cells. Knocking out GFR α 1 at day 10.5, he found that mutant precursor cells lost most of the migratory activity seen in control ENS precursors. Knockout of the same gene at day 15.5, during secondary migration, caused a near-complete failure of submucosal ganglia formation. These results indicated that not only does GDNF signaling play important roles throughout ENS development, but that its activity changes over time.

Looking at temporal changes in GDNF expression, the team found that while GDNF was highly expressed in both the longitudinal and circular myenteric layers, by day 18.5, when MG formation was complete, its expression was con-

finned mainly to the circular muscle layer, highlighting the possibility that this shift in the site of GDNF expression might be responsible for inducing secondary migration.

The formation of submucosal ganglia subsequent to MG formation indicates that even during their differentiation, the MG population must retain some undifferentiated cells. Here again, the team found that differential GDNF expression plays a role, as shown by the higher levels of a GDNF downstream factor in differentiated enteric neurons, and lower levels in undifferentiated precursors at day 12.5. Uesaka tested this hypothetical role for GDNF signaling in controlling cell state by knocking out RET, which interrupts GDNF signaling. Whereas most precursors would have differentiated into neurons within a few days, he found that in the RET knockout many remained undifferentiated. Recovery of GDNF signaling rescued the defects in migration and differentiation, sealing the case for its broad functionality.

"This work presents a very reasonable system for the long-term maintenance of precursors in an undifferentiated state, and show why even seemingly minor changes in GDNF signaling receptors can lead to major developmental defects," says Enomoto. "At the same time, we know that many other factors are also involved in regulating ENS development, so we will be curious to learn more about these are orchestrated in the embryonic gut."



(Left) ENS precursors migrate down digestive tract at embryonic days 9.5–14 (primary migration) and then radiate inward from the myenteric layer to the submucosa (secondary migration). (Right) In a condition knockout in which GDNF signaling is blocked during secondary migration (E15.5), submucosal ganglia (green) nearly entirely fail to form. (Pink shows neuronal marker PGP9.5)

Spontaneous fluctuations in cells support chemotaxis



Tatsuo SHIBATA

Chemotaxis refers to movement by cells to or away from chemical stimuli in their environments. In the multicellular body, this process plays a central role in directing immune responses by white blood cells and the formation of nervous system tissues. For more primitive eukaryotes, such as the slime mold *Dictyostelium*, chemotaxis plays what is perhaps an even more fascinating role, guiding individual cells in food-poor environments to assemble into a multicellular aggregate, or 'slug,' capable of reproduction. The mechanism underlying this chemotaxis resembles that seen in mammalian immune cells, and allows tiny cells just a few micrometers in diameter to respond to minute differences of a few percent in the concentration of a chemical stimulus. However, cells do not need an excuse to move; even in the absence of a chemotactic signal, cells move in random direction under the control of internal fluctuations. The question then is, how do cells override these random internal signals so as to be able to detect, amplify, and respond to gradients of external stimuli?

In a report published in *Journal of Cell Science*, Research Unit Leader Tatsuo Shibata and colleagues in the Lab for Physical Biology used fluorescence imaging to reveal a mechanism for controlling the spontaneous polarization of *Dictyostelium* cells, from which they developed a quantitative model able to reconstruct the phenomenon. A second paper by the same lab, published in *Biophysical Journal*, reports a model of how this self-organizing mechanism robustly facilitates the exquisite sensitive of these cells to chemical stimuli. Both studies were conducted in collaboration with researchers from the RIKEN Quantitative Biology Center.

Dictyostelium cells respond chemotactically to gradients of cAMP by initiating inositol phospholipid metabolism at the cell surface. Cell surface regions presented with a high cAMP concentration begin to produce the phosphatidylinositol lipid PIP3 from PIP2, while regions facing lower concentrations elicit the opposite reaction, catalyzed by the enzyme PTEN. Regions of high PIP3 mobilize actin fiber formation to extend a pseudopod in the direction of the gradient, driving chemotaxis.

Shibata wondered, however, how would cells behave in environments lacking cAMP, and simultaneously prevented from generating cAMP themselves. He found that PIP3 domains appeared to move at random on the surface of such

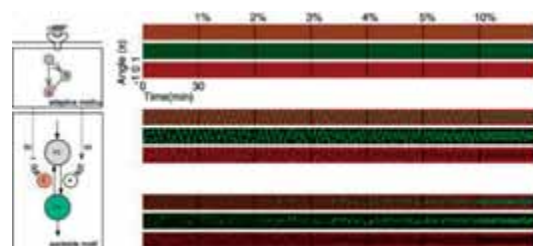
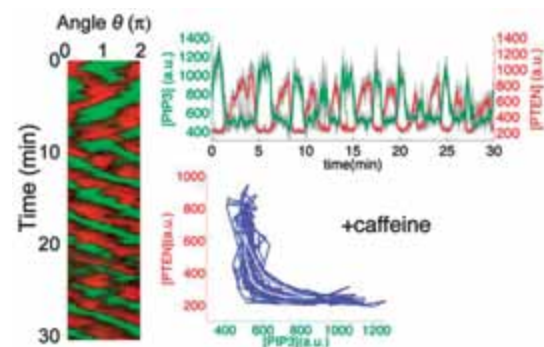
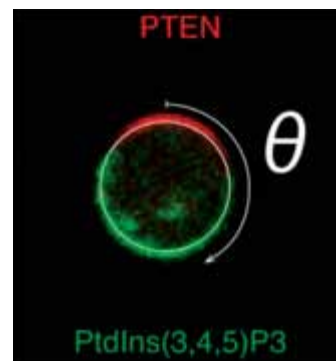


cells, indicating an internal mechanism for establishing polarity, ensuring that PIP3 and PTEN localized in a mutually exclusive fashion. Statistical analysis revealed that this activity could not be explained simply as the result of interactions between enzymes and substrates, but that rather PTEN seemed to be ‘attracted’ to areas in which PIP3 levels were low. Using a reaction–diffusion model to simulate this phenomenon, Shibata et al. were able to show how domains of persistent PIP3 expression, in agreement with their experimental observations. Adjusting the simulated parameters, he was able to evoke cycles of PIP3 oscillation, resembling the behavior of cells in which cAMP was not blocked. These validations of this quantitative model point to possible broader applications in the analysis of spontaneous polarization, such as seen in neurons, which can exhibit robust responses to even seemingly minor stimuli.

In the second study, the team looked at cells confronted with chemotactic stimuli. Using ordinary Dictyostelium cells, they observed that while some would manifest stable domains in response to cAMP, others showed transient domain patterns oriented toward higher cAMP concentrations. They next made a stochastic reaction–diffusion model of these two patterns, along with that of an inactive state absent the self-organized polarity formation, and tested how they would respond to stepwise increases in cAMP gradient steepness. The persistent and transient domain models responded much more strongly than did that of the inactive state, indicating that the self-organizing system plays a role in promoting the response to cAMP. They next turned to quantitatively analyzing PIP3 domain formation, and found that such domains formed more frequently in the presence of a stimulus and became distributed with increasing precision in the direction of the gradient as the chemotactic signal rose.

In following cAMP gradients, Dictyostelium cells need to move toward increases in concentration, and avoid pursuing lower levels of cAMP. Shibata suspected that this might be possible through the combined effect of the extracellular gradient, which introduces a small bias in the PIP3 domain induction on the side of the cell facing the higher concentration, and self-organizing activity which establishes front–back orientation. In line with his predictions, the model showed that the self-organizing system lies behind the robust, sensitive response by these cells to even shallow gradients.

“In these studies, we were able to construct quantitative models that closely recapitulate the results of experiments using known molecules observed by fluorescence imaging,” says Shibata. “These models have led to new insights into molecular mechanisms and gradient sensing, which we next hope to validate using live cells. We are also hopeful that the self-organizing activity we have described will play a role in the explication of other biological functions.”



(Top) Self-organization of polarity formation in Dictyostelium cells; PIP3 shown in green, PTEN in red. (Center left) Kymograph of PIP3 and PTEN along cell surface. (Center right) Alternating cell surface concentrations of PIP3 and PTEN. (Bottom) Simulation of mathematical model in three cases: No self-organization activity, persistent domain formation, or transient domain formation.

The case of the missing nasopalatine: The maxillary nerve in jaw evolution



Shigeru KURATANI, Hiroki HIGASHIYAMA

As its name implies, the trigeminal nerve has three major branches in vertebrates: a first division into the ophthalmic nerve and the maxillomandibular, which further bifurcates into the maxillary and mandibular nerves. The trigeminal governs sensation and muscle control in the face and jaw. In humans, it develops from a primordium just above the pharyngeal arches, from which the ophthalmic nerve diverges and extends toward the eye, and the maxillary and mandibular nerves toward their respective prominences. The structure of the jaw is conserved from fish to mammals, so the how its innervation patterns were acquired during evolution are a question of fundamental importance.

In a comparative study of trigeminal development in diverse gnathostome taxa published in *Journal of Morphology*, Hiroki Higashiyama and Group Director Shigeru Kuratani of the Lab for Evolutionary Morphology have discovered that a branch of the trigeminal (called the nasopalatine nerve in human) innervates derivatives of the medial nasal prominence. Only diapsids, such as gecko and chicken, lacked a homolog of this nerve, suggesting it had been secondarily lost in this lineage.

Textbook accounts of maxillary nerve development typically state that it is localized to the maxillary prominence and goes on to innervate the entire upper jaw. But recent embryological studies have shown that the upper jaw arises not only from the maxillary prominence, but rather forms from the fusion of this primordial structure with the medial nasal domain, suggesting an apparent discrepancy between neural morphology and developmental pattern. The medial nasal prominence is the site of origin for the upper lip, anterior palate, and front teeth, and gives rise to the upper jaw (maxilla) by merging with the maxillary prominence. But despite the region's origins in a fusion with the medial nasal prominence, to which the maxillary nerve does not localize, the maxillary nerve appears to control the entire maxillary region.

Interested in this conundrum, Higashiyama examined the distribution of trigeminal nerve branches in mouse. At day E10.5, he noted the clear projection of the maxillary nerve to the maxillary prominence, but by day E11.5 he saw that this nerve had begun to ramify rostrally. This branch, cor-

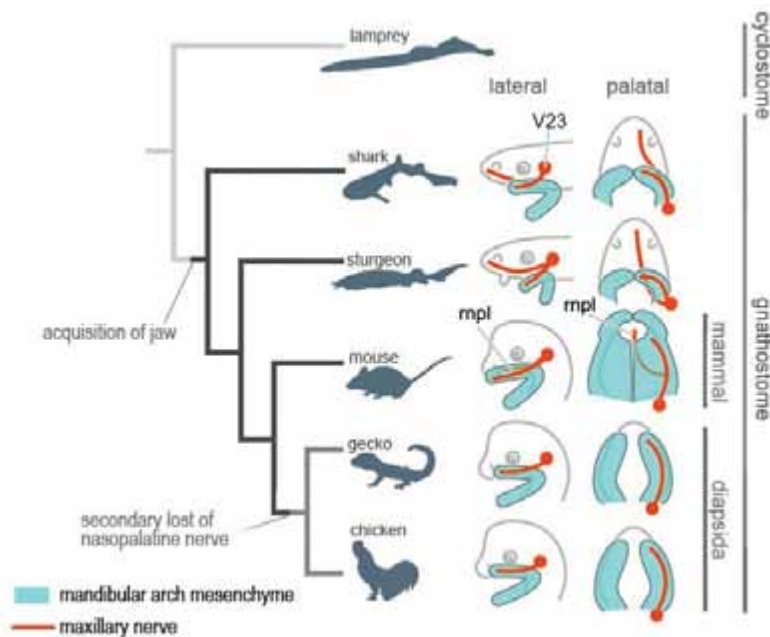


responding to the human nasopalatine nerve, follows the nasal septum to innervate the front teeth and soft palate, indicating that it is the nasopalatine that controls the medial nasal region in mammals. But what of other taxa? When Higashiyama looked at relatively closely related diapsids, such as reptiles and birds, he was surprised to find that the nasopalatine was missing, with its function assumed by posterior facial nerves.

This raised the question of which state was ancestral, and which derived. Higashiyama broadened his search to amniotes, such as cartilaginous and bony fishes, and were happy to see that the maxillary and medial nasal prominences did not fuse, considerably simplifying the investigation. In these embryos, the maxillary nerve formed two branches, extending into both the maxillary and medial nasal territories, suggesting that the arrangement seen in

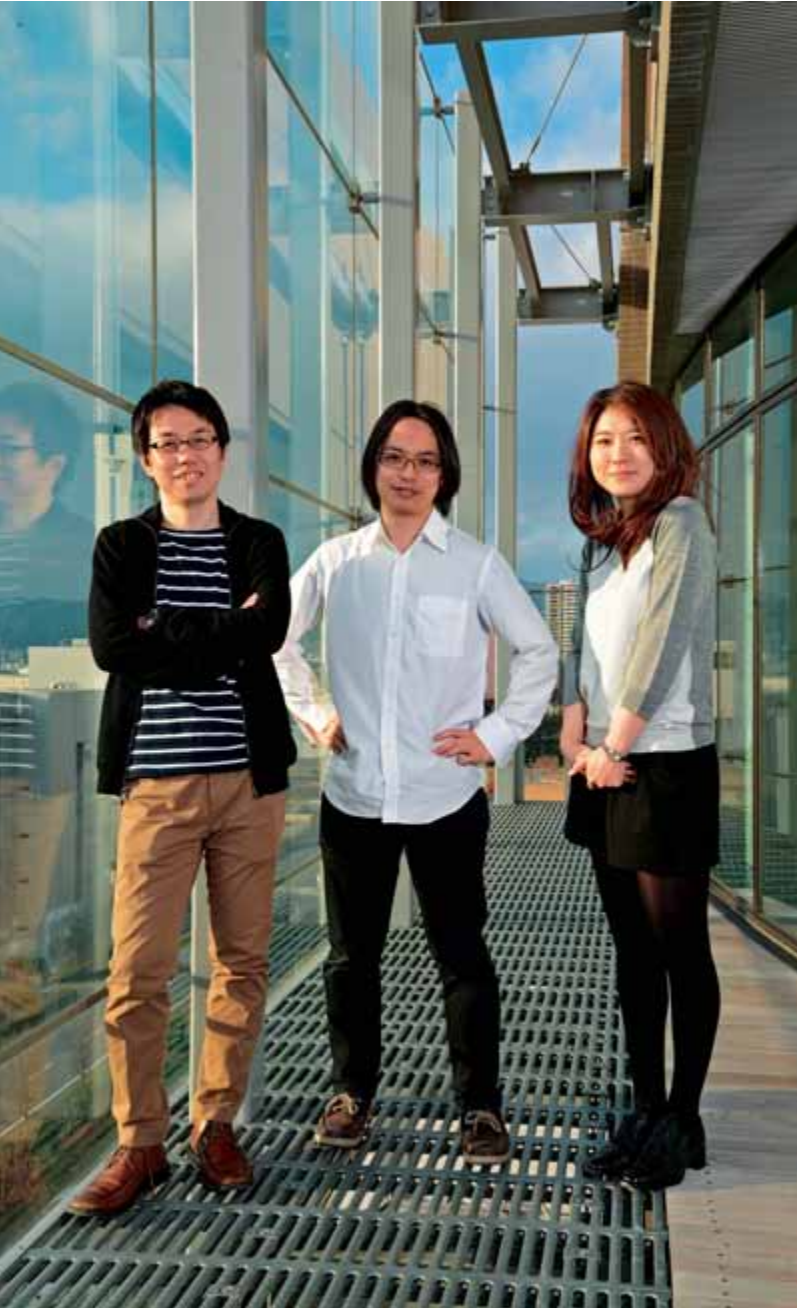
mammals is common to all gnathostomes, and only secondarily absent in diapsids. When he looked at the lamprey, a jawless fish, Higashiyama found something even more interesting – the nerve projecting to its round mouth corresponds to the nasopalatine, suggesting that it, not the maxillary, is the more ancient structure.

“Studies of vertebrate morphology often focus on hard structures, such as skeletal elements, but our bodies are mostly made up of softer bits, including the nerves we looked at in this study,” says Kuratani. “Similar to the trigeminal, the vagal nerve plays an important role in controlling enteric functions, but the relationship between this nerve and embryonic muscle development remain mysterious. By observing embryos to learn more about the making of the body, we sometimes find it teaches about its evolution as well.”



Jaw structures and innervation patterns during development. Jaw skeletal mesenchyme (prospective maxillomandibular prominence) is innervated by the maxillomandibular nerve (v23). In most gnathostomes, the medial nasal prominence is innervated by the nasopalatine nerve or a homologous branch, but this appears to have been lost secondarily during diapsid evolution.

Self-organized neocortex from human ESCs



Taisuke KADOSHIMA, Hideya SAKAGUCHI, Mika SOEN

The characteristic 'wrinkled' surface of the human neocortex is one of the keys to the brain's capacity for rational and analytic thought and language, and evolved as a means of increasing surface area to a remarkable extent. Studies of cerebral development, however, have largely been confined to model organisms, such as mouse, leaving gaps in our understanding of just this sort of human-specific feature. What is needed is a better way to study the same processes in vitro.

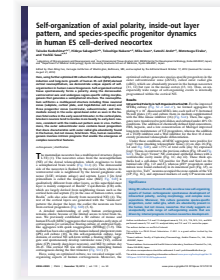
Taisuke Kadoshima and colleagues in the Lab for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director) have now succeeded in recapitulating the human neocortical development up to the equivalent of second trimester development in an in vitro environment, building on previous work by the same lab in inducing self-organized tissue differentiation from embryonic stem cells (ESCs). This work was featured on the cover of *The Proceedings of the National Academy of Sciences*, in which it was published.

The Sasai group had previously shown that a 3D culture technique known as SFEBq could be used to induce and steer the differentiation of human ESCs into a four-layered cortical-like structure, akin to that of a first-trimester fetus. Taking that study as their starting point, the group revisited the culture media and growth factors used in that protocol, and identified a number of opportunities for optimization, enabling even higher efficiency cortical induction.

Using this modified SFEBq system, they found that nearly all ESC aggregates became positive for the telencephalic marker gene *foxg1* on day 26, and rapidly began to differentiate into neuroepithelium by day 34. Following progress by a combination of live imaging and marker gene expression, the group observed the spontaneous onset of dorso-caudal–ventrororsal polarity in the neuroepithelium. The dorsocaudal epithelium further began to exhibit a rolling form of morphogenesis, resulting in curvature of the inward tissue surface, evaginating to form a hemispheric, enclosed cavity, closing resembling the process of brain formation in vivo.

©2013 National Academy of Sciences

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)

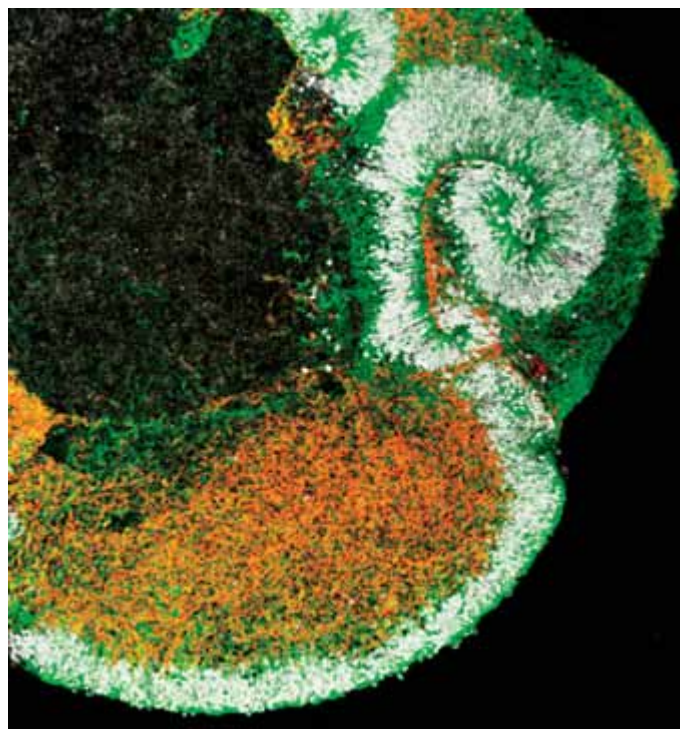


The previous SFEBq method had made culture possible for up to around 45 days, but tweaks to the modified approach resulted in a dramatic lengthening of this time span, making it possible for the hESC-derived brain-like tissue to mature even more in vitro. By around day 70 of culture, the neuroepithelium had thickened to more than 200 μm , and showed a six-layer laminar structure corresponding to that seen in the fetal neocortex. By day 91, the tissue had deepened to 350 μm , and each layer had grown morphologically similar to that seen in a second-trimester fetus.

Tracing of the movements of labeled cells over time revealed another distinctive feature of neocortical development, in which later born superficial neurons localized more

superficially than older, deeper layer neurons, resembling a similar inside-out pattern seen in fetal corticogenesis. Another intriguing finding was that region of the in vitro tissue corresponding to the outer subventricular zone developed large numbers of basal progenitors akin to outer radial glia found abundantly in human.

“These new refinements to the SFEBq culture system will allow us to begin to more closely study important questions, such as the mechanisms underlying the spontaneous acquisition of polarity by the neuroepithelium, or the processes involved in its curvature and rolling morphogenesis,” says Sasai. “These are exciting times for the study of human neurodevelopment.”



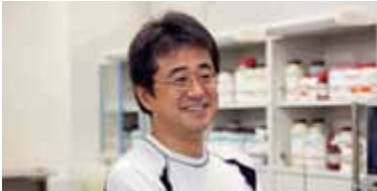
Neuroepithelium derived from human ESCs shows rolling morphogenesis (day 35 of culture)



Yasuhide FURUTA Ph.D.

Animal Resources and Genetic Engineering

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.



Staff

Unit Leader

Yasuhide FURUTA

Senior Advisor

Shinichi AIZAWA

Research Specialist

Takaya ABE

Go SHIOI

Visiting Scientist

Toshihiko FUJIMORI

Technical Staff

Kana BANDO

Naoko HATAMOTO

Michiko HIGASHIKAWA

Yoshiko MUKUMOTO

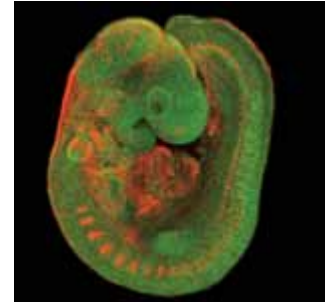
Megumi WATASE

Yui YAMASHITA

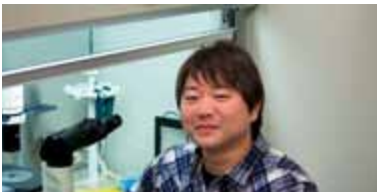
Genetic Engineering Unit

Yasuhide FURUTA Ph.D.

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.



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



Staff

Unit Leader

Hiroshi KIYONARI

Attending Veterinarian

Naoko KAGIYAMA

Research Consultant

Kazuki NAKAO

Technical Staff

Karin AKIYAMA

Kenichi INOUE

Mari KANEKO

Yuki KANEKO

Takuya KAWADA

Masako NODA

Miho SATO

Mayo SHIGETA

Aki SHIRAIISHI

Tomoko TOKUNAGA

Sachi YAKAWA

Assistant

Yuki TSUJI

Animal Resource Unit

Hiroshi KIYONARI Ph.D.

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. The unit has recently established colonies of a metatherian species, the gray short-tail opossum (*Monodelphis domestica*), and a reptilian species, the gecko (*Paroedura picta*). We are currently in the process of expanding these colonies, and determining their optimal environment and breeding conditions, with the aim to establish a distribution service of these new model animals.



Injection of C57BL/6 ES(HK3i) cells into 8 cell stage embryo for chimeric mouse production



Gecko



Opossum

Recent Publications

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

Hoshina N, et al. Protocadherin 17 regulates presynaptic assembly in topographic corticobasal ganglia circuits. *Neuron* 78.839-54 (2013)

Imuta Y, et al. Generation of knock-in mice that express nuclear enhanced green fluorescent protein and tamoxifen-inducible Cre recombinase in the notochord from Foxa2 and T loci. *Genesis* 51.210-8 (2013)

Lee J, et al. Angiopoietin-1 guides directional angiogenesis through integrin alphavbeta5 signaling for recovery of ischemic retinopathy. *Sci Transl Med* 5.203ra127 (2013)

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

Sakaguchi M, et al. The phosphatase Dullard negatively regulates BMP signalling and is essential for nephron maintenance after birth. *Nat Commun* 4.1398-1407 (2013)

Takao D, et al. Asymmetric distribution of dynamic calcium signals in the node of mouse embryo during left-right axis formation. *Dev Biol* 376.23-30 (2013)

Electron Microscope

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.



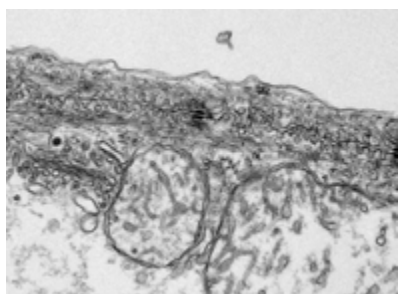
Shigenobu YONEMURA Ph.D.

Electron Microscope

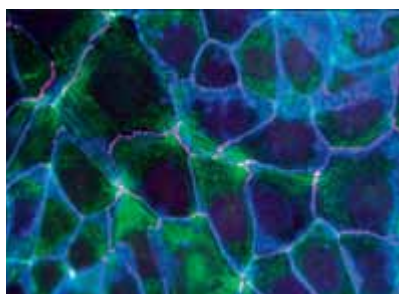
Shigenobu YONEMURA Ph.D.

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.



Cells organizing the notochord of zebrafish embryo



α -catenin molecules stretched by forces produced by Myosin II (green) are selectively labeled with α 18 antibody(red).



Staff

Laboratory Head
Shigenobu YONEMURA

Research Scientist
Hanako HAYASHI

Technical Staff
Kisa KAKIGUCHI
Kazuyo MISAKI

Visiting Scientist
Masatsune TSUJIOKA

Research Fellow
Akira ONODERA
Ayuko SAKANE

Student Trainee
Yu AMANO

Assistant
Mai SHIBATA

Recent Publications

Ikenouchi J, et al. Sphingomyelin clustering is essential for the formation of microvilli. *J Cell Sci* 126.3585-92 (2013)

Ebrahim S, et al. NMII forms a contractile transcellular sarcomeric network to regulate apical cell junctions and tissue geometry. *Curr Biol* 23.731-6 (2013)

Tsujioka M, et al. Talin couples the actomyosin cortex to the plasma membrane during rear retraction and cytokinesis. *Proc Natl Acad Sci USA* 109.12992-7 (2012)

Yonemura S. A mechanism of mechanotransduction at the cell-cell interface: emergence of alpha-catenin as the center of a force-balancing mechanism for morphogenesis in multicellular organisms. *Bioessays* 33.732-6 (2011)

Yonemura S. Cadherin-actin interactions at adherens junctions. *Curr Opin Cell Biol* 23.515-22 (2011)

Yonemura S, et al. alpha-Catenin as a tension transducer that induces adherens junction development. *Nat Cell Biol* 12.533-42 (2010)



Shigeo HAYASHI Ph.D.

Bio-Imaging

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.



Optical Image Analysis Unit

Yuko KIYOSUE Ph.D.

This unit runs the CDB's common-use 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.

In addition, we aim to shed light on the molecular mechanisms controlling the microtubule cytoskeleton in the cell/tissue morphogenesis by making full use of the available technology.

Staff

Unit Leader

Yuko KIYOSUE

Part-Time Staff

Emiko MAEKAWA

Technical Staff

Tomoko HAMAJI

Agency Staff

Shinobu SHIMAHARA

Recent Publications

van der Vaart B, et al. CFEOM1-Associated Kinesin KIF21A Is a Cortical Microtubule Growth Inhibitor. *Dev Cell* 27.145-60 (2013)

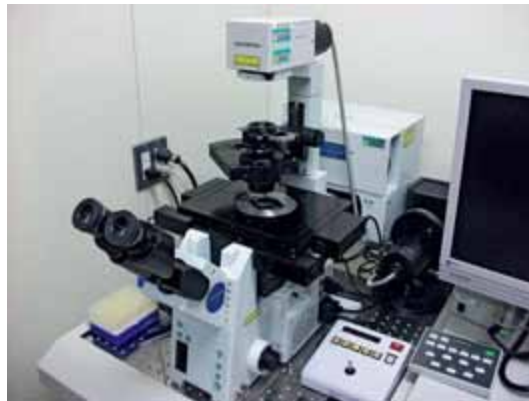
Shimozawa T, et al. Improving spinning disc confocal microscopy by preventing pinhole cross-talk for intravital imaging. *Proc Natl Acad Sci USA* 110.3399-404 (2013)

Nakamura S, et al. Dissecting the Nanoscale Distributions and Functions of Microtubule-End-Binding Proteins EB1 and ch-TOG in Interphase HeLa Cells. *PLoS One* 7.e51442 (2012)

Mimori-Kiyosue Y. Shaping microtubules into diverse patterns: molecular connections for setting up both ends. *Cytoskeleton (Hoboken)* 68.603-18 (2011)

Kosodo Y, et al. Regulation of interkinetic nuclear migration by cell cycle-coupled active and passive mechanisms in the developing brain. *EMBO J* 30.1690-704 (2011)

Hotta A, et al. Laminin-based cell adhesion anchors microtubule plus ends to the epithelial cell basal cortex through LL5alpha/beta. *J Cell Biol* 189.901-17 (2010)



Inverted microscope maintained by the Optical Image Analysis Unit

Genomics

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.

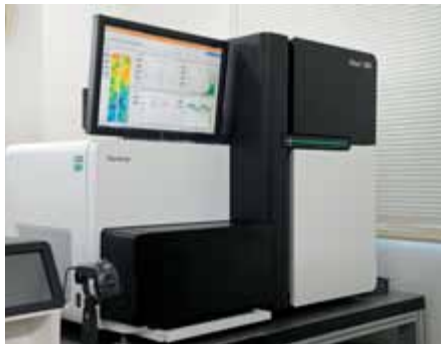


Fumio MATSUZAKI Ph.D.

Genome Resource and Analysis Unit

Shigehiro KURAKU Ph.D.

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



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



Microfluidics-based equipment for single cell gene expression profiling newly installed



Staff

Unit Leader

Shigehiro KURAKU

Research Specialist

Mitsutaka KADOTA

Research Scientist

Yuichiro HARA

Munazah ANDRABI

Miyuki NORO

Technical Staff

Tetsutaro HAYASHI

Kazu ITOMI

Kana MIYAGISHI

Osamu NISHIMURA

Chiharu TANEGASHIMA

Kaori TATSUMI

Kenichiro UNO

Assistant

Sayo SAITO

Recent 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-B (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)

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)

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

Functional Genomics Unit

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

The Functional Genomics Unit (FGU) has two missions: to provide functional genomics services to the laboratories within the CDB, and develop and introduce cutting-edge technologies related to functional genomics in order to accelerate the Center's research. We are striving to implement two types of technologies: 1) expression analysis, and 2) high-throughput measurement and perturbation. For expression analysis, we first introduced GeneChip technology, which is mainly used to measure expression profiles of genes in cells or tissues. For high-throughput measurement and perturbation, we introduced cell-based screening technology, which examines gene functions in cells. We will integrate technologies for expression analysis and high-throughput measurement and perturbation to develop new functional genomics methods. In particular, we will focus on strengthening single-cell expression analysis, and developing three-dimensional expression analysis in organs.



Staff

Unit Leader

Hiroki R. UEDA

Special Postdoctoral Researcher

Yohei SASAGAWA

Research Specialist

Takeya KASUKAWA

Itoshi NIKAIDO

Technical Staff

Kenichiro UNO

Assistant

Yuka TOKUMINE

Recent Publications

Sasagawa Y, et al. Quartz-Seq: a highly reproducible and sensitive single-cell RNA sequencing method, reveals non-genetic gene-expression heterogeneity. *Genome Biol* 14. R31 (2013)

Kasukawa T, et al. Human blood metabolite timetable indicates internal body time. *Proc Natl Acad Sci USA* 109. 15036-41 (2012)

Kasukawa T, et al. Quantitative expression profile of distinct functional regions in the adult mouse brain. *PLoS One* 6. e23228 (2011)

Masumoto K. H, et al. Acute induction of Eya3 by late-night light stimulation triggers TSH β expression in photoperiodism. *Curr Biol* 20. 2199-206 (2010)



Shigeo HAYASHI Ph.D.

Proteomics

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.



Staff

Lab Head

Shigeo HAYASHI

Research Specialist

Reiko NAKAGAWA

Recent Publications

Tsutsui T, et al. Mediator complex recruits epigenetic regulators via its two cyclin-dependent kinase subunits to repress transcription of immune response genes. *J Biol Chem* 288.20955-65 (2013)

Kamata K, et al. C-terminus of the Sgf73 subunit of SAGA and SLIK is important for retention in the larger complex and for heterochromatin boundary function. *Genes Cells* 18.823-37 (2013)

Kuroha K, et al. The Upf factor complex interacts with aberrant products derived from mRNAs containing a premature termination codon and facilitates their proteasomal degradation. *J Biol Chem* 288.28630-40 (2013)

McCloskey A, et al. hnRNP C tetramer measures RNA length to classify RNA polymerase II transcripts for export. *Science* 335.1643-6 (2012)

Shiomi Y, et al. Two different replication factor C proteins, Ctf18 and RFC1, separately control PCNA-CRL4Cdt2-mediated Cdt1 proteolysis during S phase and following UV irradiation. *Mol Cell Biol* 32.2279-88 (2012)

Terabayashi T, et al. Phosphorylation of Kif26b promotes its polyubiquitination and subsequent proteasomal degradation during kidney development. *PLoS One* 7.e39714 (2012)

Mass Spectrometry Analysis Unit

Shigeo HAYASHI Ph.D.

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.



LC-MASS spectrometry system used in the Mass Spectrometry Analysis Unit

Human Stem Cell Technology

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.



Yoshiki SASAI M.D., Ph.D. (Deputy Chief Hitoshi NIWA M.D., Ph.D.)

Human Stem Cell Technology Unit

Yoshiki SASAI M.D., Ph.D.

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.



Staff

Unit Leader

Yoshiki SASAI

Deputy Unit Leader

Masatoshi OHGUSHI

Research Specialist

Hiroyuki KITAJIMA

Visiting Scientist

Tokushige NAKANO

Daiki NUKAYA

Technical Staff

Michiru MATSUMURA-IMOTO

Maki MINAGUCHI

Junior Research Associate

Chikafumi OZONE

Part-Time Staff

Yoshinori NAKAI

Recent Publications

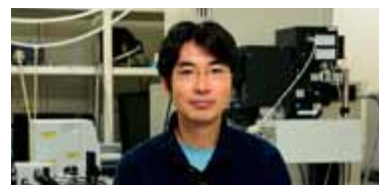
Nakano T, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 10.771-85 (2012)

Ohgushi M, et al. Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. *Cell Stem Cell* 7.225-39 (2010)

Four-Dimensional Tissue Analysis Unit

Mototsugu EIRAKU, Ph.D.

Recent advances in stem cell technology have enabled the generation of various potentially medically useful cell types from ES and iPS cells, but the extent to which such cells mimic their in vivo function when plated on culture dishes is limited. The Four-dimensional Tissue Analysis Unit seeks to develop new approaches to cell culture that will allow for more realistic in vitro recapitulation through the formation of three-dimensional tissue from stem cells. We will establish efficient 3D culture of ES cell-derived brain and retinal tissues, and develop cutting-edge live imaging technologies and optic devices for the 4D analysis of large tissues. We also support and work with users of these technologies within and outside the CDB.



Staff

Unit Leader

Mototsugu EIRAKU

Visiting Scientist

Hidetaka SUGA

Technical Staff

Eriko SAKAKURA

Junior Research Associate

YuiKO HASEGAWA

Recent Publications

Eiraku M, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 472.51-56 (2011)

Eiraku, M. and Sasai, Y. Mouse embryonic stem cell culture for generation of three-dimensional retinal and cortical tissues. *Nature Protocols* 7.69-79 (2011)

Science Policy and Ethics Studies Unit

Douglas SIPP

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.



Staff

Unit Leader

Douglas SIPP

Recent 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

CDB 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 pharmaceuti-

cal 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
- RIKEN Kobe Safety Center

RIKEN CDB Administrative Support Offices

- RIKEN CDB Administrative Support Offices
 - Developmental Biology Planning Office
 - Information Networks Office
 - Office for Research Communications
 - Library Office

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

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.

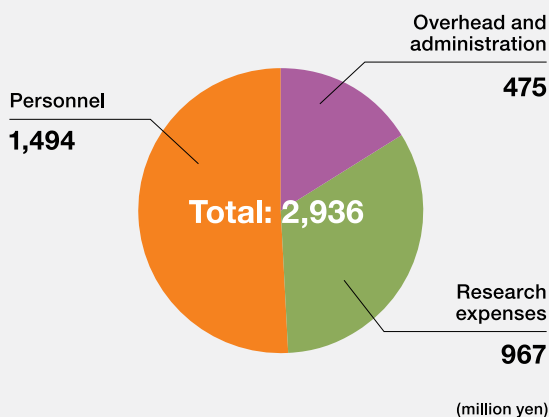
Kobe Administrative Services

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 Office for Research Communications assists in public outreach, meeting logistics, and assistance with language and relocation issues for research staff. The Library Office manages the CDB research literature collections and interlibrary loans.

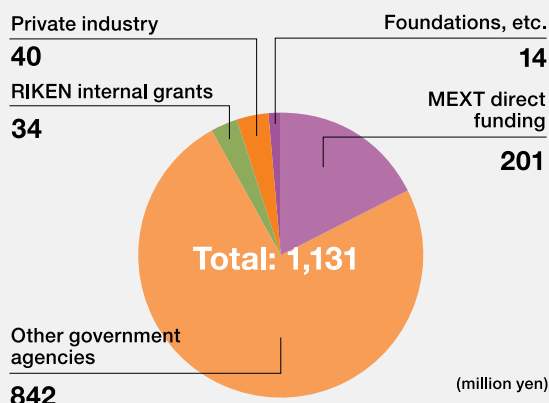
Safety Center

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.

2013 CDB Budget



In addition 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.



2013 CDB Staff

Laboratory heads	32	<div style="width: 32%;"></div>
Deputy unit leaders	4	<div style="width: 4%;"></div>
Research scientists	85	<div style="width: 85%;"></div>
Research associates	9	<div style="width: 9%;"></div>
Technical staff	110	<div style="width: 110%;"></div>
Assistants	22	<div style="width: 22%;"></div>
Visiting scientists	90	<div style="width: 90%;"></div>
Student trainees	34	<div style="width: 34%;"></div>
Part-time staff	47	<div style="width: 47%;"></div>
Other	21	<div style="width: 21%;"></div>
<hr/>		
Kobe Branch Administration	65	<div style="width: 65%;"></div>
RIKEN CDB Administrative Support Offices	22	<div style="width: 22%;"></div>
Total	541	



2013 CDB Symposium

The Making of a Vertebrate

March 4–6, 2013

The RIKEN Center for Developmental Biology held its 11th annual symposium on March 4–6 in the CDB Auditorium. More than 180 scientists from around the world met to introduce their work and discuss new developments relating to this year's theme, "The Making of a Vertebrate," echoing the title of Peter Lawrence's famous text on fly development. Topics ranged from discussions of the evolutionary roots of the vertebrate lineage to distinct features of vertebrate embryology.

This year's event was organized by Shin Aizawa and Shigeru Kuratani of the CDB and Denis Duboule of the University of Geneva and EPFL (Switzerland). In addition to the three days of invited talks, there were nearly 100 poster presentations and ample time for relaxed discussion. More than 10 international participants received travel support through the CDB travel fellowship program.

The CDB symposium series, launched in 2003, was established as a forum for addressing diverse aspects of developmental and regenerative biology, and aims to promote the free, timely and borderless exchange of research achievements.

Session 1

Nicholas D. Holland (UC San Diego, USA)
Naoki Irie (RIKEN CDB, Japan)
Billie J. Swalla (University of Washington, USA)
Stephen Green (CALTECH, USA)
Maria Antonietta Tosches
 (EMBL Heidelberg, Germany)

Session 2

Detlev Arendt (EMBL, Germany)
Andreas Hejnol (Sars International Centre for Marine Molecular Biology, Norway)
Christopher Lowe (Stanford University, USA)
Norio Miyamoto
 (Japan Agency for Marine-Earth Science and Technology, Japan)
Bo Dong (RIKEN CDB, Japan)
Manuel Irimia (University of Toronto, Canada)

Session 3

Hitoshi Niwa (RIKEN CDB, Japan)
Miguel Manzanares (CNIC, Spain)
Kunimasa Ohta (Kumamoto University, Japan)
Tom Humphreys (University of Hawaii, Manoa, USA)

Session 4

Edward M. De Robertis (HHMI/UCLA, USA)
Hidehiko Inomata (RIKEN CDB, Japan)
Masahiko Hibi (Nagoya University, Japan)
Bernard Thisse (University of Virginia, USA)
Hiroshi Hamada (Osaka University, Japan)
Shinichi Aizawa (RIKEN CDB, Japan)



Session 5

Denis Duboule

(University of Geneva & EPFL, Switzerland)

Hiroyuki Takeda (The University of Tokyo, Japan)

Toshihiko Shiroishi (NIG, Japan)

Atsushi Kuroiwa (Nagoya University, Japan)

Haruhiko Koseki

(RIKEN Center for Allergy and Immunology, Japan)

Fumitoshi Ishino (Tokyo Medical and Dental University, Japan)

Edith Heard (CNRS, France)

Session 6

Koji Tamura (Tohoku University, Japan)

Mikiko Tanaka (Tokyo Institute of Technology, Japan)

Arkhat Abzhanov (Harvard University, USA)

David Kingsley (Stanford University, USA)

Shigeru Kuratani (RIKEN CDB, Japan)

Zhikun Gai (Chinese Academy of Sciences, China)

Per Erik Ahlberg (Uppsala University, Sweden)

Hiroshi Wada (University of Tsukuba, Japan)

Kinya Yasui (Hiroshima University, Japan)

Luis Puelles (University of Murcia, Spain)

Tatsumi Hirata (NIG, Japan)

Tadashi Nomura

(Kyoto Prefectural University of Medicine, Japan)

2014 CDB Symposium

Regeneration of Organs: Programming and Self-Organization

March 10–12, 2014

The twelfth annual symposium “Regeneration of Organs: Programming and Self-Organization” will be held on March 10–12, 2014 in the CDB Auditorium. This symposium will focus on the regeneration of organs to reconsider how different the regeneration of tissues from their developmental processes, why our higher mammals have a low ability of tissue regeneration, how we can incorporate ideas from other fields into regeneration biology.

Self-organization can be defined by clear logics based on the identification and quantification of parameters. By learning such approaches from studies of development and by extending to those of regeneration, we believe that we will be able to quantitatively understand highly-coordinated background logics of regeneration. Further, it may reveal the distinct mechanisms to govern the shape and the size of organs formed by development or regeneration, or the convergence of the process triggered by different ways in development and regeneration.

Invited Speakers

Taiji Adachi (Kyoto University, Japan)

Richard Adams (University of Cambridge, UK)

Kiyokazu Agata (Kyoto University, Japan)

Naama Barkai (Weizmann Institute of Science, Israel)

Daniel Goldman (University of Michigan, USA)

Kenneth Irvine (Rutgers University and HHMI, USA)

Pierre Léopold (Institute of Biology Valrose, France)

Hans Meinhardt

(Max Planck Institute for Developmental Biology, Germany)

Ginés Morata (Universidad Autónoma de Madrid –CSIC, Spain)

Yoshihiro Morishita (RIKEN CDB, Japan)

Kenneth Poss (Duke University Medical Center, USA)

Yoshiki Sasai (RIKEN CDB, Japan)

Ashley W. Seifert (University of Kentucky, USA)

Manuel Serrano

(Spanish National Cancer Research Centre, Spain)

Andrés Simon (Karolinska Institute, Sweden)

Kaoru Sugimoto (California Institute of Technology, USA)

Takashi Takeuchi (Tottori University, Japan)

Koji Tamura (Tohoku University, Japan)

Elly Tanaka

(Center for Regenerative Therapies Dresden, Germany)

Takashi Tsuji (Tokyo University of Science, Japan)

CDB 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 700 such talks, in addition to numerous meetings,

distinguished lectures, internal forums, and colloquia. The following speakers presented CDB Seminars in the period from January to December 2013.

Date	Title	Speaker
01-07	Dissecting computations in the dopamine reward circuit: beyond stamp collecting	Naoshige UCHIDA
01-24	Wnt signaling interactions and anterior-posterior neuroectoderm patterning in deuterostome embryos	Ryan RANGE
01-24	Reconstitution of cell patterns that are driven by Delta-Notch signaling	Miki EBISUYA
01-25	How changes in cell polarity orchestrate the folding of a tissue	Yu-Chiun WANG
01-31	Coded polymer in thermal gradients: from the early life to pattern formation	Yusuke T. MAEDA
02-01	Chromatin remodeling by the <i>CHD7</i> protein is impaired by mutations that cause human developmental disorders	Karim BOUAZOUNE
02-01	Engineering developmental plasticity: Mechanisms of germ cell and multipotent cell development	Mamiko YAJIMA
02-07	Insights into intestinal homeostasis from <i>Drosophila</i> : stochastic fate allocation and stress-induced exit from quiescence	Joaquín de NAVASCUÉS
02-13	Importin α subtypes determine the differential localization of transcription factors to maintain the undifferentiated state of embryonic stem cell	Noriko YASUHARA
02-22	Mechanism of the nuclear oscillation in fission yeast meiosis	Ikumi FUJITA
02-25	Reproductive organ formation, homeostasis, and regeneration	Richard R. BEHRINGER
03-11	Reprogramming and nuclear dynamics	Yusuke MIYANARI
03-13	Induction of pluripotency	Hans R. SCHÖLER
03-13	Developmental regulation of nuclear genome organization	Ichiro HIRATANI
03-14	LIF/STAT3 signalling in human and rabbit pluripotent stem cells	Pierre SAVATIER
03-29	Cell identity transitions during induced reprogramming to pluripotency	Knut WOLTJEN
04-16	Hippo signaling in cardiomyocyte renewal and regeneration	Yuka MORIKAWA
04-22	Transcriptional regulation in 3D: models of gene expression during fly development	Nick LUSCOMBE
04-25	Rounding up on cell shape: Mitotic cell rounding as model problem in cell shape	Martin STEWART
05-13	Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF syndromes	Karen LIU
06-03	Spatio-temporal activation of ERK through Vegfrs regulates identity of segmental artery in zebrafish	Masahiro SHIN
06-06	Shielding up! Molecular basis of beetle wing evolution	Yoshinori TOMOYASU
06-20	Neural stem and progenitor cells and the evolution of the cerebral cortex	Wieland B. HUTTNER
06-24	Evolution of cerebral cortical development	Zoltán MOLNÁR
06-26	Planar spindle orientation maintains epithelial architecture and prevents tumorigenesis	Yuichiro NAKAJIMA
06-27	Polarity-dependent distribution of Angiomotin localizes Hippo signaling in preimplantation embryos	Yoshikazu HIRATE
07-02	BMP signaling in preimplantation mouse embryos	Ken CHO

Date	Title	Speaker
07-04	OA publishing at BioMed Central: a pioneer's perspective	Nandita QUADERI
07-08	"Organs, Not Cells" - A vision for the future	Hiromitsu NAKAUCHI
07-09	Characterising epithelial cell movements in the visceral endoderm of the mouse embryo	Shankar SRINIVAS
07-09	Surviving pluripotency. Who picks who will live and who will die in the early mammalian embryo?	Tristan A. RODRÍGUEZ
07-26	Effective transplantation of photoreceptors derived from three-dimensional cultures of embryonic stem cells	Robin ALI
08-01	Smad-dependent BMP signaling through type IA receptor in cranial neural crest cells directs their cell fate towards chondrocytes to cause craniosynostosis	Yuji MISHINA
08-01	B cell progenitors and precursors change their microenvironment in fetal liver during early development	Motokazu TSUNETO
08-06	Evolutionary cell biology of chromosome segregation	Bungo AKIYOSHI
08-23	Local increases in mechanical tension guide cell sorting at compartment boundaries by constraining junctional dynamics	Daiki UMETSU
09-10	Developmental biology meets single-molecule detection technologies; Measurement-based mathematical modeling of PAR protein localization in <i>C. elegans</i> embryos	Yukinobu ARATA
10-22	Dynamics of transcription and histone modifications in living cells and organisms	Hiroshi KIMURA
10-25	The molecular and cellular mechanisms of the vascular reconstruction during zebrafish fin regeneration	Yoshiko KAMETANI
11-11	Using genome engineering to reinvestigate the range of Wnt signalling	Jean-Paul VINCENT
11-13	Publishing -a useful scientific literature	Esther SCHNAPP
11-26	Social evolution shapes the diversification of bacterial intercellular signaling	Avigdor EL DAR
11-26	Integration of signals acting along orthogonal axes of the neural tube determines the competence of neural progenitors and diversity of cell types	Noriaki SASAI
11-29	Epigenetic reprogramming of mouse sperm genome following fertilization	Azusa INOUE
11-29	Tet1-mediated epigenetic reprogramming in primordial germ cell	Shinpei YAMAGUCHI
12-02	Novel mechanistic insight into how the Spindle Assembly Checkpoint prevents premature anaphase	Daisuke IZAWA
12-02	Deciphering basic signaling architectures patterning animal body axes	Hiroshi WATANABE
12-09	Regulation of ligand-independent Notch signal through intracellular trafficking	Kazuya HORI
12-12	New 3D/4D visualization/analysis methods for biological data	Hideo OTSUNA
12-16	Functional analysis of lipid metabolism genes affecting developmental signaling pathways	Takeshi SASAMURA
12-20	New protist models elucidating the origin of metazoan multicellularity: ichthyosporeans and filastereans	Hiroshi SUGA

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, chem-

istry, 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 electronic resources 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.rikenresearch.riken.jp/>



Sendai

519-1399 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-0845
Tel: +81-22-228-2111 Fax: +81-22-228-2122

Tsukuba

BioResource Center
3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074
Tel: +81-29-836-9111 Fax: +81-29-836-9109

RIKEN Headquarters

Public Relations Office

Wako Administrative Headquarters

Center for Emergent Matter Science
Center for Advanced Photonics
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
Distinguished Senior Scientist Laboratories
Initiative Research Units
Special Research Units
Global Research Cluster
2-1, Hirosawa, Wako, Saitama 351-0198
Tel: +81-48-462-1111 Fax: +81-48-462-1554

Tokyo Liaison Office

2311, 23th floor, Fukoku Seimei Building,
2-2, Uchisaiwaicho 2-chome, Chiyoda-ku, Tokyo 100-0011
Tel: +81-3-3580-1981 Fax: +81-3-3580-1980

Yokohama

Center for Sustainable Resource Science
Center for Integrative Medical Sciences
Bioinformatics And Systems Engineering division
RIKEN Program for Drug Discovery and Medical Technology Platforms
1-7-22 Suehiro, Tsurumi-ku, Yokohama, Kanagawa 230-0045
Tel: +81-45-503-9111 Fax: +81-45-503-9113

Nagoya

2271-130, Anagahora, Shimoshidami, Moriyama-ku, Nagoya, Aichi 463-0003
Tel: +81-52-736-5850 Fax: 0+81-52-736-5854

Osaka

Quantitative Biology Center
OLABB, Osaka University 6-2-3, Furuedai, Suita, Osaka 565-0874
Tel: +81-06-6155-0111 Fax: 0+81-06-6155-0112

Kobe

Center for Developmental Biology
2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047
Tel: +81-78-306-0111 Fax: +81-78-306-0101
Center for Life Science Technologies
MI R&D Center, 6-7-3 Minatojima-minamimachi, Chuo-ku,
Kobe, Hyogo 650-0047
Tel: +81-78-304-7111 Fax: +81-78-304-7112
HPCI Program for Computational Life Sciences
2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
Tel: +81-(0)78-306-0111 Fax: +81-(0)78-306-0101
RIKEN Advanced Institute for Computational Science
7-1-26 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047
Tel: +81-78-940-5555 Fax: +81-78-304-4956

Harima

RIKEN Spring-8 Center
1-1-1, Kouto, Sayo-cho, sayo-gun, Hyogo 679-5148
Tel: +81-791-58-0808 Fax: +81-791-58-0800

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.



<http://www.cdb.riken.jp/>

CENTER FOR DEVELOPMENTAL BIOLOGY
2-2-3 MINATOJIMA-MINAMIMACHI, CHUO-KU
KOBE 650-0047
JAPAN
PHONE: +81-78-306-0111 FAX: +81-78-306-0101
EMAIL: cdb@cdb.riken.jp

On The Cover

3D-reconstructed chondrocranium with peripheral nervous system of a prehatching embryo of the hagfish, *Eptatretus atami*.

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