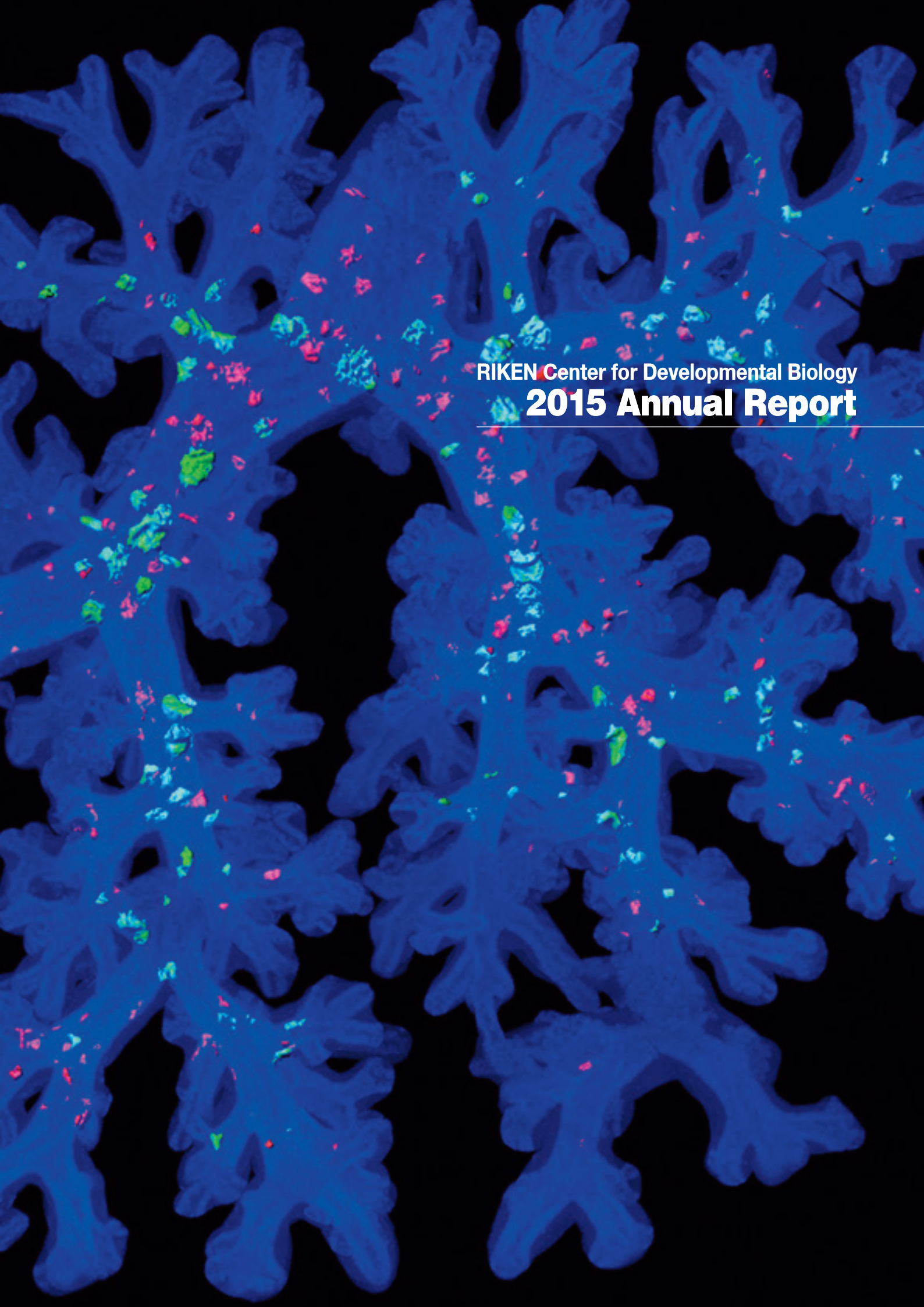


RIKEN Center for Developmental Biology

2015 Annual Report

2015 Annual Report

RIKEN
Center for
Developmental Biology

Contents

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

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

Laboratories

molecular and cell biological level, the more complex phenomena involved in organogenesis, as well as the biology of stem cells and regeneration. Scientists at the CDB conduct world-class studies in a truly international research environment, with ample 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 Center Director

It was a great sense of pride and responsibility that I joined the RIKEN Center for Developmental Biology (CDB) as Director in April of this year. My predecessor, Dr. Masatoshi Takeichi has set an incredibly high bar of achievement through his great leadership and his long career of scientific achievements, and the success of the Center over the first 15 years of its history is a testament to his vision. My task now is to guide the CDB into a new stage of its own development, one in which we continue to bring to light the mechanisms by which the body is built, and find new ways of harnessing that knowledge in rebuilding bodies that have been damaged by illness, injury, or age.

Following a reorganization of the research system last year, the CDB of 2015 is a slimmer organization, with multidisciplinary programs focused on cellular environment and response, organogenesis, stem cells and organ regeneration, and developmental biology and mathematical science, as well as its ongoing commitment to a world-renowned project in retinal regeneration. The CDB is also committed to working even more closely with other RIKEN Centers, including the Center for Life Science Technologies (CLST) and Quantitative Biology Center (QBiC), as well as the RIKEN-wide Chief Scientists program.

Our tighter research focus, however, has not slowed the pace of scientific achievement. This past year CDB labs have continued to publish outstanding work in high-impact journals, ranging from fundamental insights in cell adhesion, evolutionary developmental biology, and morphogenesis, to more application-oriented studies in stem cell differentiation and transplantation methods. The work of our research scientists continues to be recognized by awards from government agencies and private foundations, but more importantly through collaboration and interaction with their international peers.

This international focus continues to be a key feature of scientific life at the CDB, as exemplified by our annual symposium, which convened in March under the thought-provoking theme of “Time in Development.” As in previous years, this event drew a capacity audience of 140+ speakers and attendees from around the world and across the spectrum of related fields. We look forward to welcoming another great assembly of leading scientists and thinkers to the 2016 Symposium, “Size in Development: Growth, Shape and Allometry,” on March 28–30.

While maintaining our focus on conducting world-class science, the CDB will also redouble its efforts to contribute to benefiting society through translational efforts as well. Given Japan’s national emphasis on developing new medical technologies, including regenerative medicine applications, to address the challenges of an aging society, the Center is well positioned to work with national, regional and local government and private sector organizations to make this dream a reality. The city of Kobe continues to promote the incredible growth and ongoing success of its biomedical research park here on Port Island, which is now home to over 300 medical centers, research institutions, and biotechnology industry sites. Western Japan also boasts some of the world’s leading institutes in the study of stem cell technology, including the Center for iPS cell Research and Application (CiRA) at Kyoto University, with which the CDB continues to work closely in exploring the therapeutic potential of pluripotent stem cell-based approaches.

In closing, it is a new and exciting era for the CDB and for the study of development and regeneration, two interlinked processes that have been at the core of our scientific focus since the Center was first established at turn of the millennium. The potential of these fields remains enormous, but its realization will require the dedicated support and hard work of the global life science and biomedical research community, and it is to that community that I pledge we will work even harder to contribute new innovations and insights on the long road to discovery.

Hiroshi Hamada
Director, RIKEN Center for Developmental Biology

RIKEN Kobe Branch

Center for Developmental Biology

The Center for Developmental Biology (CDB) is a research center within the RIKEN Kobe Branch, which also includes the Center for Life Science Technologies (CLST), the Quantitative Biology Center (QBiC), the HPCI Program for Computational Life Sciences, the Kobe Administrative Division, which provides administrative services, and the institutional Safety Center. The CDB underwent an organizational restructuring in 2014, resulting in major changes to laboratory designations and departmental affiliations. The majority of laboratories are now designated as teams, and assigned to one of five programs, which are described in detail to the right. The CDB Director is assisted by a Deputy Director, and advised by the Advisory Council, while governance issues are discussed by the Management Committee.

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

CDB Organization 2015

Deputy Director

Cellular Environment and Response Research Program

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

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

Organogenesis Research Program

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

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

Advisory Council

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

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
  (Until March 31, 2015)

Center Director Hiroshi Hamada

Office of the Director | **Research Ethics Education Officer**

Stem Cells and Organ Regeneration Research Program

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

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

Developmental Biology and Mathematical Science Program

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

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

Research and Development Project

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

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

Senior Investigator Program

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

Management Committee

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

- Hiroshi Hamada
  Director, CDB
- Toshihiko Oguru
  Director, RIKEN Kobe Branch; Deputy Director, CDB
- Osamu Kamigaito
  RIKEN Nishina Center for Accelerator Based Science
- Makoto Taiji
  RIKEN Quantitative Biology Center
- Hisato Kondo
  Kyoto Sangyo University
- Takeshi Imai
  Lab. Sensory Circuit Formation, CDB
- Tomoya Kitajima
  Lab. Chromosome Segregation, CDB
- Erina Kuranaga
  Lab. Histogenetic Dynamics, CDB
- Tatsuo Shibata
  Coordinator, CDB
- Masayo Takahashi
  Lab. Retinal Regeneration, CDB
- Takashi Tsuji
  Lab. Organ Regeneration, CDB
- Katsutoshi Nukui
  Developmental Biology Planning Office

2015 Highlights

The 13th CDB Symposium: Time in Development

The CDB held its 13th annual symposium on March 23–25 on the theme, “Time in Development.” Close to 150 researchers from around the globe convened at the Center to share their work and engage in discussions on the different contexts of time in relation to development.

New center director takes office

On April 1, the CDB welcomed Hiroshi Hamada, a professor in the Graduate School of Frontier Biosciences, Osaka University and respected scientist known for his research on the establishment of body axes in mammals, as the new center director.

On his appointment, he stated, “On the management side, I hope that we can work to increase the transparency of the CDB. As for research, I would like to ensure that we take heed of the balance between basic and applied research and also create an environment that fosters, especially for younger scientists, the pursuit of challenging research aimed at discovering the true essence of biological phenomena.”

He established his own lab, the Laboratory for Organismal Patterning, at the CDB in October.

CDB scientists awarded MEXT prizes

In a ceremony in April, Project Leader Masayo Takahashi and her research team, and Team Leader Takeshi Imai were recognized for their scientific achievements by Japan’s Minister of Education, Culture, Sports, Science, and Technology (MEXT), receiving the Commendation for Science and Technology. Takahashi, along with lab members Michiko Mandai, Chikako Morinaga, Noriko Sakai and Chikako Yamada, received the Prize for Science and Technology for their work on the clinical development of iPSC-derived RPE for transplantation. Imai was awarded the Young Scientist’s Prize for his work on neural circuit formation using the olfactory system as a model.

Exploring the interface between developmental biology and mechanics

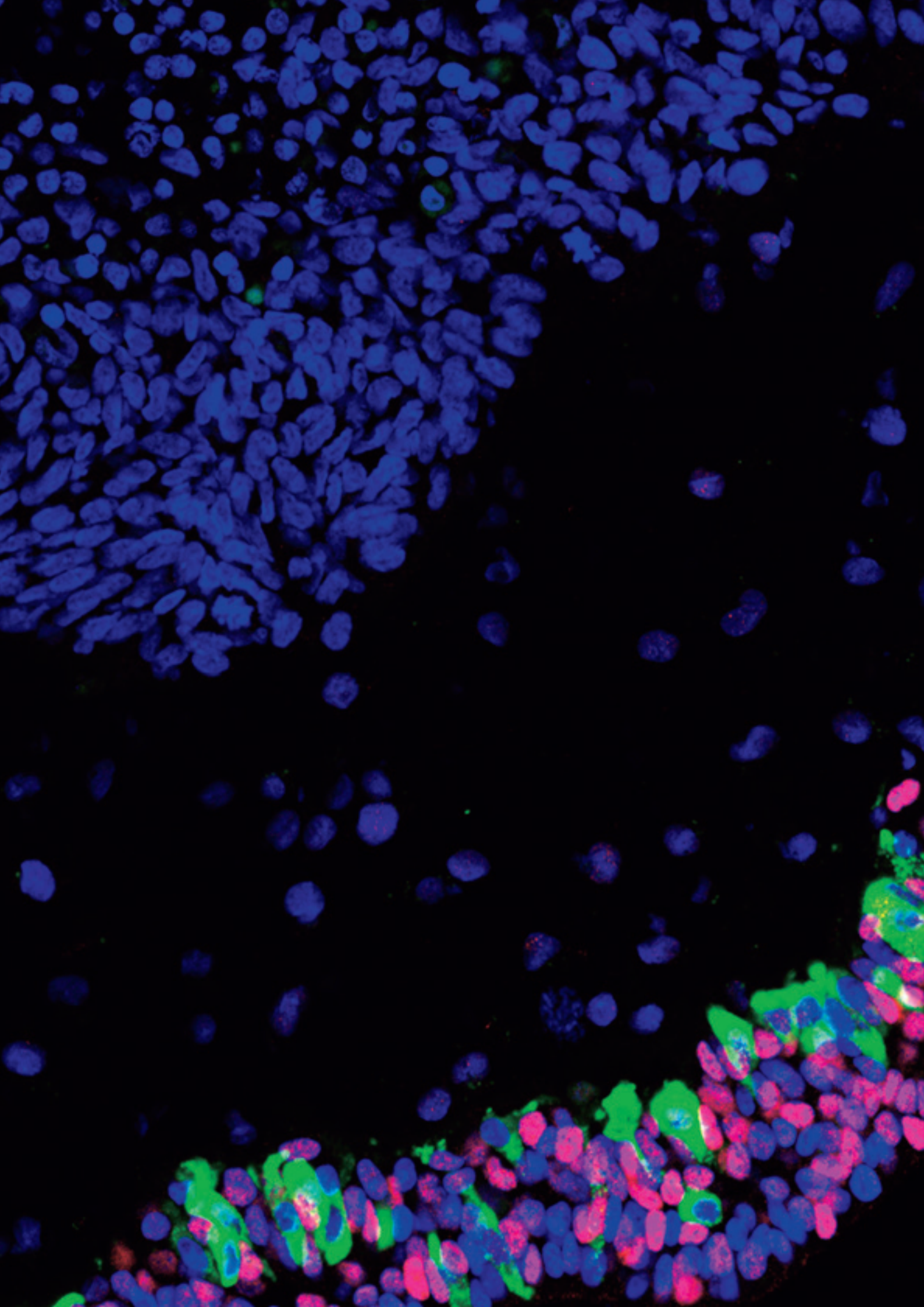
The 26th CDB Meeting, “Mechanical Perspectives of Multicellular Organization,” was held September 8–9. The multidisciplinary meeting attracted over 130 scientists with varied backgrounds including biology, medicine, physics, and engineering to share and engage in discussions on how biological fields can incorporate perspectives and technologies from physics and mechanics to understand biological processes.

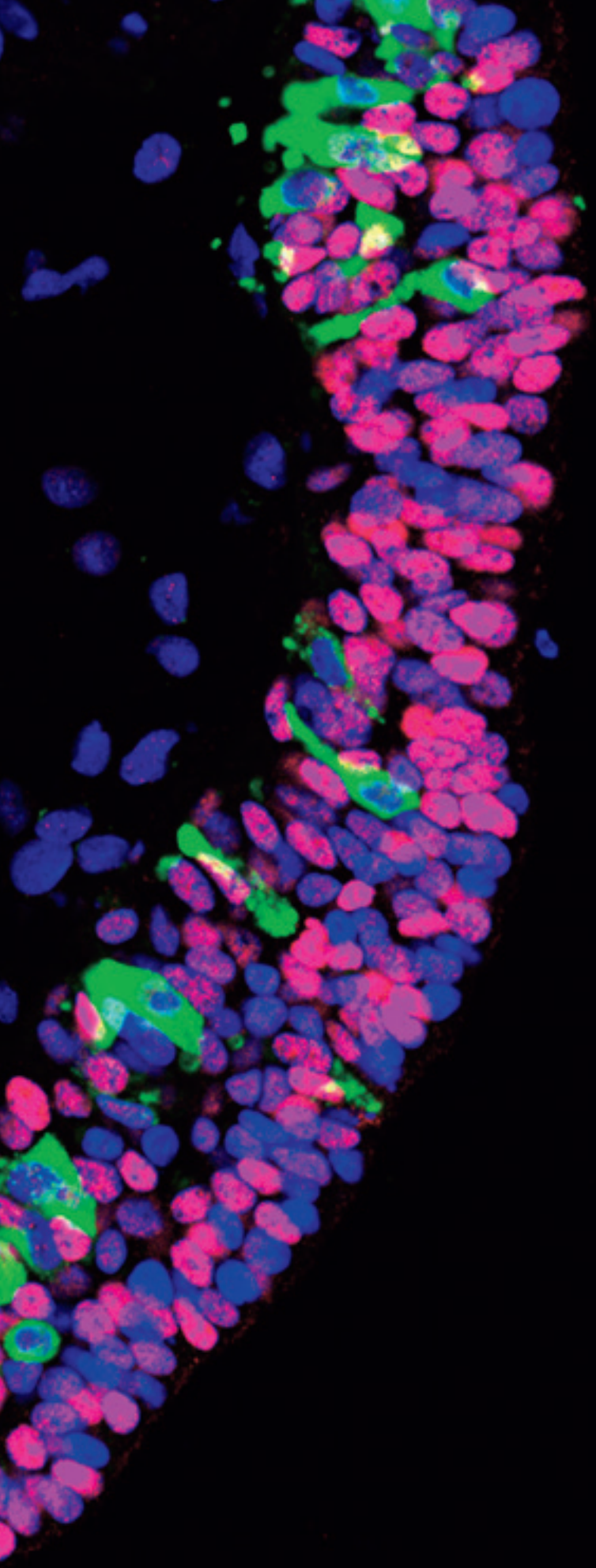
Masayo Takahashi awarded Ogawa-Yamanaka Stem Cell Prize

The Gladstone Institutes named Masayo Takahashi, project leader of the Laboratory for Retinal Regeneration, as the inaugural winner of the Ogawa-Yamanaka Stem Cell Prize for her “trailblazing work leading the first clinical trial to use induced pluripotent stem cells (iPSCs) in humans.”

Update on the first transplantation of iPSC-derived RPE cell sheet

RIKEN, the FBRI, and the Kobe Medical Center General Hospital held a joint press conference in October to report on the progress of the recipient of the first iPSC-derived RPE cell transplantation in the pilot safety study of iPSC-based intervention for wet-type age-related macular degeneration. The research team reported that the results of periodic examinations during the monitoring period appear to be good, and that no signs of tumorigenesis, abnormalities, and recurring neovascularization have been detected. The patient will continue to be monitored for the next three years.

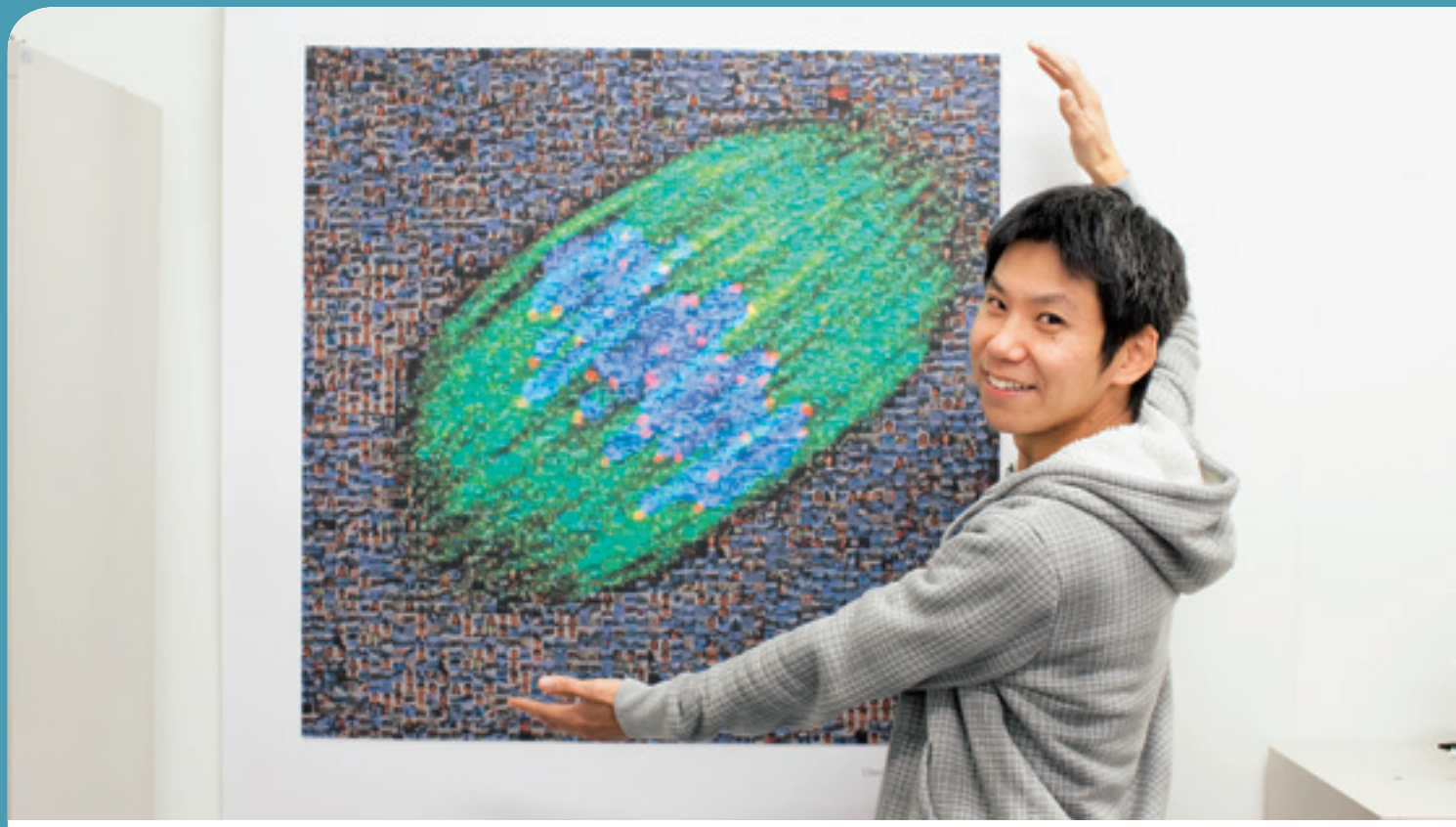




Research Highlights

Pituitary tissue generated from human ES cells.
Green, growth hormone; red, PITX1; blue, nucleus.
Image: Laboratory for Organ Regeneration

Oocyte maturation directed by PLK1



Tomoya KITAJIMA

Chromosome segregation is one of the most important events during cell division, both in somatic cells (mitosis) and in germ cells (meiosis). Improper segregation of chromosomes leads to a range of defects in the daughter cells, and nowhere is correct chromosome segregation more crucial than in the division of germ cells as they pass on genetic information to the next generation of offspring. But surprisingly, errors in chromosome segregation are known to occur with high frequencies in mammalian oocytes. Meiosis in oocytes is also unique, as the cell division cycle is arrested for an extended period in prophase I before resuming meiotic division. One factor that plays a major role in cell division is Polo-like kinase 1 (PLK1), a serine/threonine kinase, which has been implicated in multiple events during mitosis. The functions of PLK1 in oocytes undergoing maturation (meiosis), however, are not well understood.

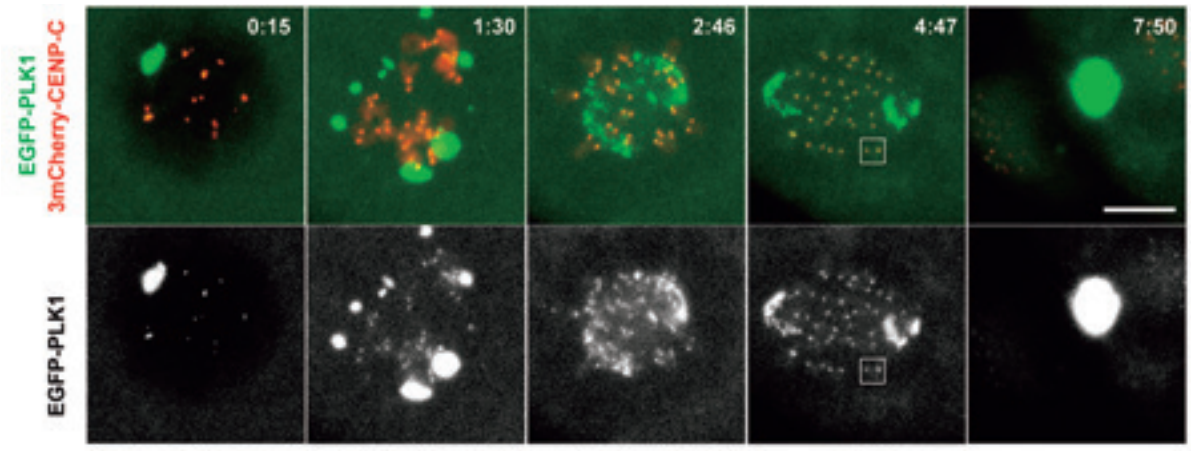
Now, a new joint study by team leader Tomoya Kitajima and colleagues in the Laboratory for Chromosome Segregation and collaborators at the Institute of Animal Physiology and Genetics (Czech Republic) and European Molecular Biology Laboratory (EMBL; Germany) looks at the role of PLK1 during meiosis, using mouse oocytes as a model. Their findings, published in *PLoS ONE*, demonstrate that PLK1 controls meiotic cell division in a manner similar to mitosis, such as promoting microtubule elongation and stabilizing microtubule-kinetochore binding. They also find that PLK1 has functions specific to meiosis such as activating the anaphase promoting complex/cyclosome (APC/C) for entry into anaphase I, for proper chromosome segregation and maintaining chromosome condensation during meiosis I-meiosis II transition.

Kitajima and colleagues first examined the normal localization patterns of PLK1 during meiosis of oocytes using immunostaining and 4D live imaging techniques. PLK1 was initially found dispersed in the cytoplasm during prophase I, and was activated and localized to microtubule organizing centers (MTOCs) before nuclear envelope breakdown (NEBD). When oocytes were treated with an inhibitor to specifically block PLK1 function, there was a marked delay in the timing of NEBD and of chromosome condensation, two events that signal the resumption of meiosis. The resumption of meiosis requires another kinase, CDK1, which is known to be activated by PLK1. Analyses by the researchers indicated that in addition to activating CDK1, PLK1 also regulates NEBD through an alternative route. PLK1 was found to contribute to MTOC formation, and to be required for proper meiotic spindle assembly after NEBD as well.

Inhibiting PLK1 function also caused chromosomal misalignment in during metaphase I. When they investigated the cause of this misalignment, the researchers discovered a reduction in phosphorylation levels of BUBR1, which is essential for kinetochore function, and an increase in the number of microtubules that were not attached to kinetochores (a region of the chromosome that binds to microtubules). As the PLK1 was seen to localize to the kinetochores after NEBD under normal conditions, their findings suggest that PLK1 stabilizes the binding between kinetochores and microtubules.

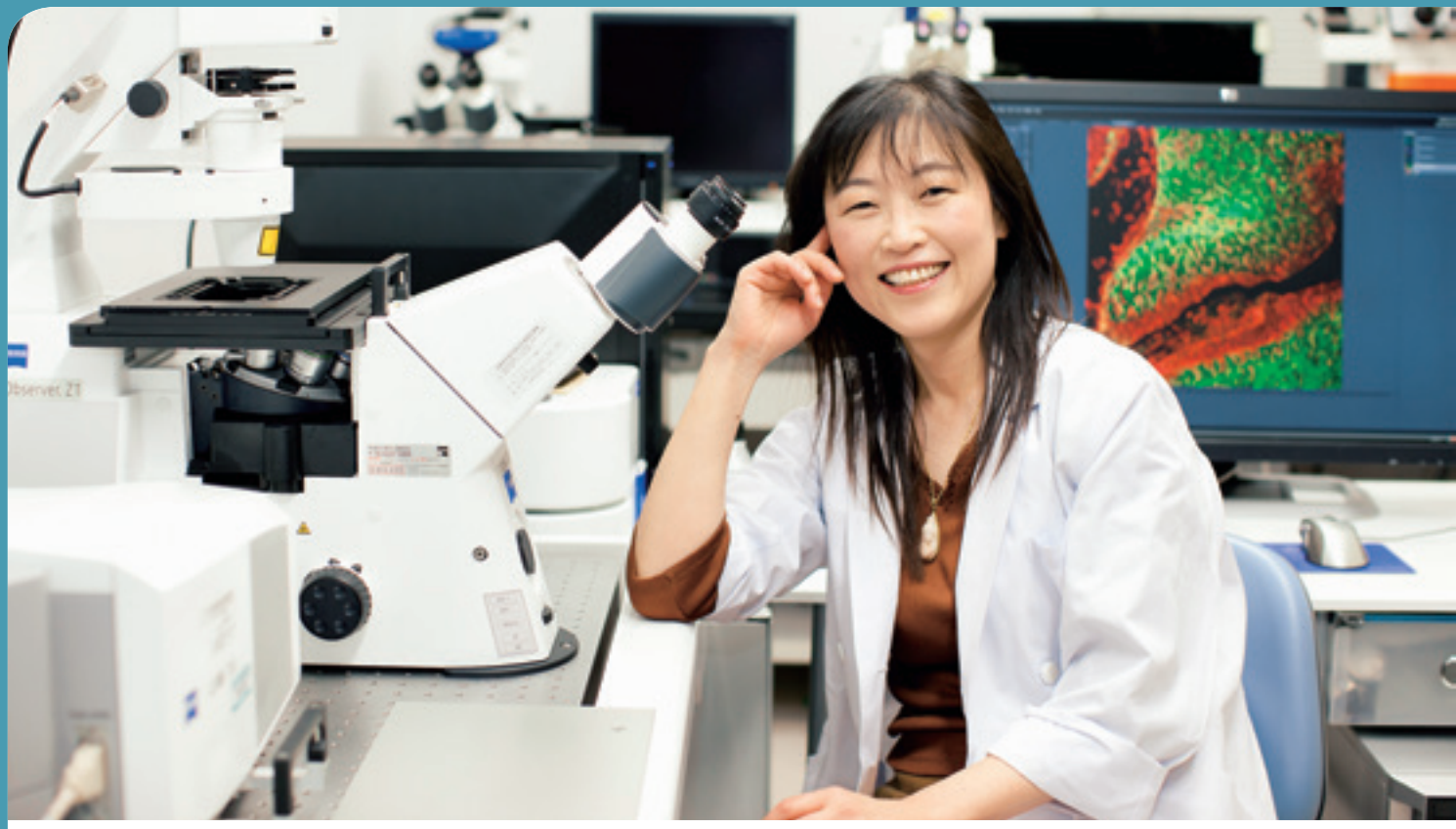
Normally, when problems occur in chromosomal alignment, the spindle assembly checkpoint (SAC), a mechanism preventing the onset of anaphase I, is activated thereby preventing oocytes from undergoing transition from metaphase to anaphase. In PLK1-inhibited oocytes, meiosis was arrested at metaphase. Upon closer examination, PLK1 was found to be essential for the degradation of EMI1, a molecule that prevents the APC/C from being activated to push oocytes to enter anaphase. In contrast to what is seen in somatic cell division, inhibiting SAC alone in oocytes does not resume cell division, thus APC/C activation via PLK1 is necessary for progression to anaphase. In addition, their experiments show that even after APC/C is activated and oocytes enter anaphase PLK1 contributes to chromosome segregation, maintaining chromosome condensation, and also cytokinesis.

"The present study shows that PLK1 is an important factor that directs multiple events during oocyte maturation, similar to PLK1 functions in mitosis; but there are also slight differences in its roles and contributions," says Kitajima. "Our joint research recently uncovered a binding partner of PLK1 called, Meikin, found in the kinetochores of oocytes which regulates meiosis-specific kinetochore function. Thus, the functions and contributions of PLK1 during meiosis appear to be regulated by factors uniquely assembled by the oocyte."



Timelapse images of oocyte undergoing first meiotic division. PLK1 (green) localizes to MTOCs and kinetochores (red). By anaphase I (far right panel), PLK1 accumulates at the spindle midzone.

Generating 3D cerebellar structure and functional cerebellar neurons



Keiko MUGURUMA

The brain's cerebellar cortex, which is associated with motor control, consists of multiple cell types arranged in a layered structure; from top to bottom, the molecular layer, the Purkinje cell layer and the granule cell layer. Purkinje cells, found in the thin Purkinje cell layer, have large cell bodies with highly arborized dendrites fanning out toward the molecular layer above and a long axon extending down to the deep cerebellar nuclei; they play a central role in processing information received from outside the cerebellum. Granule cells in the granule cell layer are much smaller and transmit information received from around the body to the Purkinje cells. These two cell types arise from two distinct regions of the developing cerebellum and later mesh together to form the layered structure of the cerebellum. While successful induction of certain cerebellar neurons in vitro have been reported, recapitulating the developmental processes to generate three-dimensional (3D) cerebellar structure has proven to be a challenge.

New work led by research specialist Keiko Muguruma of the Laboratory for Organogenesis and Neurogenesis (Masatoshi Takeichi, Team Leader), former group director Yoshiki Sasai (dec.), and other colleagues details a method to generate functional cerebellar neurons from human embryonic stem cells (hESCs) in vitro. They also show that these induced neurons can self-organize to form a 3D structure resembling the human cerebellar cortex seen in the early stages of development. Published in *Cell Reports*, their work demonstrates how the addition of essential growth factors at key time points can produce cerebellum-like structure in a culture system.

In a previous study led by Muguruma, the group successfully generated cerebellar neurons from mouse ESCs (mESCs) at relatively high efficiencies. There, using the SFEBq (serum-free culture of embryoid body-like aggregate) method developed by the lab, they induced the formation of Purkinje neurons by adding FGF2 and insulin to the medium. Further addition of BMP4 to the system produced granule cells; however, they were not able to generate Purkinje neurons and granule cells simultaneously.

In the current study, they initially attempted to induce cerebellar neurons from hESCs with the mESC method but met with difficulties; whether or not the ROCK inhibitor which promotes cell survival and reaggregation of hESCs was added to the 3D culture system, the resulting floating aggregates were extremely fragile and difficult

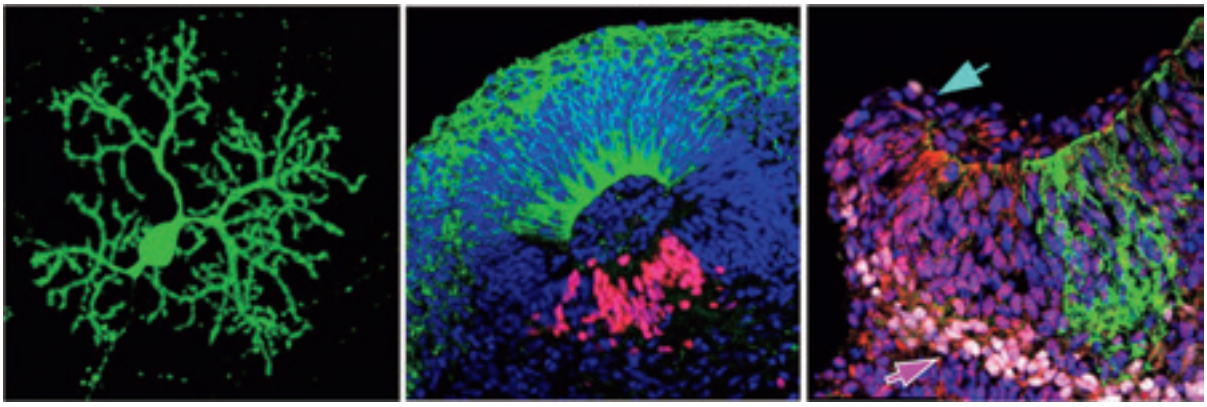
to culture long-term. This problem was solved by adding TGF-β-receptor blocker, a known neuroectoderm differentiation promoter. As with mESCs, Muguruma et al. then confirmed that adding FGF2 leads to the induction of isthmus organizer-like tissue in the hESC aggregates within 14 days in culture, and that by day 35, these aggregates also formed hollowed rosette structures within, which expressed markers in a pattern typically seen in cerebellar plate neural epithelium (CPNE) that later gives rise to cerebellum-specific neurons.

They next examined whether these CPNE cells have the potential to generate mature cerebellar neurons. As synaptogenesis between granule cells and Purkinje cells promotes Purkinje cell maturation, FGF2-treated aggregates were dissociated and cocultured with granule cells isolated from mouse tissues. Cells expressing an early differentiation marker for Purkinje cell progenitors differentiated into mature Purkinje cells, identified by their characteristic morphology and expression of late Purkinje-cell markers. Electrophysiological experiments on these hESC-derived Purkinje cells also demonstrated they function similar to naturally born Purkinje cells.

A closer look at the hollowed rosettes formed in the aggregates at day 35 revealed that while many were small and round, a few were relatively large and elliptical in shape. The elliptical rosettes expressed dorsal-specific markers on the outside and ventral-specific markers on the inside, resembling the dorsal-ventral axis seen in the neural tube. The team tested a range of conditions and found that adding FGF19 from day 14 promoted the formation of these elliptical structures. The further addition of SDF1, a factor linked to cerebellar development, at day 28 led to the formation of a large continuous neuroepithelial structure resembling the CPNE, rather than the formation of multiple elliptical structures inside the aggregate. SDF1 also promoted differentiation of the continuous neuroepithelium to generate a three-layered structure—the ventricular zone, Purkinje cell precursor zone and rhombic lip-derived zone—similar to normal cerebellar development. The periphery of one end of the hESC-derived neuroepithelium was also curled, and exhibited gene expression patterns seen in the rhombic lip which normally generates the granule cells in vivo.

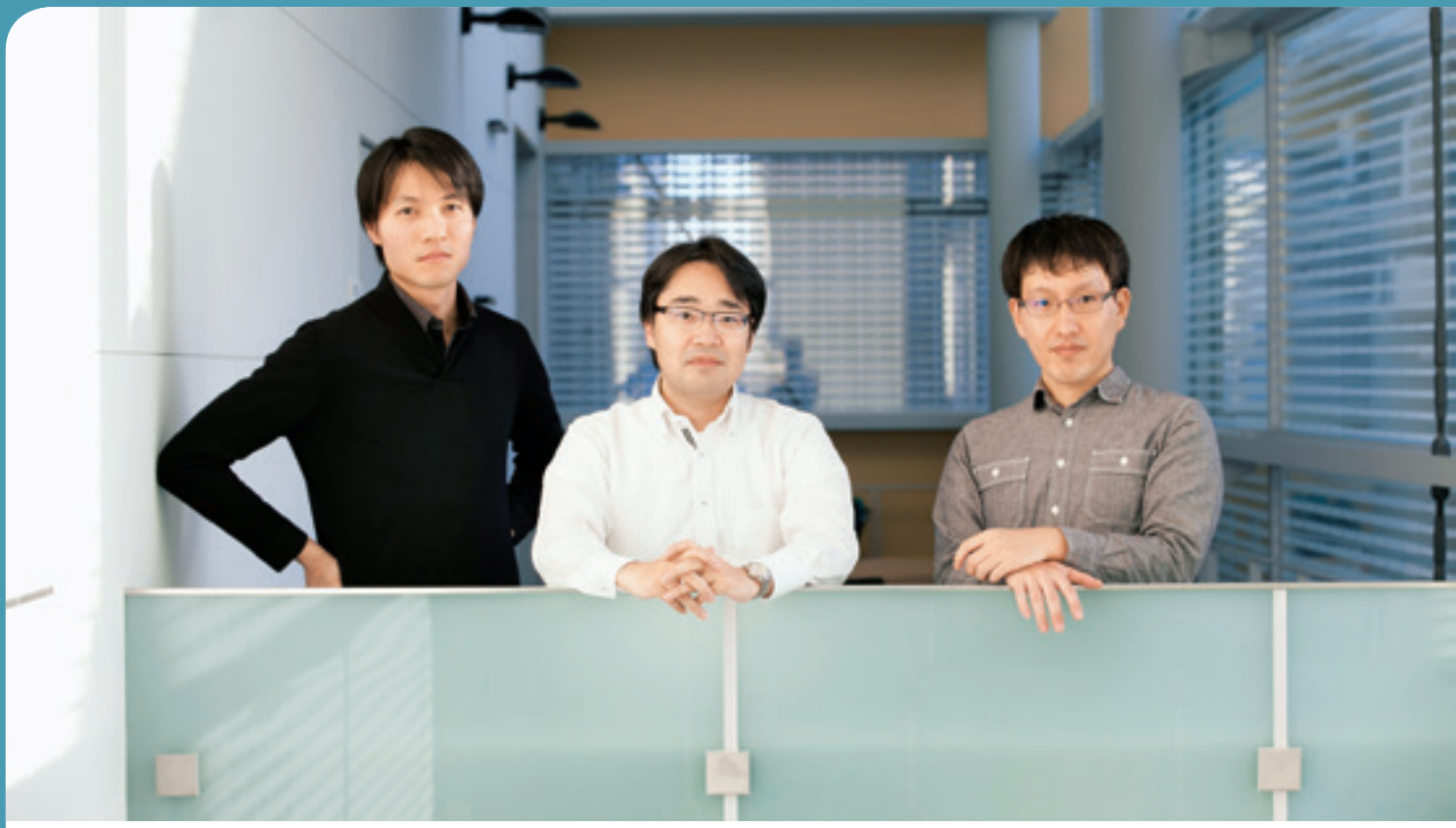
Muguruma and her colleagues are also attempting to establish cerebellar disease models from patient-derived hiPS cells by adapting their current protocol for human iPS cells. These attempts to generate cerebellar tissue in vitro could lead to understanding disease pathology or to applications in drug screening.

“Cerebellar development involves very dynamic changes in tissue morphology. So far we have only been able to generate embryonic cerebellum, roughly equivalent to the first trimester,” says Muguruma. “But, if we can produce a more mature cerebellum tissue in vitro, it may reveal the mechanisms underlying the dramatic morphological changes taking place during development.”



Left: hESC-derived Purkinje cell. Middle: Elliptical structures formed inside hESC aggregate, with D-V axis resembling the neural tube. Green, dorsal marker; magenta, ventral marker. Right: hESC-derived cerebellar cortex-like structure. Curled rhombic lip-like structure (blue arrow) generates granule cells, which migrate (pink arrow) to align with Purkinje cells (green).

Induction of retinal tissue with ciliary margin from hESCs



Chikafumi OZONE, Atsushi KUWAHARA, Tokushige NAKANO

During retinal development, a section of the neuroepithelium balloons outwards to form a sac-like optic vesicle, which then folds inwards to produce a two-layered optic cup. Cells in the outer layer of the optic cup differentiate into retinal pigment epithelium (RPE), while those in the inner layer differentiate into neural retina which includes the light-sensing photoreceptor cells. At the peripheral margin of the embryonic retina lies the ciliary margin (CM), the junctional region where the neural retina and the RPE converge. In birds, fishes, and some reptiles, the CM is known to maintain a stem cell population that contributes to retinal development and regeneration. Whether or not a similar stem cell population exists in the CM of the mammalian eye is unclear, and in humans, where access to the embryonic retina is difficult, even less is known about this region.

A new study carried out by Atsushi Kuwahara in the Laboratory for Organogenesis and Neurogenesis (Masatoshi Takeichi, Team Leader; laboratory closed March 2015), Mototsugu Eiraku, team leader of the Laboratory for In Vitro Histogenesis, and others shows evidence that strongly suggests the CM of the embryonic human retina also fosters a stem cell population which plays a key role in the growth of the retina. The team developed a protocol allowing them to drive human embryonic stem cells (hESCs) to spontaneously self-organize to form a three-dimensional (3D) retinal structure that includes CM-like regions at high efficiencies. They then found that the CM-like region is a niche for retinal stem cells and serves as a source of progenitors to produce new neural retinal cells. This work, published in *Nature Communications*, was a joint project with the Environmental Health Science Laboratory, Sumitomo Chemical Company Ltd.

The Laboratory for Organogenesis and Neurogenesis, under the direction of former group director Yoshiki Sasai, previously developed the SFEBq method, a 3D cell culturing method that can efficiently induce differentiation of pluripotent stem cells to form a neuroepithelial sheet through the addition of a cocktail of signaling factors. The signaling factors stimulate cells to spontaneous self-organize, further differentiate and produce 3D neural tissues, partially recapitulating in vivo early embryonic development processes. To date, they have generated tissues resembling cerebrum, hypothalamus, pituitary gland, and cerebellum. They also successfully generated 3D stratified retinal tissue from both mouse and human ESCs, which included a region at the neural retina–RPE

boundary containing cells distinct from both cell types. In the current study, the team tried to determine whether CM could indeed be generated in hESC-derived retinal tissue, and to uncover the role of the CM.

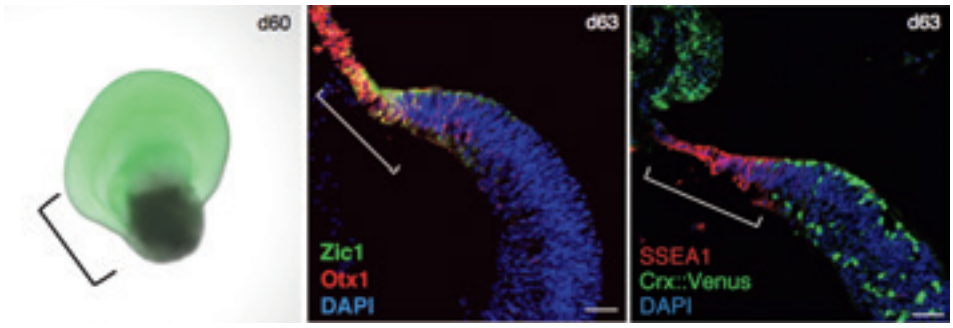
Kuwahara et al. first focused on improving the induction efficiency of the initial differentiation step from ESCs to retinal tissues. They found that the transient addition of BMP4 to the culture system when differentiation begins could drive ESCs to differentiate into retinal structures at markedly higher efficiencies and with greater stability, naming this improved differentiation method, the BMP method. This method also eliminated the need to add extracellular matrix proteins to the culture media, the addition of which poses difficulties when considering large scale production and future clinical applications. Analyses of retinal tissues generated with this method showed that most had gene expression profiles characteristic of neural retina, and not RPE.

They next speculated that if both neural retina and RPE could be produced inside the cultured cell aggregates, it might be possible to generate a CM, which in normal development, forms at the junction between neural retina and RPE. Past reports have indicated the importance of Wnt signals for RPE differentiation as well as maintenance, and FGF signals for neural retina differentiation and maintenance. Focusing on these two signaling pathways, the team attempted to create a two-domain retinal tissue containing both neural retina and RPE. They succeeded in establishing a protocol which could generate the said retinal tissue with high efficiency and reproducibility; BMP-method derived retinal tissue is treated for six days with Wnt agonists and FGF inhibitors to tip the differentiation bias toward RPE cell fate, and then placed again under conditions favorable for inducing neural retina. Interestingly, analyses of the derived tissue indicated that it was initially biased toward a neural retina fate, but then became transiently biased to an RPE fate after treatment with Wnt agonists and FGF inhibitors before resuming a neural retina fate. They called this the 'induction-reversal' method because of the swinging of cell fates between neural retina and RPE in the aggregates.

When retinal tissue generated via induction-reversal method was cultured over a two-month period, both the neural retina and RPE continued to grow, with the tissue eventually taking on a turnip-shaped form. The junctional region between the neural retina and the RPE of the derived retinal structure was distinctly thin and tapered, and expressed genes specific to the CM. Following three months of culture, the neural retina comprised numerous photoreceptor progenitors, and by five months, differentiated photoreceptor cells were present. The tapered CM-like region was also found to contain a large number of stem cells with high sphere-forming abilities, serving as a source of progenitors for the growing retinal tissues.

"Our results are consistent with the current view that RPE and neural retina are capable of fate transition. We hope to further unveil the mechanisms involved human retinal development through examining retinal formation in culture," says Eiraku.

Lead author Kuwahara adds, "The protocol developed here allows us to generate retinal tissue that more closely resembles the biological retina at higher efficiencies and with greater stability. It is a step closer to realizing regenerative medicine for retinal disorders, and we hope to continue advancing our research in this regard."



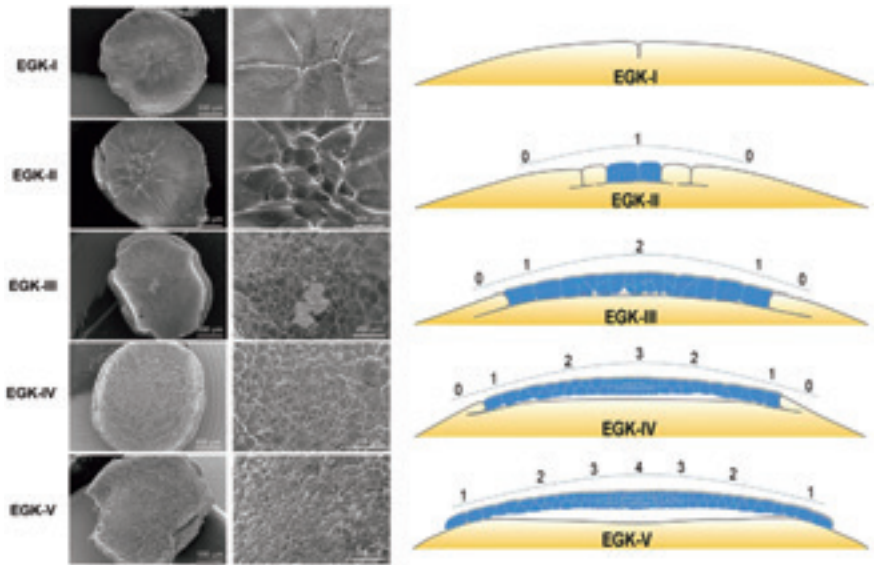
Left: Turnip-shaped hESC-derived retinal tissue using induction-reversal method at differentiation day 60. (Green, neural retina; Dark region, RPE) Middle and right: Formation of CM-like zone (white brackets) at border region between neural retina and RPE via spontaneous self-organization (differentiation day 63).

Chick embryo reveals conserved developmental mechanisms among vertebrates

The chick has long been a cherished model for studying development, owing to the ease with which the embryo can be accessed and manipulated inside the eggshell. However, much of its first day of development following fertilization, which occurs inside the hen before the egg is laid (oviposition), remains shrouded in mystery. Studies of fish and mouse embryos show that initial cleavage processes immediately after fertilization heavily influence cell fate decisions and morphogenesis at later stages, but whether similar developmental processes unfold in avian embryos in the early stages before egg-laying has not been closely examined before.

A new study by technical staff Hiroki Nagai of the Laboratory for Early Embryogenesis (Guojun Sheng, Senior Investigator) along with other collaborators, including Shigenobu Yonemura, head of the Ultrastructural Research Team at the RIKEN Center for Life Science Technologies (CLST), closes in on the early avian developmental processes that take place after fertilization but before egg-laying. Using both light and scanning electron microscopy (SEM), they carried out detailed observations of the developmental patterning of pre-oviposition chick embryos, providing the first look at early embryonic development that occurs before egg-laying. Their findings, published in *Development*, reveal several surprising similarities in early developmental patterning among birds and other vertebrate species.

In the 1970s, scientists Eyal-Giladi and Kochav created a developmental staging system for chick embryos (EGK stages), which classifies early embryonic stages based on morphological characteristics observed under the light microscope; the staging system divides developmental processes between post-fertilization to pre-primitive streak formation into 14 stages, from EGK-I to -XIV, with EGK-I to -X covering early embryonic stages before oviposition. In the 1990s, with the help of electron microscopy, Etches and colleagues were able to observe ultrafine structures of the later pre-ovipositional EGK stages (EGK-IV onward) in chick embryo. For the current study by Nagai and colleagues, they examined the morphological characteristics of early pre-ovipositional EGK stages (EGK-I to -V: post-fertilization to 8 hours) in detail using both light and electron microscopy, retrieving the fertilized eggs from the hens with a non-invasive, abdominal massage method.



Left: Images captured by SEM of chick embryos between EGK-I to -V. Cellularization of blastoderm as development progresses. Right: Schematic view of cellularization process. Subgerminal cavity beneath the blastoderm gradually expands as blastomeres become completely cellularized (blue).

Zebra finch embryo at EGK-VIII/IX. Syncytial nuclei forms near the boundary between the yolk membrane and subgerminal cavity.

Nagai H, et al. Cellular analysis of cleavage-stage chick embryos reveals hidden conservation in vertebrate early development. *Development* 142.1279-86 (2015)

During EGK-I, several cleavage furrows appear on the surface of the yolk on the animal pole side, forming multinucleate cells. At EGK-II, cells of varying sizes that have completed cellularization (compartmentation) are found near the center, and by EGK-III, these cells begin a series of rapid divisions and a space emerges between cellularized cells and yolk cells, called the subgerminal cavity.

The team then focused on two important events that occur in early developmental stages to look for possible similarities with other vertebrate species. One was zygotic gene activation (ZGA), when the embryo, which is initially dependent on maternal transcription products accumulated in the egg for development, switches over to its own developmental regulatory system by activating transcription of its own genes. ZGA is thought to be closely linked to epigenetic reprogramming mechanisms in cells, but when and how this occurs has not been well studied in avian species. Upon examining the chick embryos, they discovered that ZGA takes place between late EGK-II and early EGK-III (64- to 128-cell stages), similar to zebrafish embryos in which ZGA occurs at a the 128-cell stage. That ZGA occurs at similar developmental timing in chick and zebrafish, which both have yolk-rich eggs, suggests there is a common embryonic molecular mechanism at work.

The second event was one specific to unevenly distributed yolk-rich eggs such as chick and zebrafish, the formation of the yolk syncytial layer (YSL). Research in zebrafish have revealed that the YSL forms during the cleavage stage near the boundary where the embryo (blastoderm) contacts the yolk and contributes to the dynamic collective migration of blastoderm cells; whether a similar YSL forms in avian embryos has remained unclear. Nagai et al. carefully examined pre- and post-oviposition chick embryos and found YSL was present between stages EGK-V to -XI. Analyses of other avian species, such as finch and quail, also showed YSL formation at similar developmental timings as in the chick. These findings indicate that YSL formation is a common developmental phenomenon among birds and fishes.

“Several features of early embryonic development in chick closely resemble what is seen in zebrafish embryos,” says Sheng. “It will be interesting to determine whether the features conserved across species are a result of convergent evolution or of evolutionary constraints.”

Hiroki NAGAI

Improved donor organ preservation method for transplantation

Takashi TSUJI, Jun ISHIKAWA

Organ transplantation is an approach considered by medical specialists to replace an organ that can no longer carry out essential physiological functions. Despite many advances in transplantation research, there are still several hurdles that need to be overcome to improve success rates and access to organ transplantation. Static cold preservation systems are currently used to preserve donor organs prior to transplantation, but they can only maintain organs in healthy states for relatively short periods—from a few hours to less than a day depending on the organ. New methods to store organs in healthy conditions for longer periods are thus needed as this will improve transplant success rates. There is also a worldwide shortage of organs available for transplantation, as the number of patients waitlisted for transplants continues to grow with no corresponding rise in the number of suitable donors or donor organs, necessitating the development of methods to expand the use of organs from marginal donors, whose organ health may not be considered ideal for transplantation, to increase the donor organ pools.

Now, research associate Jun Ishikawa of the Laboratory for Organ Regeneration (Takashi Tsuji, Team Leader) together with researchers at the Tokyo University of Science, Keio University, and Organ Technologies Inc. have developed a new technique that can preserve donor organs for longer periods and can resuscitate organs retrieved from donors after cardiac arrest. Using the rat liver as a model organ for transplantation, they reveal a set of conditions that can preserve donor livers for up to two days while ensuring that liver physiological functions are recovered after transplantation. Their findings, published in *Scientific Reports*, imparts important insights toward establishing an efficient perfusion culture system to extend the storage period of donor organs, as well as to increase the donor organ pool by resuscitating organs donated from marginal donors, such as those who died from cardiac arrest.

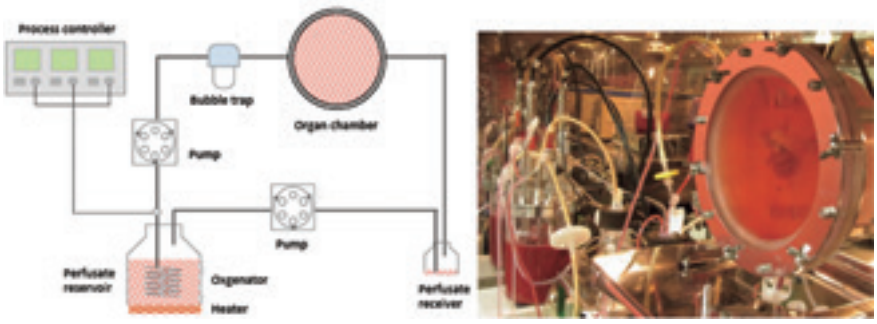
The group first designed a perfusion culture system, which simulates the circulatory system of the body, and placed a liver extracted from a rat into the system's organ chamber filled with culture medium, ensuring that the entire tissue was sufficiently perfused with medium. The liver was connected via its portal vein and inferior vena cava to their perfusion culture system. They tested a range of experimental conditions and found that a combination of cooling the culture to 22°C and adding red blood cells (RBCs), which serves as oxygen trans-

porters, to the culture media maintained liver functions for up to two days as confirmed by measuring concentrations of byproducts of physiological liver functions, such as albumin synthesis, urea, and bile. Under this condition, the levels of alanine aminotransferase, a protein marker indicative of hepatic disorders when high, were low, and tissue structures also remained intact, indicating that liver damage was suppressed. The team also determined that liver cells could survive and recover proliferative abilities after being cooled down to 22°C and then warmed back to body-temperature (37°C) conditions, whereas under other colder temperatures, the cells did not recover proliferative activities or died when returned to 37°C conditions.

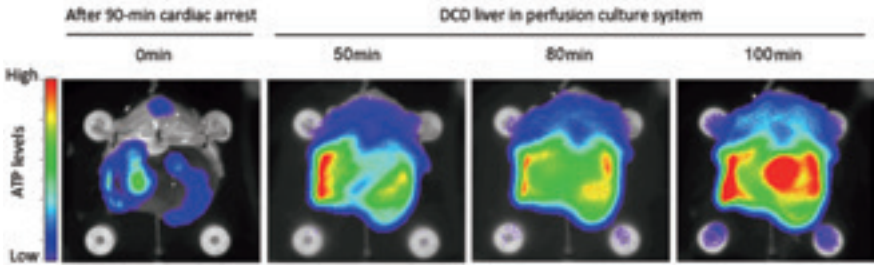
Next the group examined whether transplanting a liver cultured in their 22°C perfusion system for 24 hours could replace the function of the recipient's natural liver. They carried out an auxiliary liver transplantation, and compared effectiveness of using their system with or without addition of RBCs and of using conventional cold storage at 4°C on liver preservation period. Survival rates of rats transplanted with livers stored at 4°C or cultured in perfusion system without RBCs dropped dramatically after transplantation, whereas recipients of livers cultured in RBC-added perfusion system all survived. One week after the transplant, they removed most of the recipient's natural liver to confirm its regenerative abilities. Most recipients of livers stored at 4°C or in the perfusion system without RBCs did not survive, while those transplanted with livers cultured in RBC-added perfusion system showed 100% survival rates for an additional week. Normal metabolic functions were also maintained in the livers cultured in their RBC-added system.

Ishikawa et al. also tested whether their perfusion system could be used to resuscitate livers obtained from donors after cardiac arrest. Organs from individuals who have died from cardiac arrest are often not used for transplantation as oxygen and energy (ATP) needed for physiological functions are rapidly depleted due to lack of blood circulation to organs (ischemia), leading to tissue damage. They isolated the liver from a rat that had been in cardiac arrest for 90 minutes and placed it in their RBC-added perfusion culture system for 100 minutes. ATP levels in the liver dropped dramatically after cardiac arrest as expected, but after it was placed in the perfusion culture system, there was a marked increase in ATP levels. Survival rates of recipients of the perfusion culture-treated livers was 100% after two weeks, and even showed regeneration of tissue mass after partial removal of the recipient's natural liver. Thus, their findings indicate livers extracted from cardiac arrest donors can be resuscitated by the addition of RBCs to their perfusion culture system maintained at 22°C.

"This study demonstrates that longer organ preservation periods and resuscitation of ischemia-damaged organs is possible using our perfusion culture system," says Tsuji. "Our next step is to modify the system for pig models, which are genetically closer to humans, with the final goal of making this available for human clinical applications."

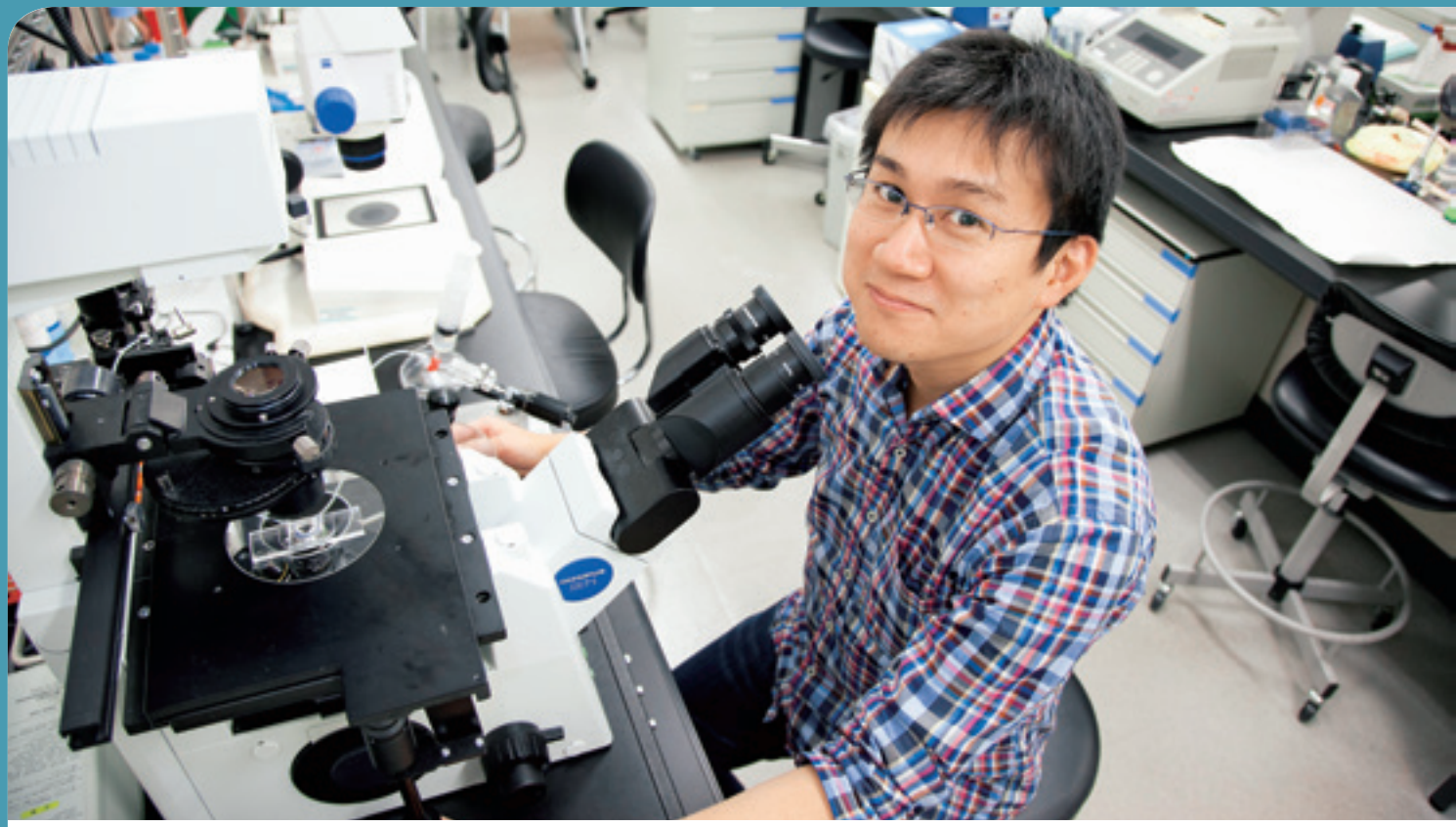


Setup of perfusion culture system. Image on right shows rat liver placed in organ culturing chamber.



ATP levels in liver drop after cardiac arrest, but recover when placed in perfusion culture system.

Surprising instability of meiotic cell division



Shuhei YOSHIDA

Oocytes display a surprisingly high frequency of errors in cell division despite being a vital means for animals, including humans, to pass on their genes to the next generation. During meiosis in oocytes, sister kinetochores (KTs), one located on each chromosome making up a homologous chromosome pair (bivalents), must correctly form stable attachments to the microtubules (MTs) and spindle apparatus for the bivalents to be properly segregated into two future daughter cells. Errors in the chromosomal segregation process in oocytes are known to cause congenital diseases such as Down syndrome or lead to miscarriages in humans. The mechanisms and reasons for the high rate of errors in chromosome segregation in oocytes remain largely unknown.

A new study by Shuhei Yoshida and colleagues in the Laboratory for Chromosome Segregation (Tomoya Kitajima, Team Leader) has now uncovered one of the causes of the high rate of errors seen in the first meiotic division (MI) in oocytes. They carried out a detailed analysis of the events during MI in mouse oocytes, specifically around the KT-MT attachment sites, and discovered that correct KT-MT attachments are initially relatively unstable due to the prolonged presence of Aurora B and C (B/C) kinase, an attachment destabilizer, around the attachment sites, contrary to what is observed in mitosis. Their findings, published in *Developmental Cell*, suggest that the modified features of KTs and chromosomes in MI make it difficult for the oocyte to pry the KTs spatially away from the influence of Aurora B/C, thus contributing to errors in chromosome segregation.

Both mitosis and meiosis require the formation of correct and stable KT-MT attachments for proper segregation of chromosomes. Past studies of mitosis suggest that stability of KT-MT attachments are linked to the regulation of KT phosphorylation, such as by Aurora B/C kinase which localizes to the inner centromere. In mitosis, MTs extending from opposite spindle poles form an attachment to KTs on the sister chromatids (bipolar attachments). The resulting tension draws the sister KTs apart, causing Aurora B/C kinase to become spatially separated from the KTs, leading to a drop in KT phosphorylation levels and stabilizing the attachment site. For MI of meiosis, MTs extending from the same pole form attachments with KTs on the bivalents (monopolar attachments), but the mechanisms at work to ensure correct, stable KT-MT attachments has not been closely examined. Despite the inherent differences in KT-MT attachments, a stabilizing

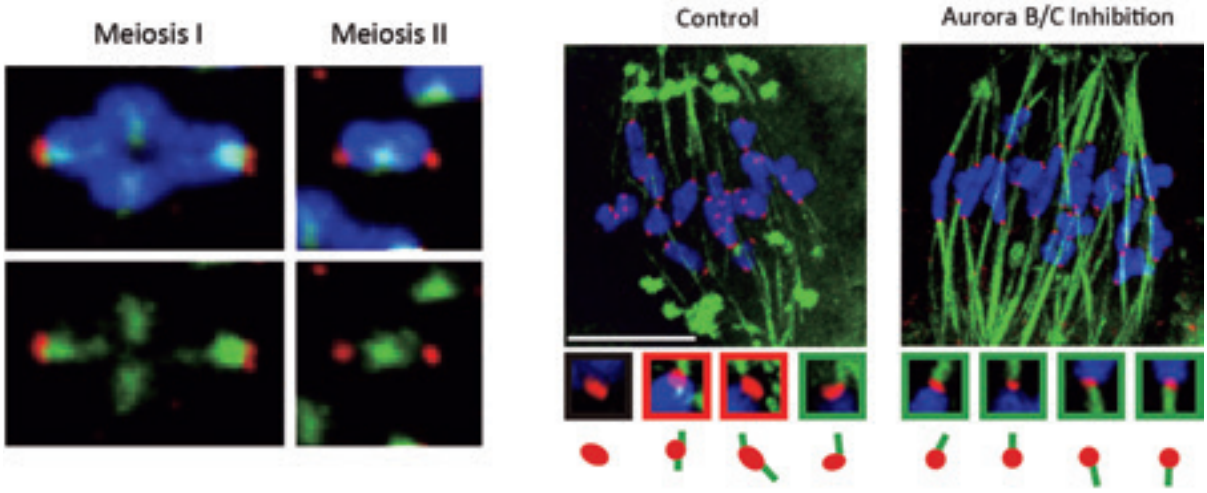
mechanism similar to that seen in mitosis has been proposed for KT-MT attachments in MI.

Yoshida et al. first turned their attention to the localization of KT-MT attachment destabilizer Aurora B/C kinase during meiosis. During early MI, MTs emanating from an apolar spindle attach to sister KTs on the bivalents and the spindle then begins to bipolarize causing the bivalents to be pulled and stretched in opposite directions, eventually forming a belt-like spatial arrangement. But, at this point, the KT-MT attachment remains unstable. Aurora B/C remained localized in the vicinity of the KT-MT attachments even after the KTs were pulled apart by the MTs. In contrast, during meiosis II, which unfolds similarly to mitosis, Aurora B/C kinase was localized at some distance from the KT-MT attachment sites when KTs were being pulled apart. When the oocytes were treated with an Aurora B/C kinase inhibitor as bivalents are stretched in MI, there was a marked rise in the number of correct KT-MT attachments formed, suggesting that the lingering Aurora B/C around the KT-MT attachment sites after bivalent stretching, as seen in MI, is a direct cause of the instability of KT-MT attachments.

How then are KT-MT attachments stabilized in the later MI stages? Close examination of changes in phosphorylation levels of KT-MT attachments during MI revealed high phosphorylation levels for some period after bivalent stretching, which then dropped markedly four to six hours later. These results suggested the workings of a molecular mechanism in MI that decreases phosphorylation levels at KT-MT attachment sites over a prolonged period leading to a gradual stabilization of these attachments without having to physically distance Aurora B/C from the KT-MT attachment sites.

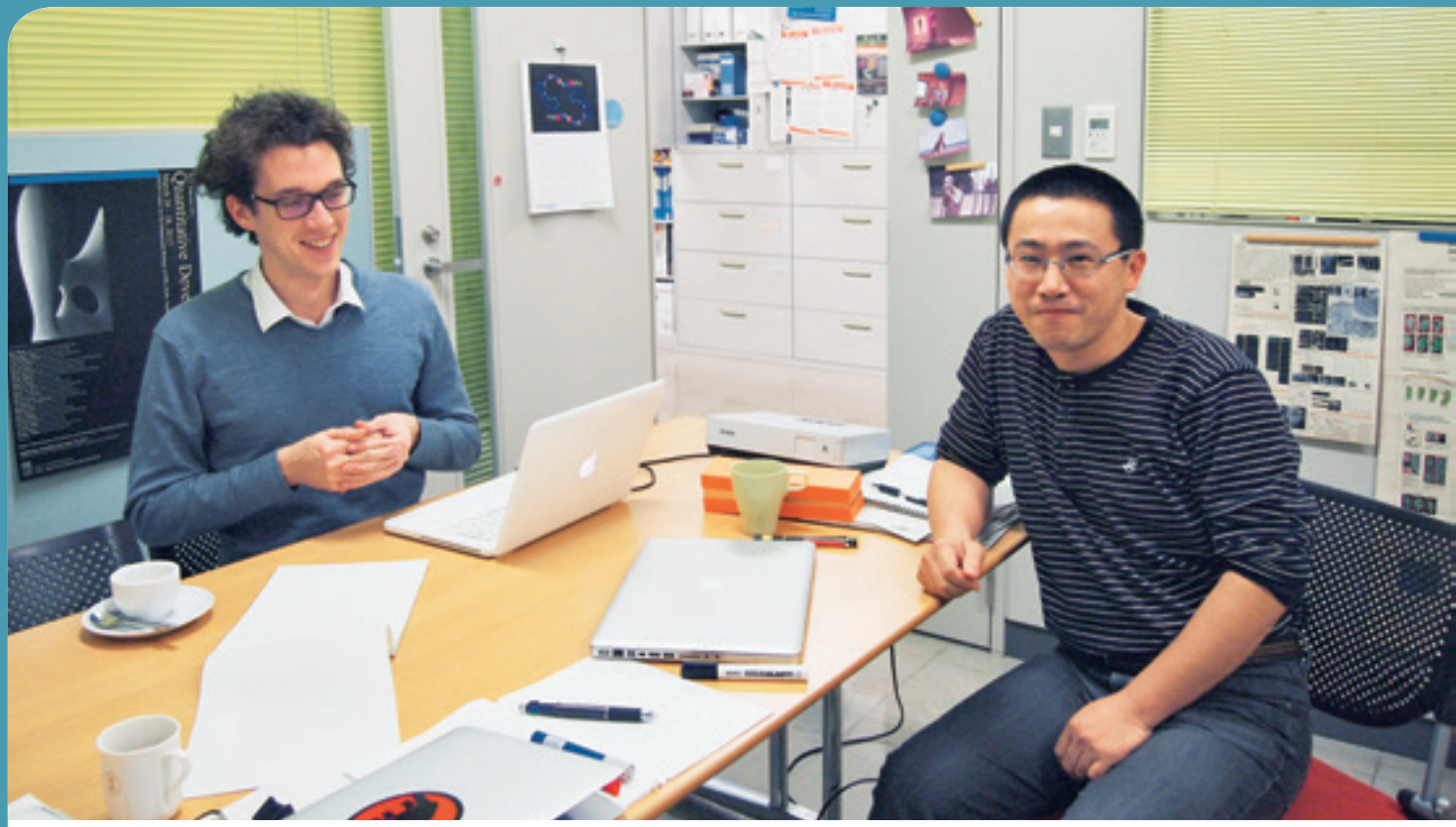
To search for the factor blocking the phosphorylating activity of Aurora B/C around KT-MT attachments, Yoshida et al. closed in on PP2A-B56, a phosphatase known to antagonize Aurora B/C activity by dephosphorylating KTs during mitosis. When localization patterns of PP2A-B56 during MI were analyzed, they found its concentration levels gradually increased over several hours around the KT-MT attachment sites. They next searched for the mechanism underlying the regulation of PP2A-B56 levels and found that Cdk1-dependent phosphorylation of BubR1, known in mitosis as a checkpoint protein, assists in the recruitment of the phosphatase to the vicinity of KTs. Gradual PP2A-B56 accumulation at KTs counters Aurora B/C activity, resulting in dephosphorylation of KTs and progressive stabilization of KT-MT attachment sites.

“Our study reveals the surprising instability of chromosome segregation in oocytes, and also a mechanism for stabilizing KT-MT attachments that is different from mitosis,” says Kitajima. “As the oocyte is the starting point of development, it has evolved many unique features. But we may be looking at an example of a biological paradox, if these features have unwittingly created a weak spot in basic intracellular activity.”



Left: Aurora B/C remains localized close to KT-MT attachment sites in MI than compared with MII, after chromosomes begin being pulled in opposite poles. (red, KTs; green, Aurora B/C complex; blue, chromosomes) Right: Inhibiting Aurora B/C activity results in an increase in correct KT-MT attachments. (red, KTs; green, MTs; blue, chromosomes).

Self-organized actin pattern in the tracheal tube



Edouard HANNEZO, Bo DONG

Periodic structural patterns, as seen in centipedes or woodlice, are fundamental body pattern forms that are also commonly observed in humans and other animals. How these periodic structural patterns arise is one enduring fundamental question of developmental phenomena. In the 1950s, Alan Turing, a brilliant mathematician and founder of the concept for modern computing, proposed that a reaction between two molecules diffusing at different speeds could produce a striped pattern autonomously without preexisting positional information, and that this molecular level self-organization was the basis of morphogenesis in living organisms. Many groups have since attempted to validate his theory through biological experiments, and to date it has been demonstrated at the cellular level to produce striped patterns in fish. However, little progress has been made to validate the theory in vivo at the molecular level.

New work by former research scientist Bo Dong in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader), now a professor at the Ocean University of China, in collaboration with Edouard Hannezo at the Institut Curie in France reveals a self-organizing mechanism of actomyosin in the cell cortex that produces the actin ring patterns in the tracheal tube of *Drosophila*. They demonstrate through mathematical modeling and experimental observations that actin ring patterns are self-organized via actin flows created by myosin contractility-induced forces and that these actin rings are stabilized by extracellular mechanical factors. Their study, published in *Proceedings of National Academy of Sciences*, establishes the relevance of Turing's theory in understanding biological phenomena.

In *Drosophila* tracheal tube formation, the epithelial cells are aligned with the apical side facing the lumen (interior) of the tube. As lumen formation begins, evenly spaced actin rings are generated along the tracheal tube, straddling the apical cell cortex (just below the cell membrane) of epithelial cells. A hard cuticular structure is then assembled around these actin rings after the larva hatches from the egg. Similar periodic structures are also seen in mammalian trachea and *C. elegans* embryos, which suggests that this is a fundamental mechanism for maintaining tubular or cylindrical structures.

How then is the actin ring pattern formed? To maintain the evenly spaced patterns beyond cell boundaries, the team speculated that there must be a mechanism functioning at the tissue level. Hannezo and Recho adopted

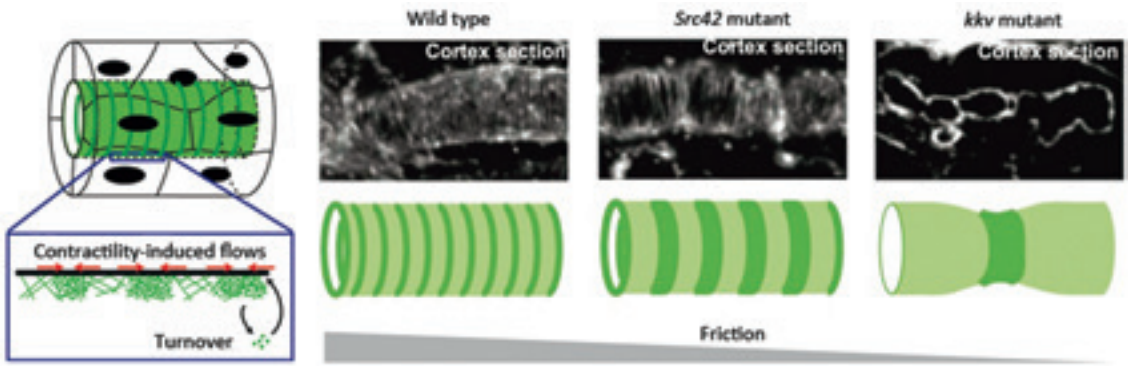
a theoretical approach using mathematical modeling to predict that in a viscous cytoplasmic environment, the repeated contractility-driven forces of the myosin motor protein causes actin flows, eventually giving rise to a periodic actin ring pattern.

Dong then set out to validate the model experimentally. They first analyzed *Drosophila* tracheal tube development using fluorescence live imaging, which revealed a gradual increase in actin concentration and appearance of circumferential supracellular actin rings, each cell with 15 to 20 actin rings. Because myosin II colocalizes with actin, the rings were speculated to arise from the binding of actin and myosin II to form the contractile actomyosin complex. Fluorescence recovery after photobleaching (FRAP) experiments confirmed that actin flow and turnover did take place in the cortex, and when developing embryos were treated with a drug inhibiting myosin contractility, the actin rings disappeared, indicating that myosin activity is required for tracheal actin ring formation. Thus, together these experiments validated that the basic assumptions of the mathematical model were supported in vivo.

In their model, friction within the cell cortex was postulated as a hypothetical factor influencing actin flows, playing a large role in orienting or positioning actin within the cortex. It predicted that actin would be assembled in a circumferential ring pattern when circumferential friction was slightly higher than axial friction, and that a decrease in friction would lead to wider spacing between the actin rings. Thus, Dong et al. proposed that the source of friction was the binding between the actin filaments, intercellular adhesion complex and cell cortex. They examined flies with mutations in a gene encoding a factor important for stabilizing the adhesion complex, and found that intervals between actin rings were wider in mutants than seen in the wildtype. In another experiment where actin-cell cortex binding was inhibited, they also found wider actin ring intervals. These results were consistent with the predictions made by their model, and demonstrate that cell cortical friction contributes to ring pattern formation in the tracheal tube.

Another potential source of friction was the binding between the cell cortex and solid extracellular matrix (ECM) that initially fills the tracheal tube. Chitin fibers, the main ECM component, are aligned in the tube in an anteroposterior direction, distributed in a manner enabling directional friction. They examined a fly mutant strain that cannot synthesize chitin and consequently has no chitin fibers in the developing tube, and observed that contractile forces exerted by actin rings were sufficient to cause localized constrictions of the tube. Intervals between actin rings were uneven and irregular, resulting in a tube with non-uniform circumference and several kinks along its length. These results suggest that under normal conditions where ECM fills the lumen, friction between the ECM and the cell cortex contributes to the stabilization of actin rings and uniformity of the tracheal tube. Thus, the actin dynamics observed in the tracheal tube are consistent with the behavioral predictions of their model when values for factors such as friction are manipulated.

“It was surprising to discover that molecular level self-organization, as proposed by Turing over 50 years ago, is one mechanism maintaining the uniform circumference of the tracheal tube throughout its entire length,” says Hayashi. “We hope to further investigate the physical laws associated with tissue level morphogenesis when supracellular structures that span the cells, such as actin rings, are controlled by extracellular factors.”



Left: Model of actin ring formation. Right: Actin rings formed along the cortex of tracheal tube. In mutants for a factor involved in stabilizing adhesion complex (*Src42* mutant) and those unable to produce ECM (*kkv* mutant), cortical friction decreased and intervals between actin rings were wider than seen in wild type. This is consistent with predictions of the mathematical model.

Anchoring the signaling complex needed for cell elongation

Tetsuhisa OTANI

Almost all cell types display some sort of polarity, resulting in a diversity of cell shapes and functional differentiation. Extensive and robust changes in morphology can arise from polarization of cellular components during development, which requires tightly regulated intracellular transport systems capable of shuttling and localizing proteins and other molecular cargo to specific destinations within the cell. One widely observed cell morphogenetic phenomenon is cell elongation, in which a cell elongates in one direction during its development. The distal tip of an elongating cell serves as a hub for the signaling complexes that regulate molecular cargo trafficking as well as cytoskeletal organization. While motor proteins are known to shuttle molecular cargo to the distal tip along the polarized cytoskeleton, how the signaling molecules that coordinate elongation are localized and maintained at the tip during elongation remain poorly understood.

A new study published in *Development* by Tetsuhisa Otani in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader) and his colleagues offers new insights into how distal tips of elongating cells are organized and maintained. Using the mechanosensory bristle of the *Drosophila* fruit fly as a model system, they demonstrate a mechanism regulating the correct transport and positioning of IκB kinase ε (IKKε), a protein kinase that plays a central role sorting molecular signals needed for elongation, near the distal tip of the elongating bristle.

In a previous work also led by Otani using the same model, they reported that once IKKε was localized and activated at the distal tip, it acts as a signaling center to sort the various molecular cargos transported to the tip. To solve the mystery of how IKKε is robustly maintained at the distal tip during elongation, the group combed through past publications for molecules that reportedly bind to IKKε.

Their search led them to the Spindle-F (Spn-F) protein, which has been shown to interact with IKKε as well as contribute to the localization of active IKKε. These two proteins were speculated to function as a pair due to similar polarity defects observed in oocytes of *spn-F* and of *ikkε* mutants. Upon closer examination of the respective mutants, Otani et al. discovered bristle morphology defects in both mutants; bristles were shorter, with some displaying multiple branching near the tip or bulges along the bristle. In normal bristles, activated IKKε and Spn-F

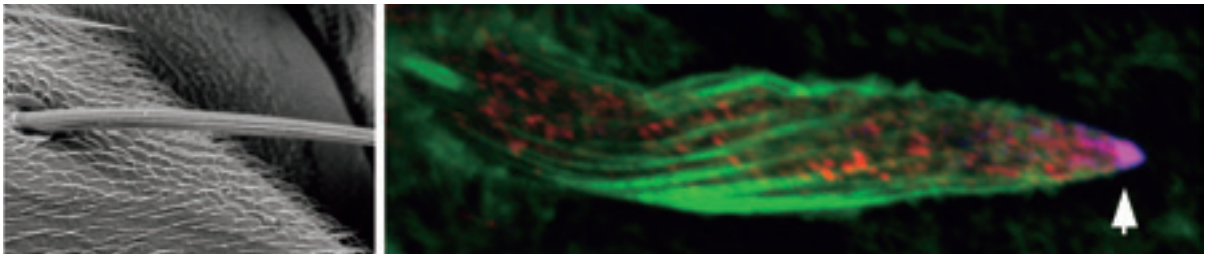
are found co-localized at the tip of the bristle, but Spn-F is also found dispersed in the cytoplasm. Time-lapse imaging experiments tracking cytoplasmic Spn-F behavior revealed that the protein moved along microtubules to the tip, and fluorescence recovery after photobleaching (FRAP) experiments also showed that Spn-F was stably localized to the distal tip once transported there.

How then are IKKε and Spn-F transported to the tip? Cytoplasmic dynein, a minus-end motor protein, was a prime candidate because microtubules in the elongating tip are oriented with the minus-ends facing the distal tip. Indeed, when dynein activity was inhibited in the bristle, both IKKε and Spn-F were unable to localize to the distal tip. When the domain regions of the Spn-F were analyzed, they found that cytoplasmic dynein and IKKε could bind simultaneously to Spn-F through distinct binding regions and that this simultaneous binding is crucial for cell elongation. Thus, Spn-F appears to serve as an adaptor protein linking IKKε to dynein, which transports them both along the microtubules to the distal tip.

Next, Otani et al. turned their attention to the mechanism underlying the sustained localization of IKKε at the tip after being transported there by cytoplasmic dynein. They examined the possible role of a protein called Javelin-like (Jvl) at the distal tip as it is known to interact with Spn-F and also contributes to the polarization of active IKKε in oocytes. In *jvl* mutants, IKKε and Spn-F colocalized at the distal tip during early stages of elongation, but in the later stages, they were no longer localized at the tip, and bristle tips showed morphological defects. Time-lapse imaging tracking the molecular dynamics of Jvl and Spn-F revealed that each molecule actively moves along the microtubules independently of the other, but when both proteins colocalize together, they stop moving. The interaction of Jvl and Spn-F thus results in their immobilization, and consequently immobilizes IKKε, which is bound to Spn-F.

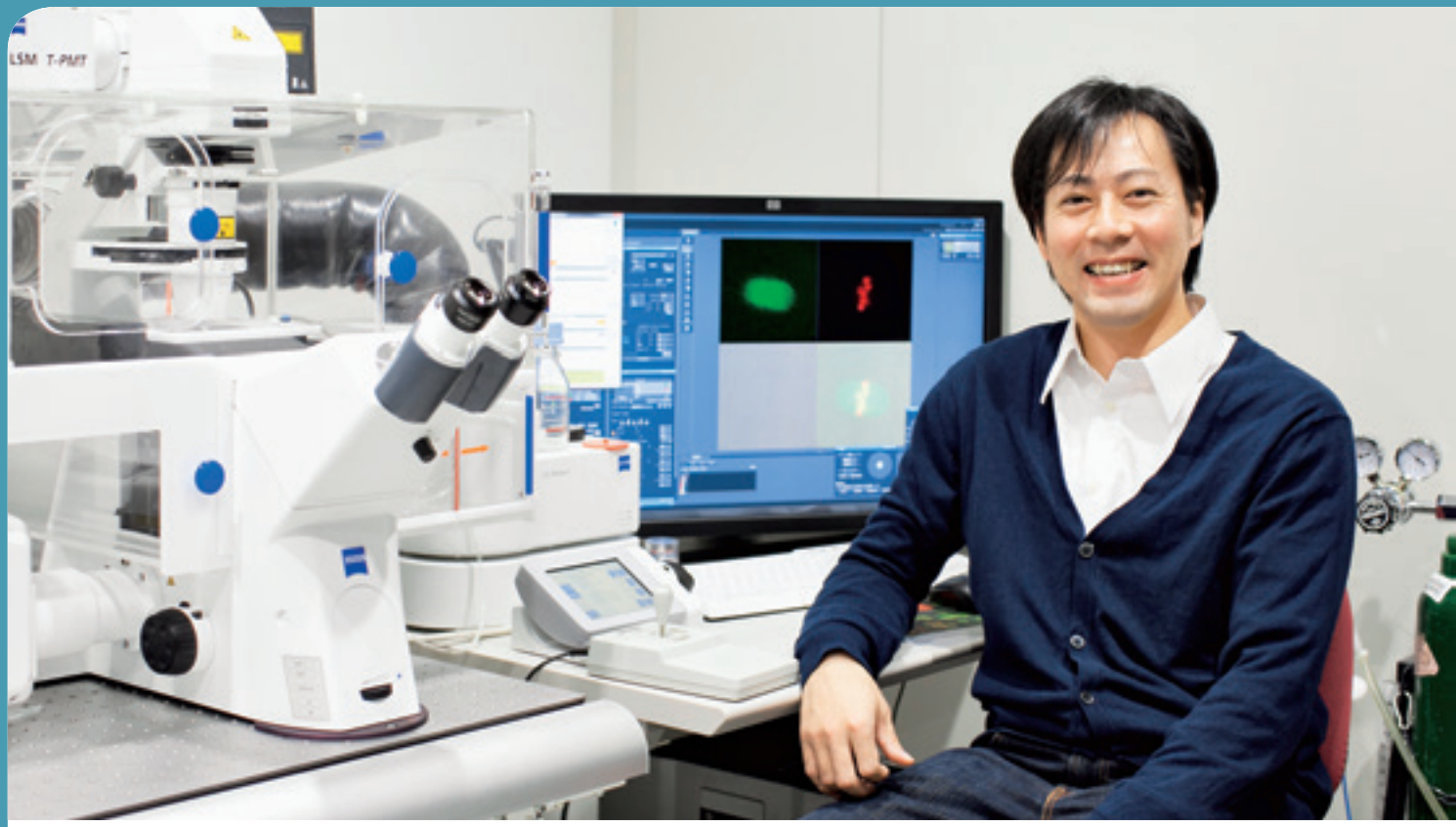
Based on their findings, they proposed that the IKKε-Spn-F complex and Jvl are transported independently to the distal tip in a unidirectional manner, and when Jvl interacts with Spn-F at the tip, the IKKε-Spn-F complex becomes anchored there.

“Spn-F is not just an adaptor molecule linking the motor protein to IKKε. It also serves as a signal to recruit factors that regulate dynamics of IKKε after being transported to the tip,” says Hayashi. “Examining different adaptor molecules may help us understand the elaborate intracellular transport system underlying the morphogenesis of more complex cell shapes such as mammalian neurons.”



Left: Electron microscopy image of *Drosophila* bristle. Right: Pupal stage bristle cell. IKKε (blue) and Spn-F (red) are colocalized near elongating tip (white arrow).

Leading cause of age-related aneuploidy in oocytes revealed



Yogo SAKAKIBARA

The germ cell, more commonly referred to as oocyte or sperm, is the only cell type in the animal body that contributes to the next generation by passing on its genetic information. Despite its importance in reproduction, oocytes display a surprisingly high rate of chromosome segregation errors as maternal age increases. When an oocyte with abnormal chromosomal numbers (aneuploid) is fertilized, it often results in a miscarriage or in congenital disorders, such as Down Syndrome, if it develops to term. While chromosome segregation errors are known to occur during the first meiotic division (MI) of oocytes, the exact causes and mechanisms remain unclear.

A new study published in *Nature Communications* by Yogo Sakakibara, a special postdoctoral researcher in the Laboratory for Chromosome Segregation (Tomoya Kitajima, Team Leader) and collaborators at the IVF Namba Clinic, Japan, and the Karolinska Institute, Sweden, takes a closer look at the chromosome dynamics during MI in both mouse and human oocytes. Using high-resolution 3D imaging microscopy to track chromosome movement, they demonstrate that premature separation of bivalent chromosomes during MI is a major cause of chromosome segregation errors observed in aging oocytes.

Germ cells arise from meiosis, a specialized type of cell division which reduces the chromosome number by half through two rounds of cell division, meiosis I (MI) and meiosis II (MII). During MI, maternal and paternal chromosomes pair and undergo synapsis to form homologous chromosome sets (bivalents); these bivalents then separate into univalents and are evenly divided into two daughter cells. In MII, sister chromatids of univalents are separated and are again evenly divided into two cells, resulting in haploid cells. A study published by Henderson and Edwards in 1968 reported the observation of a high frequency of univalents in chromosome spreads taken from aged oocytes undergoing MI, and proposed that this was likely the cause of chromosome segregation errors. Many studies following their work appeared to support this hypothesis; however, it remained difficult to prove directly due to small sample sizes and limitations in experimental methods.

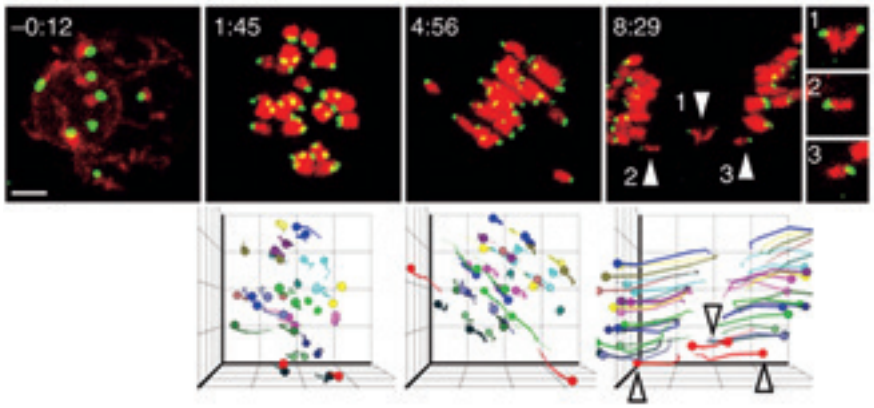
In the present study, Sakakibara and his collaborators successfully used high-resolution 3D imaging technology to examine and analyze chromosome dynamics in live oocytes during MI. They began by analyzing 275 oocytes obtained from naturally aged mice (16 months), and found that 20 of those oocytes exhibited errors in

chromosome segregation. They identified three distinct patterns of segregation errors. The first was balanced predivision (45%), in which both pairs of sister chromatids making up a bivalent are separated prior to MII. The second was unbalanced predivision (35%), in which one pair of the sister chromatids of a bivalent is segregated prematurely. In the third pattern, both pairs of sister chromatids fail to separate, resulting in nondisjunction (20%). In cases of balanced predivision, the oocyte appears as though normal chromosomal segregation has taken place after MI; however, errors will arise during MII. In contrast, aneuploidy can be detected after MI in oocytes that undergo unbalanced predivision or nondisjunction. These patterns of segregation errors in the mouse, are consistent with reports from human genetic studies and analysis of aneuploidy in human oocytes. Chromosome segregation errors were not observed in the 167 oocytes obtained from young mice (two months old).

Next, the group re-examined the chromosome dynamics to determine the underlying cause of these segregation errors. Univalents were found in 80% of oocytes exhibiting segregation errors during a period of MI when bivalents should be present. They discovered that while bivalents need to maintain their structure until segregation occurs in MI, some are unable to withstand the bipolar forces generated by the microtubules and consequently become hyperstretched until they eventually separate prematurely into univalents. The same bipolar forces bioriented the univalents, and in many cases, the sister chromatids of the univalents were mistakenly segregated in MI. These findings suggest that the glue holding the bivalent together until segregation is weakened, corresponding with recent reports of reduced levels of cohesin, a protein complex mediating cohesion between sister chromatids of bivalents, in aged oocytes.

Sakakibara and his colleagues also analyzed chromosome segregation dynamics in human oocytes. They obtained oocytes that would otherwise be disposed of from a fertility clinic, with the donors' consent, and found that, similar to the results seen in mice, oocytes from relatively aged donors exhibited premature bivalent chromosome separation during MI. The resulting univalents were then pulled to opposite poles, strongly suggesting that premature segregation of bivalents is also a cause of chromosomal segregation errors seen in humans.

"While our study clearly demonstrated the major cause of age-related chromosomal segregation errors observed in the mouse, we need to be careful when trying to interpret the data from human oocytes," explains Kitajima. "We are planning to examine the process of premature bivalent separation from a molecular perspective. In particular, we hope to reveal the mechanism underlying the reduction of cohesin in aged oocytes."



Chromosome dynamics in aged mouse oocyte during MI. Chromosome (red) localization was traced by tracking movements of kinetochores (green). One of the bivalents was hyperstretched by microtubule-generated bipolar forces and eventually separated prematurely into univalents (arrowheads). Univalents then underwent balanced predivision of sister chromatids.

Three patterns of segregation errors observed in oocytes from naturally aged mouse (16 months) during MI.

Just being naive? Early pluripotency in a bird model of development

Siu-Shan MAK, Anna WRABEL

Embryonic stem cells (ESCs) are perhaps the most recognized type of pluripotent stem cell derived from mammalian embryos, but they have a lesser known cousin as well, called epiblast stem cells (EpiSCs). Whereas mouse ESCs are obtained from the inner cell mass (ICM) at the blastocyst stage of development, and can give rise to all three germ layers as well as reproductive germ cells, their epiblast counterparts are derived from a later stage, after the embryo has implanted into the uterine lining, and no longer have the capacity for germ cell differentiation. In recent years, these variant states of pluripotency have come to be described as naive and primed, respectively, and the transition to a primed state in mouse is now known to be controlled by the activin and FGF pathways. Human ESCs in contrast are already in a primed state, and efforts to obtain naive ESCs from the ICM have not borne fruit, highlighting interspecies differences in pluripotency.

In a report published in *eLife*, Siu-Shan Mak (lead author) and Anna Wrabel (technical assistant), in a joint study by the Laboratories for Sensory Development (Raj Ladher) and Early Embryogenesis (Guojun Sheng) identified naive pluripotency in cells obtained from zebra finch eggs immediately after laying. This lays the groundwork for expanded studies into the establishment and maintenance of pluripotency across amniote species, not only mammals. Ladher and Sheng have respectively since moved to the National Centre for Biological Sciences (Bangalore) and Kumamoto University.

Chicken and quail are the most commonly used avian species in developmental research, but their embryos have already developed substantially by the time the egg is oviposited. In this study, however, the Ladher and Sheng labs recognized that the comparatively less advanced development of zebra finch embryos at the time of oviposition afforded an opportunity to look for naive pluripotent stem cells in bird.

The team first observed embryos at this stage and determined that they represented a state of development (stages 6–8) roughly comparable to the mouse blastocyst prior to epithelialization. Chicken embryos from laid eggs in contrast have already undergone this transition and correspond more closely to mouse epiblast.

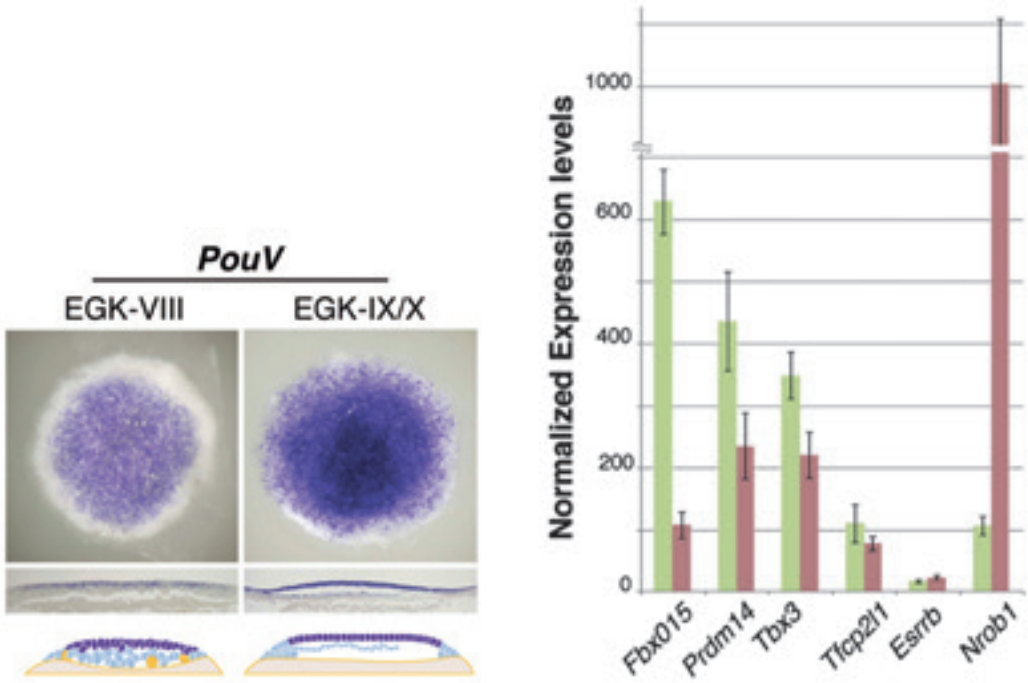
Looking at the molecular profiles of zebra finch embryos confirmed what their morphology suggested; not only did they express canonical markers of pluripotency such

Mak S-S, et al. Characterisation of the finch embryo supports evolutionary conservation of the naive stage of development in amniotes. *eLIFE* 4.e07178 (2015)
Mak S-S, et al. Zebra finch as a developmental model. *genesis* 53.669-667 (2015)

as *Nanog*, *PouV* and *DNMT3b*, but numerous markers of naive pluripotency as well. The expression of the latter markers dropped precipitously after stage 10, consistent with a clearly timed transition. Importantly, stage 6–8 embryos also expressed germline markers, which lines up well with another feature of naive pluripotency in mouse ESCs. Interestingly, markers of naive pluripotency were also observed, although at generally lower levels, in cells from chicken embryos at a later stage (~10) of development.

These findings point to the prospect that naive and primed pluripotency may be conserved across amniote taxa, not only in mammals. “We’re hoping to one day identify bona fide pluripotent stem cells from zebra finch embryos, which would enable the study of mechanisms behind the establishment and maintenance of pluripotency in a much wider range of model systems,” says Ladher.

This new work involved not only performing cell and molecular biology experiments in zebra finch embryos, but establishing the bases for rearing and housing zebra finches under laboratory conditions. The same joint team published their methods for zebra finch breeding in the October issue of *genesis*, in the interests of adding new experimental options for avian embryological research.



Left: Expression of *PouV* in stage 8–10 zebra finch egg. Right: Expression levels of naive pluripotency markers in cells from zebra finch (green) and chicken (red) eggs.

Nutrient-dependent regulation of growth control hormone revealed

Naoki OKAMOTO and Takashi NISHIMURA (second row, fourth and third from the right)

Insulin/insulin-like growth factor (IGF) signaling plays an important role in the regulation of biological processes such as growth, metabolism, reproduction and longevity, and is known to be widely conserved across species. In mammals, major players of this pathway are insulin and IGF, which have distinct roles in metabolism and growth, respectively, whereas in the *Drosophila* fruit fly, there are eight insulin-like peptides (Dilps) that fulfill these roles. The function and regulation of these endocrine hormones must be capable of readily adapting to environmental changes, particularly to nutrient availability, but the mechanisms by which organisms can sense these changes to regulate hormone levels remain poorly understood.

In new work published in the journal *Developmental Cell* by former research scientist Naoki Okamoto and team leader Takashi Nishimura in the Laboratory for Growth Control Signaling, they examine the function of nutrient-dependent growth control hormone, Dilp5, and the mechanisms regulating its production in *Drosophila*. They pieced together their findings to reveal a complex signaling relay and positive feedback mechanism at work to control the expression of *dilp5* in the insulin-producing cells of the fly brain. Okamoto has since moved to the University of California, Riverside, to continue his research.

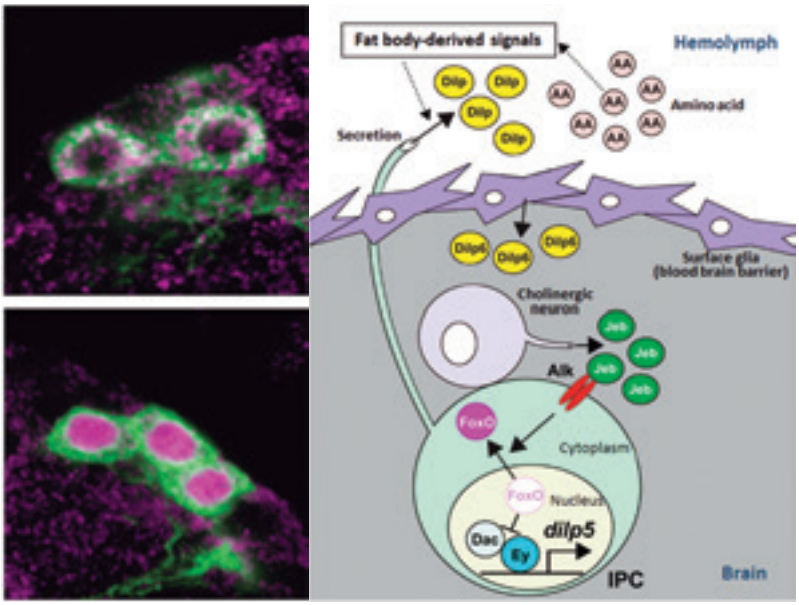
In *Drosophila*, Dilps are primarily secreted by insulin-producing cells (IPCs) in the brain in response to a signal from the fat body (insect equivalent of vertebrate liver) which acts as a sensor of nutrient availability. The secreted Dilps then circulate throughout the body to act on target tissues. One of the main Dilps produced by IPCs is Dilp5, an important regulator of body growth. Expression levels of *dilp5* are initially low immediately after larval hatching, but rise sharply as larvae begin to feed, and expression levels are also known to fluctuate depending on availability of nutrients in the surrounding environment. A past study by Okamoto and his colleagues demonstrated that *dilp5* expression was stimulated through the synergistic action of two transcription factors, Eyeless (Ey) and Dachshund (Dac), in IPCs. However, it remained unclear how Ey and Dac were tied to nutrient-dependent changes in *dilp5* expression.

In this study, the team first tested different nutrient mixtures to determine the critical nutrient source regulating *dilp5* expression was amino acids. Target of rapamycin (Tor) signaling, a well-known amino acid sensing path-

way, was analyzed as a potential upstream signal regulating IPCs, but the disruption of various factors in this pathway showed no effects on *dilp5* expression. They shifted their focus to search for a nutrient-dependent molecule that could regulate the Ey-Dac protein complex, and transcription factor FoxO surfaced as a prime candidate because its activity is known to be regulated by nutrient availability, and can both activate and down-regulate expression of a diverse array genes. Starvation experiments revealed FoxO localization moved from cytoplasm to nuclei of IPCs, and that nuclear FoxO can interact directly with Ey, thereby competing with Dac for binding to Ey. Thus, localization of FoxO within the cell can switch expression of *dilp5* on and off.

How then is FoxO localization regulated? They screened for potential signaling factors in the IPC to uncover the activation of anaplastic lymphoma kinase (Alk) leads to FoxO localization in the cytoplasm and promotes dilp5 expression. Alk was in turn found to be activated by a secreted ligand called Jelly belly (Jeb), discovered to be produced by cholinergic neurons. Thus, Jeb secreted by cholinergic neurons surrounding the IPC regulated FoxO localization indirectly. Further experiments showed that Dilp6 produced by surface glial cell layer of the *Drosophila* central nervous system, which functions similarly to the blood brain barrier of mammalian brain, was responsible for regulating *Jeb*. Surface glial cells are exposed to the hemolymph, and IPC-derived Dilps (Dilp2, 3, 5) released and circulating in the hemolymph stimulate *dilp6* expression in glial cells, initiating the indirect signaling relay to enhance *dilp5* expression in the IPC through a positive feedback loop.

"It's puzzling why *Drosophila* use such a complicated mechanism. Under poor food conditions, *dilp5* mutants showed reduced growth rates and smaller adult body sizes than wildtype, and required longer time for larval development, suggesting that the multistep *dilp5* regulatory mechanism is important to maintain insulin signals needed for promote growth, even under nutrient-restricted conditions," says Nishimura. "The fact that FoxO and cholinergic neurons are also involved in insulin regulation in mammals suggests the mechanism revealed in this study has been conserved throughout evolution. We plan to further explore the elaborate survival strategies that organisms have developed to adapt to environmental changes."



Left: FoxO in IPCs is shuttled from cytoplasm to the nucleus under starved conditions (green, IPC (dilp2); pink, FoxO). Right: Model of the regulatory mechanisms of nutrient-dependent *dilp5* expression.

Hippocampal neurons from hES cells

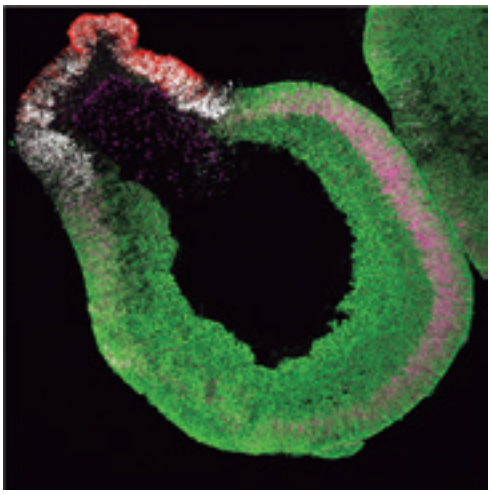
The hippocampus is the seat of memory formation and learning, and disturbances of hippocampal function are known to lead to cognitive and psychiatric disorders, making this a brain region of fundamental importance in a range of biomedical research fields. In developmental terms, the hippocampus emerges through the activity of an organizing center on neighboring tissues, but its location deep in the embryonic brain makes it difficult to study experimentally, and the mechanisms by which hippocampal cells are induced remain unclear.

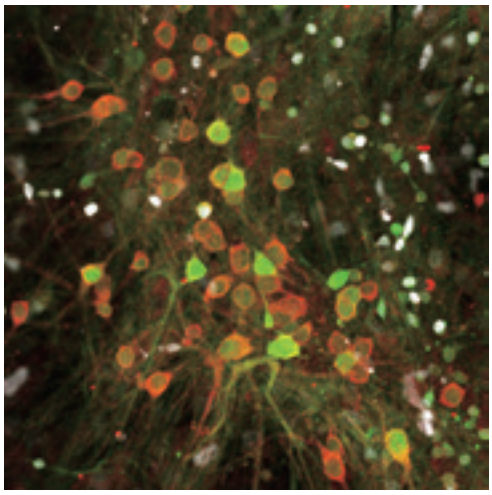
Now, junior research associate Hideya Sakaguchi and others from the Laboratory for In Vitro Histogenesis (Mototsugu Eiraku, Team Leader) and the former Lab for Organogenesis and Neurogenesis (Yoshiki Sasai, dec.) report the induction of self-organizing hippocampus-like tissue from human embryonic stem cells (hESCs). Published in *Nature Communications*, this work provides a new experimental resource for studies ranging from hippocampal development to Alzheimer's disease.

This new report represents the most recent in a series of experiments aimed at inducing neuronal differentiation from pluripotent stem cells stretching back more than a decade, and makes use of the SFEBq (serum-free floating culture of embryoid body-like aggregates with quick reaggregation) method developed by the same group. In this study, the group sought to adjust SFEBq conditions to steer hESCs toward the primordial tissue in the embryonic brain (called medial pallium) from which the hippocampus arises.

Using a previously reported SFEBq technique for inducing self-organized cerebral cortex-like tissue, Sakaguchi sought to develop a cocktail of factors delivered at the right times and dosages to trigger hippocampal differentiation from hESCs. By tweaking the levels of Wnt and BMP4, dorsalizing factors that strongly influence neuronal differentiation, the group was able to induce the expression of genetic markers of choroidal plexus, the neighboring cortical hem, and finally medial pallium, suggesting that a self-organized form of differentiation was occurring in the same chronological order seen in normal development.

They next attempted to generate hippocampus-like tissue from the self-organized medial pallium. By experimenting with different culture media and conditions, they gradually discovered a reliable method for directing the medial pallium into hippocampus, as evidenced by marker gene expression. As these cell colonies begin to destabilize by about 100 days in vitro, they dissociated them into individual neuronal cells between 73 and 84 days of culture and followed their developmental course. After isolation, these individual cells re-aggregated, and by day 197 around 75% expressed markers, indicating hippocampal differentiation. Moreover, markers of two distinct neuronal cell types—pyramidal and granular—were each observed in about one-third of the total popu-





Neuroepithelium at day 42 in vitro. Cortical hem (negative for TTR and Lmx1a) and medial pallium (green, Foxg1::Venus; magenta, Lef1) form sequentially adjacent to the choroid plexus (red, TTR; white, Lmx1a) (left). At day 179 of culture, granule (Prox1, white) and pyramidal (KA1, red) neurons are seen (right).

Sakaguchi H, et al. Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. *Nat Commun* 6.8896 (2015)

lation, and glial- and astrocyte-like cells were present as well.

Tests of neuronal function by patch-clamping confirmed that the differentiated neuronal cells possessed working synapses, and calcium imaging at day 143 showed synchronized depolarization, suggesting that the hippocampal tissue was exhibiting network-like activity.

“The ability to recapitulate hippocampal development in vitro is a significant step forward,” says Eiraku. “We look forward to the use of this system not only to study biological phenomena such as patterning and regionalization, but as a resource in biomedical research into diseases like Alzheimer’s and schizophrenia as well.”

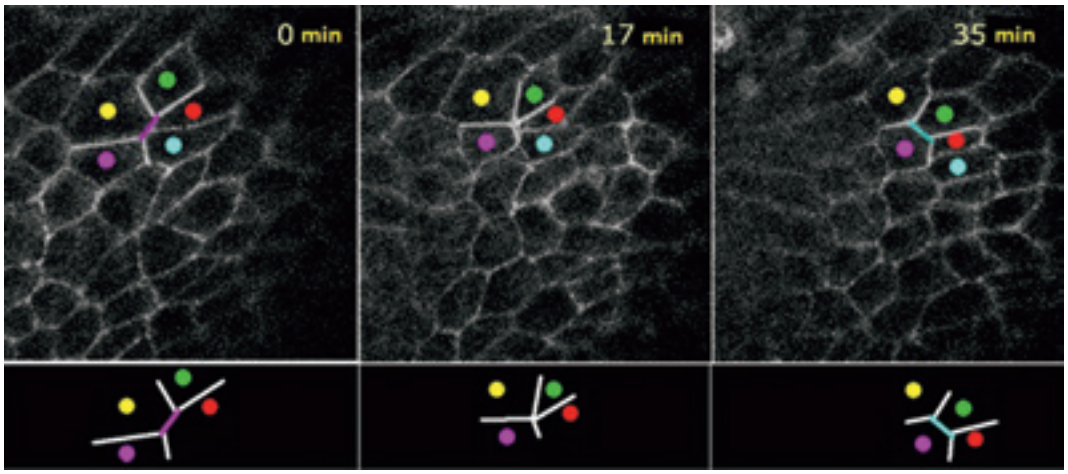
Hideya SAKAGUCHI, Mototsugu EIRAKU

Putting a new spin on autonomously organized epithelial movement

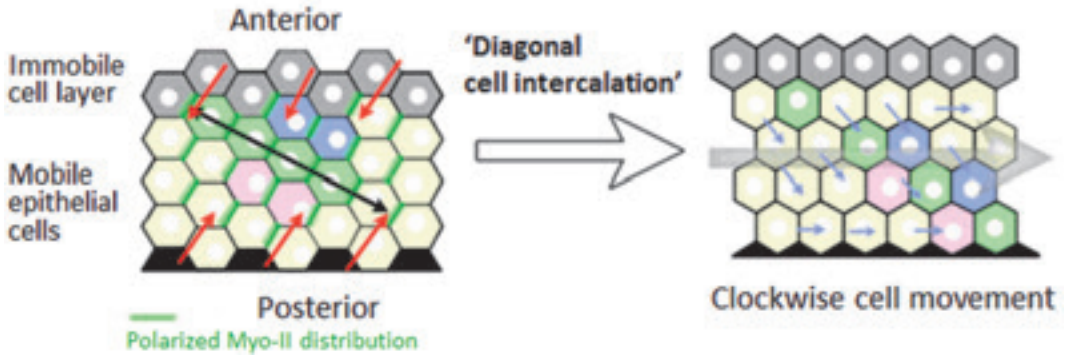
The bending and stretching of sheets of epithelial cells into channels, tubes, and other topologies is a central process throughout much of development. In the pupa of *Drosophila* fruit flies, an interesting phenomenon occurs in which the cells of the male reproductive organ (terminalia) rotate consistently in the same direction with respect to the anterior-posterior axis, resulting in the wrapping of the spermiduct around the hindgut. In order for the terminalia to make its circuit, the surrounding epithelial cells must also move as a group in the same direction. But how they do this without a clear 'conductor' to coordinate their movements has been a mystery.

A new report in *Nature Communications* from the Laboratory for Histogenetic Dynamics (Erina Kuranaga, Team Leader) working with the Laboratory for Physical Biology (led by Tatsuo Shibata) in the RIKEN Quantitative Biology Center (QBiC) shows how epithelial left-right asymmetry can give rise to such autonomous cellular movements.

The pupal genitalia are located in the tail region, and their rotation is made possible by the coordinated movement of epithelial cells in abdominal segment eight (A8) that connect the terminalia with the abdomen. This A8 segment is divided into anterior (A8a) and posterior (A8p) components. Over 38 hours following the first 24 hours after puparium formation, first A8p makes a 180° turn, after which A8a completes the rotation by turning the remaining 180°. In this new study, the team began by isolating these tissues and observing them in culture to see whether they would retain their ability to rotate. They



Cells in adjacent epithelium show rightward-leaning positioning, as revealed by changes in the positions of cells marked by colored dots.



A model of unidirectional movement by left-right asymmetrical repositioning of cells. Accumulation of Myo-II at right-leaning cell boundaries results in directional contraction and rearrangement of cell positions.

Sato K, et al. Left–right asymmetric cell intercalation drives directional collective cell movement in epithelial morphogenesis. *Nat Commun* 6.10074 (2015)

found that, even isolated from the rest of the abdomen, A8 epithelium still rotated autonomously.

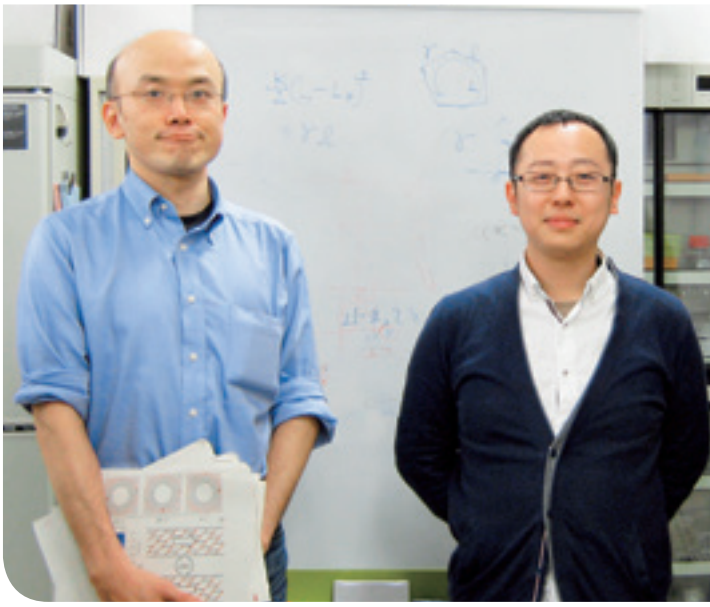
They next sought to follow the changes in the positions of individual cells by labeling cell boundaries. While these cells maintained their cell-cell junctions, what the team found was that they constantly changed their borders and positions relative to each other throughout the rotation process. And intriguingly, the rearrangement of apical cell-cell boundaries showed a distinct rightward slant with respect to the A-P axis.

When the team examined the localization of the motor protein Myo-II, known to play a role in apical contraction, they found that while its distribution was not polarized asymmetrically within a cell prior to the start of rotation, its polarization showed a clear right-handed preference before and during the rotation of A8a. Higher concentrations of Myo-II were also associated with higher levels of left-right asymmetry, suggesting that the lopsided distribution of this factor might play a part in driving epithelium to move en masse.

They looked at Myo-II in a fly mutant for Myo ID, in which the terminalia rotates opposite to the ordinary direction. In these cells, Myo-II tended to be more highly accumulated in left-leaning cell boundaries, again suggesting a role for this factor in coordinating epithelial movement at the group level. Finally, a mathematical simulation of the effects of left-right asymmetry gave further computational support to the notion that such rearrangements alone can give rise to autonomous movement by groups of cells, which can be made to be unidirectionally rightward when the cells are neighbored to the anterior by other immobile cells.

“These new results were made possible by combining advanced live imaging and quantitative modeling techniques,” says Kuranaga. “This finding of how left-right asymmetry can drive autonomous epithelial movements shows promise beyond the fruit fly, and may be of fundamental importance in a number of organogenetic processes in other species as well.”

Ayako ISOMURA, Emi MAEKAWA, Erina KURANAGA



Katsuhiko SATO, Tetsuya HIRAIWA

Pulmonary neuroendocrine cell clusters at airway branches

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

The cellular composition of respiratory epithelium varies in different sections of this highly branching organ system. The airway, for example, contains numerous ciliary cells capable of sweeping out foreign material, while Club cells that secrete surfactant protecting the airway surface dominate. A third type, neuroendocrine (NE) cells, has been found to aggregate at branch points in the airway in many different species, but the developmental process responsible for this localization has remained unknown.

A new study by research scientist Masafumi Noguchi and others in the Laboratory for Lung Development (Mitsuru Morimoto, Team Leader) uses single cell resolution 3D time-lapse imaging of an entire lung lobe to show how NE cells cluster at airway bifurcation points in the mouse lung. They also find that Notch signaling plays a critical role in inhibiting NE cell differentiation, thus limiting the numbers of such cells. This work was published in the December issue of *Cell Reports*.

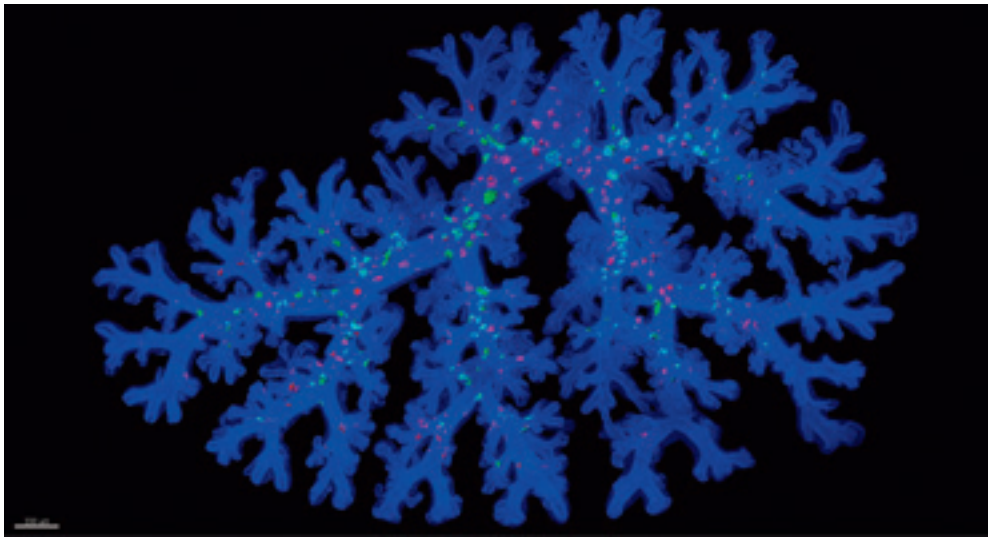
In order to overcome the historical challenge of observing minute changes in the developing lung over time, the team first generated a mouse in which epithelial cell nuclei and NE cells were labeled with different genetic fluorescent reporters. They next used two-photon microscopy to visualize whole-mount preparations of cleared fetal lung at high precision and tissue depth, and used these images to generate a 3D model of the entire airway, making it possible to analyze the sizes and spatial relationships of NE cell clusters, which they found indeed form following a consistent pattern.

Previous works by the Morimoto team and other groups have shown that Notch-Hes1 signaling is involved in NE cell cluster formation, so in the new study they used a *Hes1* knockout mouse to investigate how this would affect the differentiation of emerging NE cells. In wild type embryos, NE cells appear as solitary cells throughout the airway, not just at branch points, in a salt-and-pepper distribution from about day 13.5, but in the *Hes1* knockout the number of NE cells clearly increased, with greater numbers of neighboring cells undergoing NE cell differentiation, and much larger clusters of NE cells at bifurcation points by day 16.5. This suggests that the Notch signaling pathway plays a role in suppressing the

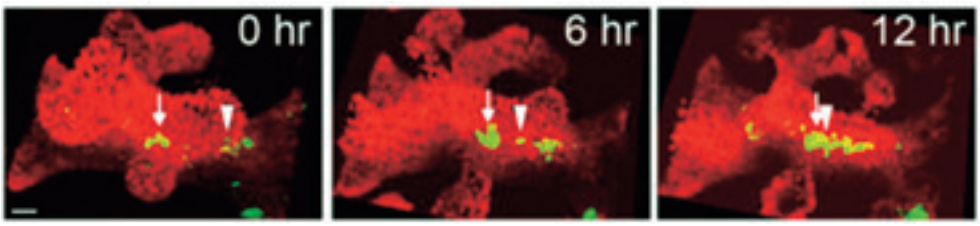
NE cell differentiation of adjacent cells as a means of restricting the NE cell numbers, and with the ultimate effect of limiting NE cell clusters to appropriate sizes.

What then leads differentiated NE cells to aggregate at airway branches? Using a 4D live imaging setup, Noguchi visualized changes in cultured fetal lung lobes for up to 15 hours, and was surprised to find that the NE cells appear to be moving autonomously toward branch points, crawling through the epithelium and taking a direct path to a distal bifurcation, an unusual example of independent epithelial cell migration in morphogenesis.

"So we know that NE cells cluster at branches, but the next question is, what controls their migration to such points. I think this would require at least three factors – a chemoattractant to draw cells from distant sites, a second to anchor them at branch points, and a third to bind similar cells to each other," says Morimoto. "If we can gain a better understanding of this process, I think it may also shed light on the mechanisms at work in the metastasis of small cell lung cancer as well."



Localization of NE cell clusters in 3D airway. Clusters at branch points shown in green, those at other sites in red.



NE cell (yellow) migration in developing airway epithelium (red). Initially isolated cell (arrowhead) converges toward bifurcation point (arrow).

Doubling teeth from single tooth germ

Following tooth loss, dental treatments using artificial materials such as dentures, dental bridges and implants are used to replace the tooth, however, these methods do not restore full physiological function of the teeth. Other tooth replacement therapies such tooth transplantation of wisdom tooth and transplantation of autologous or bioengineered tooth germs, a group of cells in early development that give rise to teeth, into regions of tooth loss, have recently been developed and shown to fully restore physiological tooth function. As humans have a limited number of tooth germs, securing biological sources that do not cause immunological rejection remains a critical issue, and thus the development of a technique to increase tooth germ numbers is heavily anticipated.

A recent research collaboration between CDB's Takashi Tsuji, team leader of the Laboratory for Organ Regeneration, and graduate student Naomi Yamamoto and others in Prof. Keiji Moriyama's laboratory at the Tokyo Medical and Dental University has led to the development of a unique approach for tooth regeneration, using a mouse model. They split a single tooth germ into two using mechanical force, which could subsequently be transplanted into the oral cavity and develop into two fully functional teeth. Their approach and findings are published in the online journal, *Scientific Reports*.

The tooth germ is derived from the embryonic ectoderm through epithelial-mesenchymal interactions. The team first extracted molar tooth germ from the jaw of an embryonic day 14.5 mouse, when the tooth germ is generated, and tied a knot (ligation) around the tooth germ using a thin nylon thread, specifically through the signaling center in an attempt to split the tooth germ. After culturing the ligated tooth germ for six days, histological staining revealed the formation of two tooth germs, each surrounded by epithelial tissue. The ligated tooth germ were then transplanted into the mouse subrenal capsule, and after 30 days of maturation, it gave rise to two teeth that contained hard tissues such as enamel and dentin, which was surrounded by periodontal ligament and alveolar bone, as seen in natural teeth. The split teeth however were half the size of natural molar teeth, and also had half the number of dental cusps on the crown of the tooth.

Next, the team carefully analyzed the germ splitting process using time-lapse imaging, and found that after ligation, the tooth germ signaling center, enamel knot, appeared in each split germ. Epithelial tissues surrounding each split germ continued to proliferate, eventually invaginating along the ligated surface resulting in two separate germs. Gene expression analyses for genes important in early tooth development showed split germs displayed similar expression patterns as those in natural tooth germ.

Past studies have shown that tooth morphology is regulated by reaction-diffusion waves of gene expression involving an activator, *Lef1*, and an inhibitor, *Ectodin*. Analyses of the expression patterns of these two genes were performed in natural and split tooth germs. In natural tooth germ, *Lef1* was expressed in the enamel knot and neighboring mesenchyme, and *Ectodin* was expressed around the region of *Lef1* expression. The split tooth germ showed smaller yet comparable expression patterns within each germ. Thus, ligation of the tooth germ leads to re-regionalization of the tooth-forming field in each split germ through reaction-diffusion waves of gene expression.

The group then implanted the split tooth germ into the jaw bone of the mouse to test whether teeth would de-

Left: Tooth germ split into two after ligation. Middle: Micro-CT of split tooth germ 50 days after transplantation into oral cavity of mouse. Right: Nerve fibers in the tooth pulp and periodontal ligament of split tooth.

Yamamoto N. et al. Functional tooth restoration utilising split germs through re-regionalisation of the tooth-forming field. *Sci Rep* 5.18393 (2015)

velop and erupt similar to natural tooth. The transplanted germs became engrafted into the surrounding tissue, and two months post-transplantation, micro-CT analysis revealed that the newly erupted teeth were aligned and in contact with the teeth on the opposing jaw. When orthodontic methods similar to braces were applied to the split teeth, the bone supporting the teeth underwent remodeling to accommodate teeth movement, indicating the presence of a functional periodontal ligament. Nerve fibers were also detected in the tooth pulp and periodontal ligament of the split teeth, as seen in natural teeth. Analyses of response to noxious stimuli, such as orthodontic force or pulp exposure, revealed both engrafted and natural teeth showed comparable responses, indicating that split teeth were properly connected to the central nervous system.

“Not only does tooth loss affect physiological tooth functions such as teeth alignment, chewing and speech, it can also affect overall health,” explains Tsuji. “In the future, our method could be used as a new form of regenerative medicine therapy for patients with tooth loss by generating multiple tooth germs from the patient’s own tooth germs and later implanting them.”

Keiji MORIYAMA, Naomi YAMAMOTO

Takashi TSUJI

Transplantation of hESC-derived retinal tissue in primate models of retinitis pigmentosa

Retinitis pigmentosa (RP) is a degenerative condition that ultimately damages the photoreceptors at the back of the eye, one of the many layers of specialized cells in the complex retina. Patients afflicted with RP suffer from loss of visual acuity, poor night vision and a narrowed visual field. Despite ongoing efforts to develop effective treatments using gene transfer and bioengineering approaches, to date RP remains resistant to therapy. The emerging paradigm of cell therapy represents another potential alternative, and the 4D cell culture techniques developed by CDB Team Leader Mototsugu Eiraku and former Group Director Yoshiki Sasai (dec.), in which embryonic stem cells (ESCs) can be steered to give rise to highly organized, three-dimensional tissue-like structures in vitro provides a promising starting point for the development of potential cellular resources for transplantation.

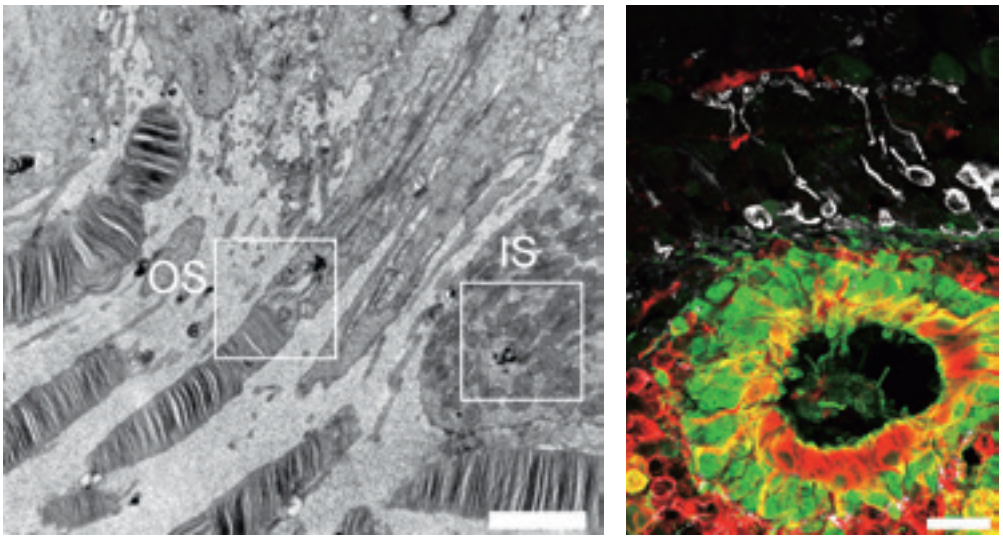
Now, a new study by Hiroshi Shirai, Michiko Mandai and others in the Laboratory for Retinal Regeneration (Masayo Takahashi, Project Leader) reports the successful transplantation of human ESC-derived retinal tissue into a non-human primate model of retinitis pigmentosa. This work, published in the *Proceedings of the National Academy of Sciences*, represents an exciting new step toward the development of cell-based treatments for RP.

The same group had previously shown that retinal tissue generated from mouse ESCs and induced pluripotent stem cells (iPSCs) is capable of maturation and integration into host tissue in mouse models, which prompted to them to move one step closer toward clinical testing by attempting a similar approach using macaque models of RP, which have the advantage of closer similarity to humans.

Before attempting transplants in macaques, the group first used a rat model to identify culture conditions for generating retina-like tissue with the ability to survive, mature, and function in the host environment. After experimenting with a range of differentiation times and culture conditions, they determined that slightly less mature cells, at 50–60 days of in vitro differentiation, produced superior grafts in terms of thickness and ability to integrate.

Next, the group needed to develop a monkey model of RP, a disease in which degeneration progresses from the peripheral part of the eye inwards, resulting in visual acuity losses in the center of the field of vision. The scientists used two different approaches to simulate this radiating degeneration, chemical injections and photocoagulation by laser light, producing selective foci of damage suitable for use in transplantation experiments and subsequent monitoring.

They next transplanted hESC-derived retina tissue after ~60 days of differentiation into the subretinal space of



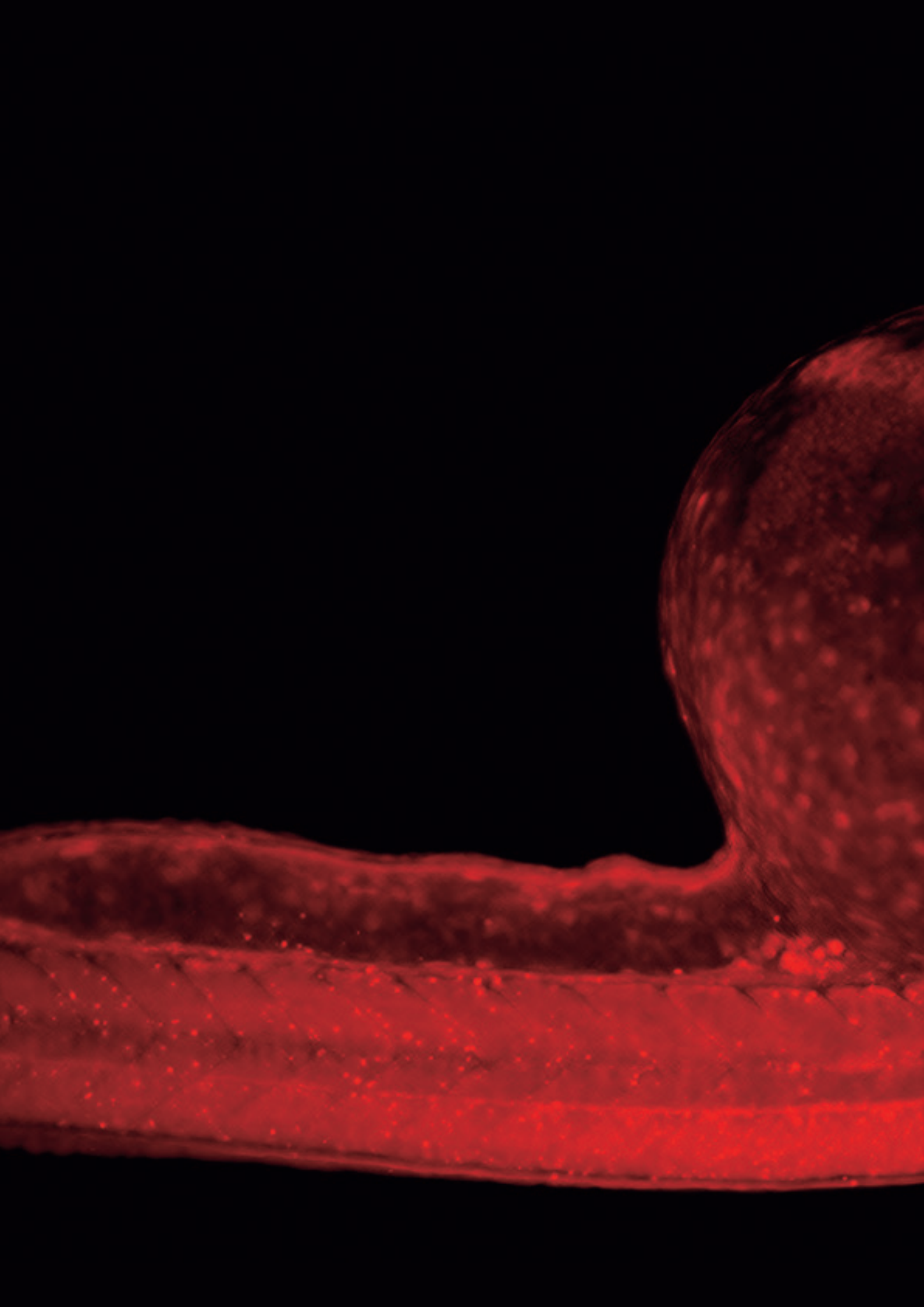
Transplantation of hESC-derived retinal precursor tissue into immunocompromised rat yields mature photoreceptor cells with both inner and outer segments (left). Transplantation of hESC-derived retinal precursor tissue into subretinal space in a primate model of RP produces mature photoreceptors (green) proximate to host bipolar cells (white) suggestive of synapse formation (right).

Shirai H. et al. Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *PNAS Plus* 113.E81-E90 (2016)

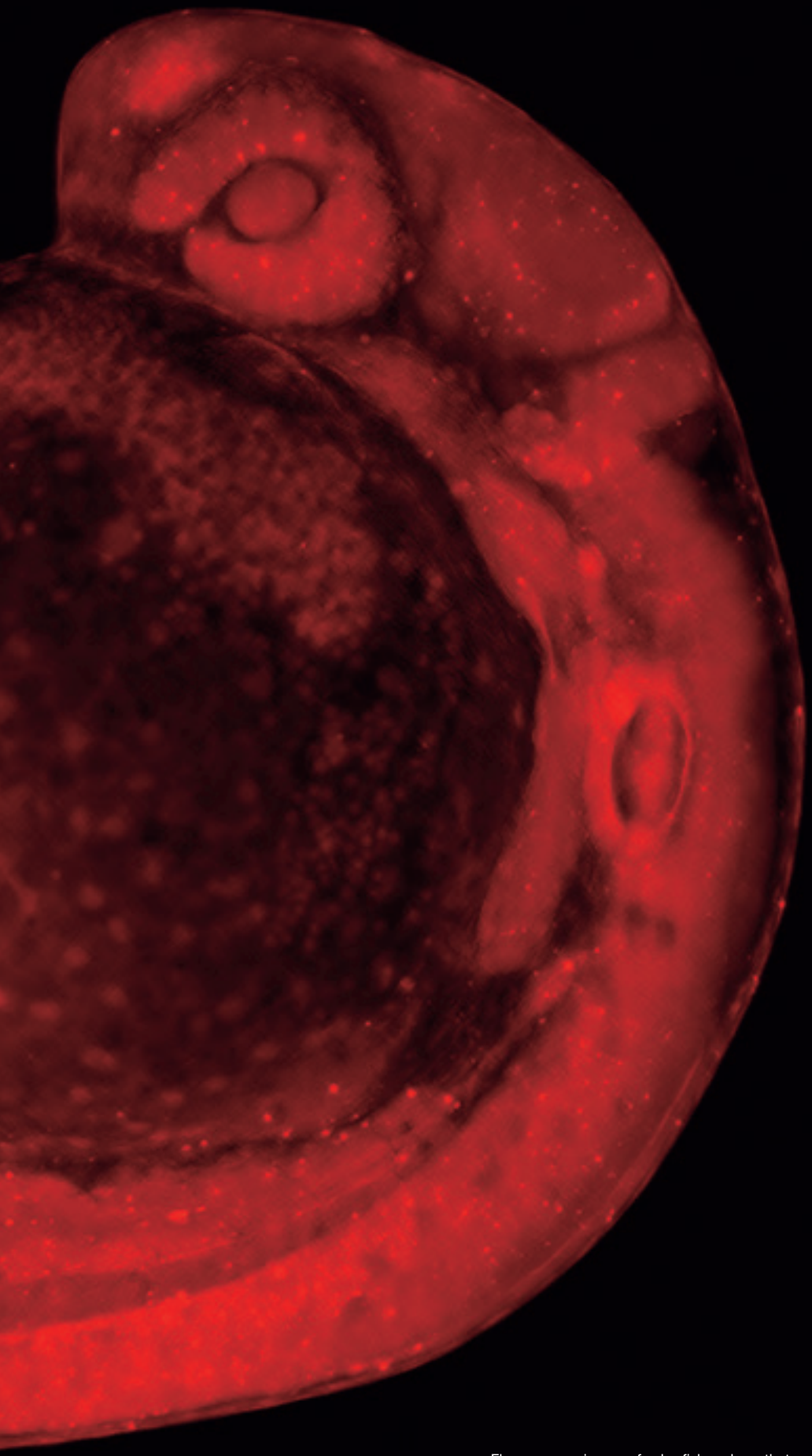
immune suppressed macaque RP models. They observed proliferative growth of the grafted tissue for up to 120 days, and ongoing differentiation as evidenced by the expression of early photoreceptor markers by day 90 and markers of rod and cone cells by day 150 of differentiation. Additionally, they observed growth patterns suggesting the formation of synaptic connections between host bipolar cells and graft photoreceptors, although at low frequencies.

In establishing primate models for preclinical studies of cell transplantation in RP, this study opens up new possibilities for testing of different culture and surgical delivery techniques. “In the future, we hope to test whether iPSC-derived tissue will show similar ability to integrate and function in the host retina,” says Mandai.

Hiroshi SHIRAI, Michiko MANDAI



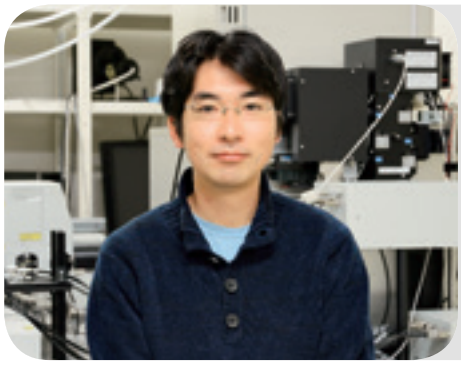
Laboratories



Fluorescence image of zebrafish embryo that was injected with fluorescent dye at the one-cell stage.
Image: Laboratory for Axial Pattern Dynamics

In Vitro Histogenesis

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



Team Leader

Mototsugu EIRAKU Ph.D.

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

Staff

Research Scientist
Shunsuke MORI
Satoru OKUDA
Nozomu TAKATA

Foreign Postdoctoral Researcher
Nicholas Robert LOVE

Research Associate
Yuiko HASEGAWA

Visiting Scientist
Atsushi KUWAHARA
Daiki NUKAYA
Hidetaka SUGA
Morio UENO

Technical Staff
Tomoko HARAGUCHI
Masako KAWADA
Eriko SAKAKURA

Junior Research Associate
Hideya SAKAGUCHI

Student Trainee
Rika YAMADA

Assistant
Fumi WAGAI

In vitro generation of a functional organ with complex structures is a major challenge of cell biology. Toward this goal, it is a reasonable strategy to recapitulate the ontogeny that is the most efficient and robust process for organogenesis acquired through evolution. Our laboratory aims to clarify molecular and cellular dynamics underlying organogenesis, and to develop new technologies for *in vitro* recapitulation, that is, 3D functional organ formation from stem cells. These researches aim to make important contributions to the field of developmental biology, stem cell biology and regenerative medicine.

Recent Publications

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

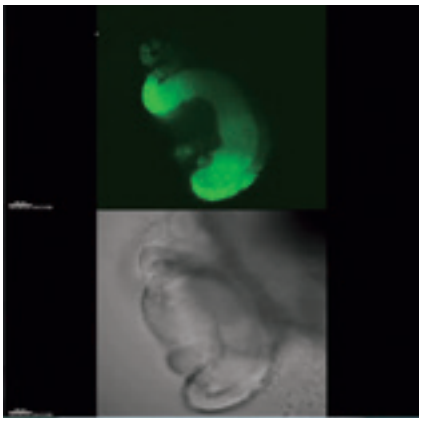
Kuwahara A, et al. Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue. *Nat Commun* 6. 6286 (2015)

Okuda S, et al. Vertex dynamics simulations of viscosity-dependent deformation during tissue morphogenesis. *Biomech Model Mechanobiol* 14.413-25 (2015)

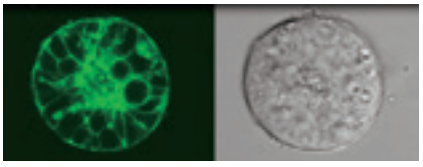
Kadoshima T, et al. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human E cell-derived neocortex. *Proc Natl Acad Sci U S A* 110. 20284-9 (2013)

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

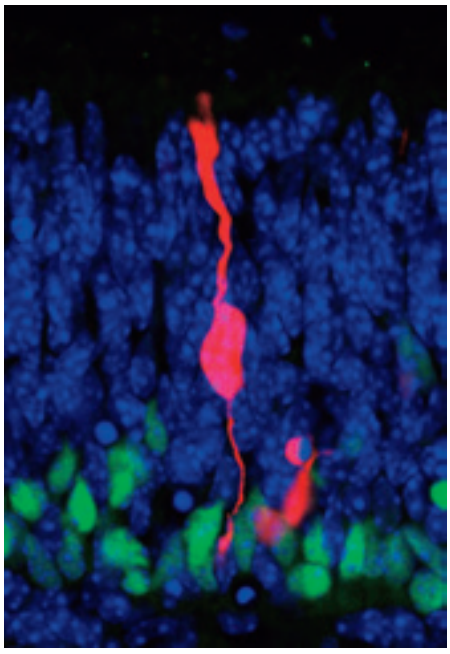
Eiraku M, et al. Relaxation-expansion model for self-driven retinal morphogenesis: a hypothesis from the perspective of biosystems. *Bioessays* 34. 17-25 (2012)



Self-organized optic cup formation from ES cell



ES cell-derived neuroepithelium (lifeact-GFP)



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

Tissue Microenvironment

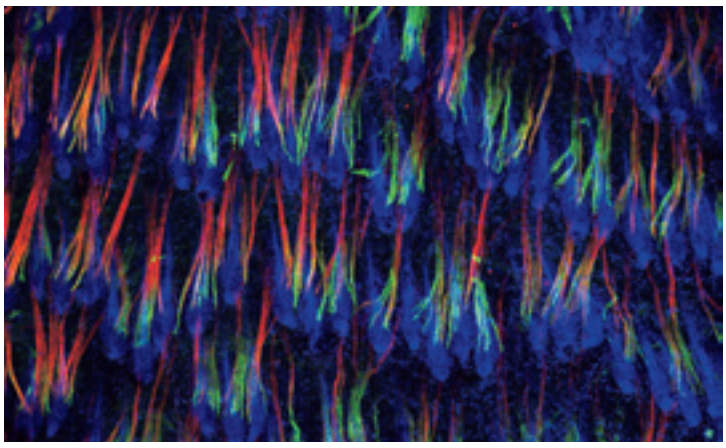
http://www.cdb.riken.jp/en/research/laboratory/fujiwara.html

Team Leader

Hironobu FUJIWARA Ph.D.

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

In our bodies, we have millions of different environments in which cells reside, which are known as cellular or tissue microenvironments. A series of recent studies has shown that these specialized tissue microenvironments instruct the fate and behaviors of cells. The aim of our lab is to gain a better understanding of the mechanisms underlying the ways in which tissue microenvironments are regionally specialized, and how these specialized microenvironments then instruct cellular behavior and communication, and the formation of organs. We are particularly interested in the role of regional specialization of the extracellular matrix (ECM) in the formation of the stem cell microenvironment, or niche, in the hair follicle. A deeper knowledge of this will provide a molecular basis to an improved understanding of the niche regulation of stem cells and organ formation, and the development of tailor-made microenvironments for different lineages of stem cells in the skin.



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

Staff

Research Scientist
Ritsuko MORITA
Ko TSUTSUI

Visiting Scientist
Natsumi SAITO
Norio UEMATSU

Technical Staff
Noriko BAN-SANZEN

Intern Student
Eloi Franco TREPAT

Part-Time Staff
Sonoko TOCHITANI

Assistant
Asako NAKAGAWA

Recent Publications

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

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

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

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

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

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

Organismal Patterning

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

Team Leader

Hiroshi HAMADA M.D., Ph.D.

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

Staff

Visiting Scientist
Katsuyoshi TAKAOKA

Assistant
Kaori SONE

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

Recent Publications

Shinohara K., et al. Absence of radial spokes in mouse node cilia is required for rotational movement but confers ultrastructural instability as a trade-off. *Dev Cell* 35.236-246 (2015)

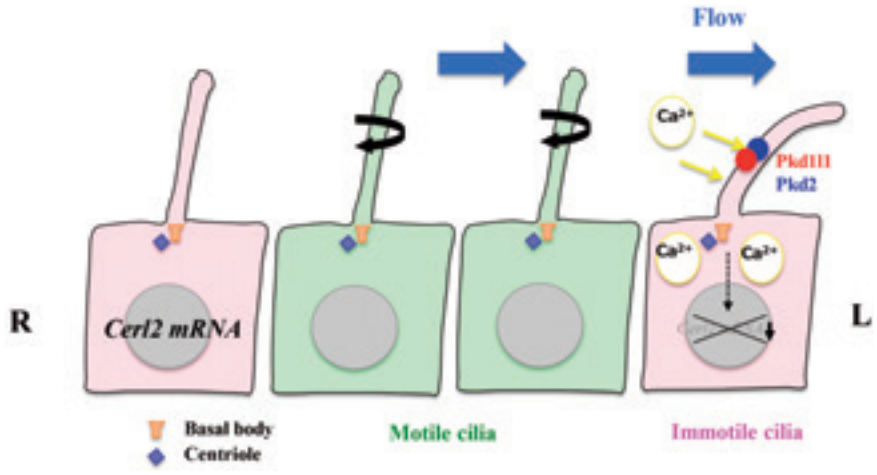
Nakamura T., et al. Fluid flow and interlinked feedback loops establish left-right asymmetric decay of *Cerl2* mRNA in the mouse embryo. *Nat Commun*. 3.1322 (2012)

Yoshiba S., et al. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science* 338.226-231 (2012)

Shinohara K., et al. Two rotating cilia in the node cavity are sufficient to break left-right symmetry in the mouse embryo. *Nat Commun*. 3.622 (2012)

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

Hashimoto M., et al. Planar polarization of the node cells determines the rotation axis of the node cilia. *Nat Cell Biol* 12.170-176 (2010)



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

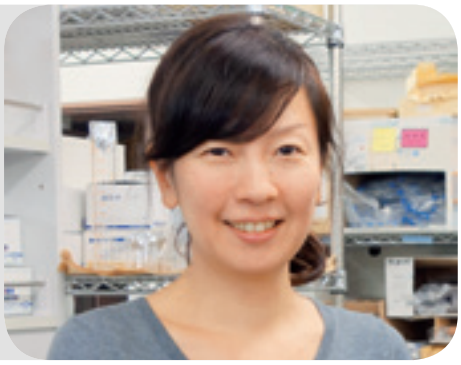
Neocortical Development

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

Team Leader

Carina HANASHIMA Ph.D.

Carina Hanashima received her Ph.D. from Waseda University School of Science and Engineering in 1999. She worked as a postdoctoral fellow in the laboratory of Eseng Lai at Memorial Sloan-Kettering Cancer Center, Cell Biology Program from 1999 to 2002, where she was engaged in 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.



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

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

Staff

Research Scientist
Yuko GONDA
Takuma KUMAMOTO
Kenichi TOMA
Tien-Cheng WANG

Visiting Researcher
Pei-Shan HOU

Technical Staff
Chihiro NISHIYAMA

Part-Time Staff
Reiko ODA

Assistant
Eri KOJIMA

Recent Publications

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

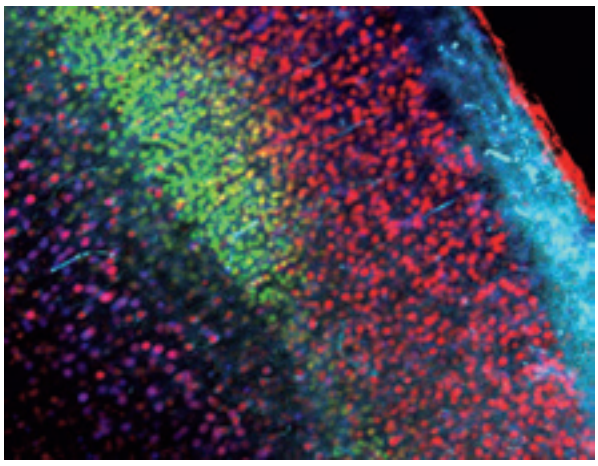
Toma, K. and Hanashima, C. Switching modes in corticogenesis: mechanisms of neuronal subtype transitions and integration in the cerebral cortex. *Frontiers in Neuroscience* 9.274 eCollection (2015)

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

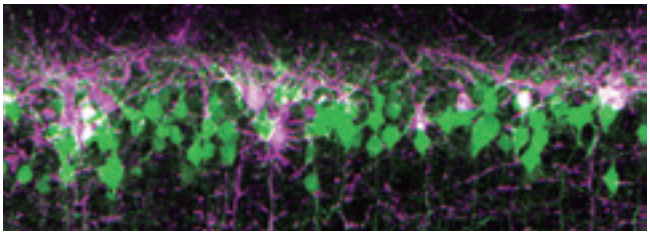
Toma K, et al. The timing of upper-layer neurogenesis is conferred by sequential derepression and negative feedback from deep-layer neurons. *J Neurosci* 34.13259-76 (2014)

Kumamoto T. and Hanashima C. Neuronal subtype specification in establishing mammalian neocortical circuits. *Neurosci Res* 86.37-49 (2014)

Yeh M. L, et al. Robo1 modulates proliferation and neurogenesis in the developing neocortex. *J Neurosci* 34.5717-31 (2014)



The neocortex is composed of distinct neuronal subtypes that establish 6 layers. Coronal section of a one-week-old mouse neocortex showing Reelin (layer I, cyan), Brn2 (layer II/III, red), RORß (layer IV, green), Ctip2 (layer V/VI, blue) expressing neurons.



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

Morphogenetic Signaling

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

Team Leader

Shigeo HAYASHI Ph.D.

Shigeo Hayashi received his B.Sc. in Biology from Kyoto University in 1982, and his Ph.D. in Biophysics from the same institution in 1987, for his work on lens-specific regulation of the chicken delta *crystallin* gene. Inspired by the discovery of the homeobox, he changed his research focus to the developmental genetics of *Drosophila* and spent three years as a postdoctoral research associate in Matthew Scott's lab at the University of Colorado before returning to Japan to work 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

Research Scientist
Toshiya ANDO
Wei-Chen CHU
Takefumi KONDO
Takuya MAEDA
Yosuke OGURA

Technical Staff
Xiaorui CAI
Akiyo KIMPARA
Tetsuhisa OTANI
Housei WADA

International Program Associate
Guangxia MIAO

Part-Time Staff
Ikuko FUKUZYOU
Noriko MORIMITU

Assistant
Ryoko ARAKI
Mai SHIBATA

Recent Publications

Dong B, et al. Cortical instability drives periodic supracellular actin pattern formation in epithelial tubes. *Proc Natl Acad Sci* 112. 8620-25 (2015)

O Tani, et al. A transport and retention mechanism for the sustained distal localization of Spn-F-IKKε during *Drosophila* bristle elongation. *Development* 142.2338-51 (2015)

Miao G and Hayashi S. Manipulation of gene expression by infrared laser heat shock and its application to the study of tracheal development in *Drosophila. Dev Dyn* 244.479-87 (2015)

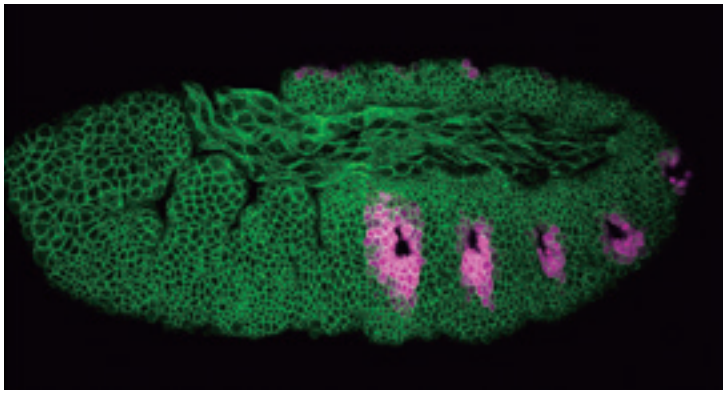
Dong B, et al. Balance between apical membrane growth and luminal matrix resistance determines epithelial tubule shape. *Cell Rep* 7.941-50 (2014)

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

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

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

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



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

Developmental Epigenetics

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

Team Leader

Ichiro HIRATANI Ph.D.

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

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.

Staff

Research Scientist
Hisashi MIURA
Rawin POONPERM
Naoyuki SARAI

Student Trainee
David MEYER

Technical Staff
Akie TANIGAWA

Recent Publications

Shang W. H. et al, Chromosome engineering allows the efficient isolation of vertebrate neocentromeres. *Dev Cell* 24.635-48 (2013)

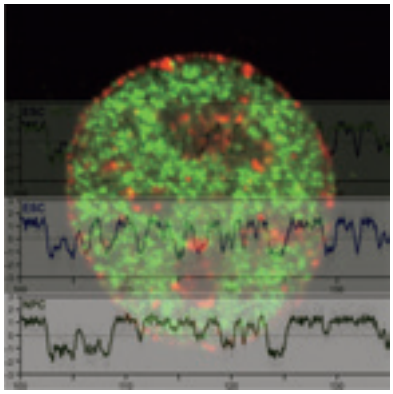
Ryba T, et al. Genome-scale analysis of replication timing: from bench to bioinformatics. *Nat Protoc* 6.870-95 (2011)

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

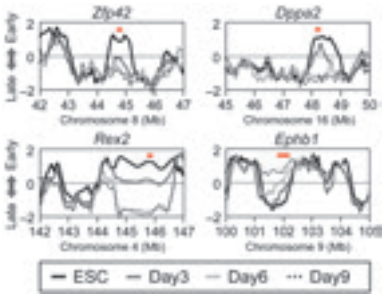
Ryba T, et al. Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. *Genome Res* 20.761-70 (2010)

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

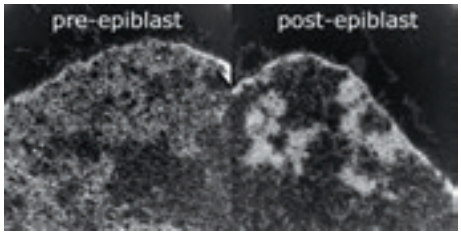
Hiratani I, et al. Replication timing and transcriptional control: beyond cause and effect--part II. *Curr Opin Genet Dev* 19.142-9 (2009)



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



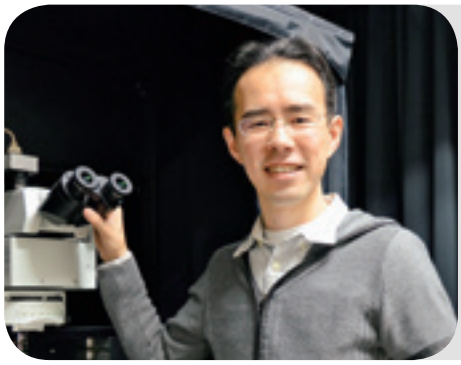
Genome-wide DNA replication profiling during ES cell differentiation can reveal domains that show large-scale changes in nuclear organization.



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

Sensory Circuit Formation

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



Team Leader

Takeshi IMAI Ph.D.

Takeshi Imai completed his Ph.D. in the laboratory of Prof. Hitoshi Sakano at the University of Tokyo Graduate School of Science in 2006. He did consecutive postdoctoral fellowships 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

Research Scientist
Satoshi FUJIMOTO
Ryo IWATA
Meng-Tsen KE
Marcus LEIWE

Technical Staff
Miwako NOMURA
Yoichi SAKASHITA

Student Trainee
Shuhei AIHARA
Aya MURAI
Richi SAKAGUCHI

Assistant
Eri YAMASHITA

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

Recent Publications

Ke M. T., et al. Super-resolution mapping of neuronal circuitry with an index-optimized clearing agent. *Cell Rep* doi:10.1016/jcelrep.2016.02.057 (2016)

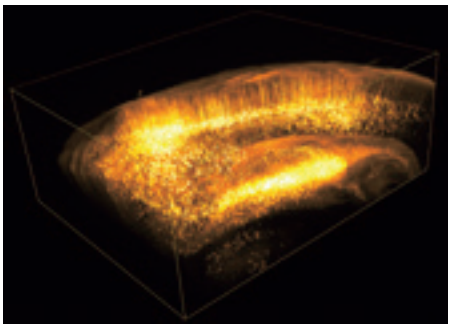
Imai T. Construction of functional neuronal circuitry in the olfactory bulb. *Semin Cell Dev Biol* 35C.180-88 (2014)

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

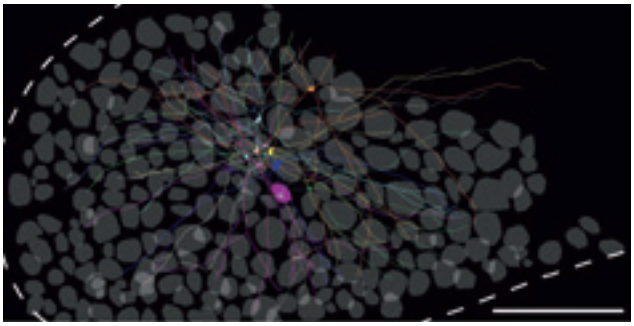
Ke M. T, et al. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nat Neurosci* 16.1154-61 (2013)

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)



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



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

Axial Pattern Dynamics

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

Team Leader

Hidehiko INOMATA Ph.D.

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

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

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

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

Staff

Research Scientist
Takehiko ICHIKAWA
Shinya MATSUKAWA

Technical Staff
Setsuko KANAMURA
Kaori NIIMI

Part-Time Staff
Mako MIYAGI
Masako SUZUKI

Recent Publications

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

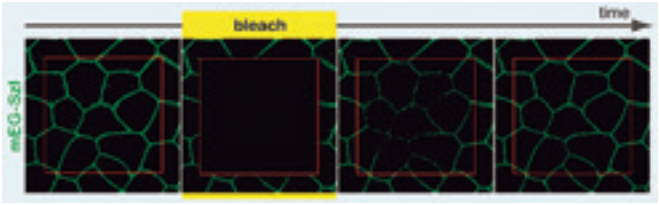
Takai A, et al. Anterior neural development requires Del1, a matrix-associated protein that attenuates canonical Wnt signaling via the Ror2 pathway. *Development* 137.3293-302 (2010)

Inomata H, et al. Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. *Cell* 134.854-65 (2008)

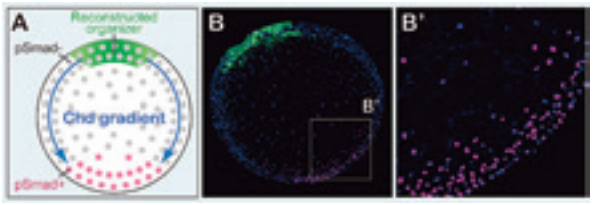
Arakawa A, et al. The secreted EGF-Discoidin factor xDel1 is essential for dorsal development of the Xenopus embryo. *Dev Biol* 306.160-9 (2007)

Onai T, et al. XTsh3 is an essential enhancing factor of canonical Wnt signaling in Xenopus axial determination. *EMBO J* 26.2350-60 (2007)

Inomata H, et al. A scaffold protein JIP-1b enhances amyloid precursor protein phosphorylation by JNK and its association with kinesin light chain 1. *J Biol Chem* 278.22946-55 (2003)



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



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

Chromosome Segregation

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

Team Leader

Tomoya KITAJIMA Ph.D.

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

Staff

Research Scientist
Aurélien COURTOIS
Yogo SAKAKIBARA
Shuhei YOSHIDA

Visiting Scientist
Hirohisa KYOGOKU

Student Trainee
Takeshi ASAKAWA
Yi DING
Namine TABATA

Assistant
Kaori HAMADA

Recent Publications

Sakakibara Y et al. Bivalent separation into univalents precedes age-related meiosis I errors in oocytes. *Nat Commun* 6.7550 (2015)

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

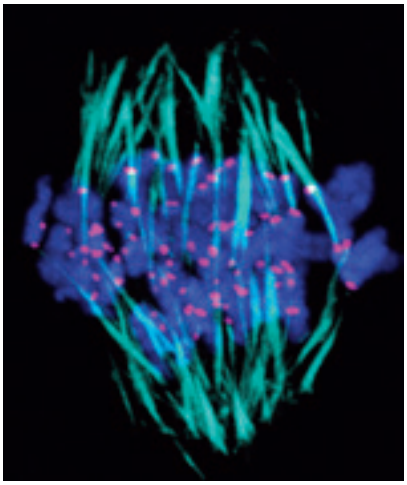
Kim J et al. Meikin is a conserved regulator of meiosis-I-specific kinetochore function. *Nature* 517(7535).466-471 (2015).

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

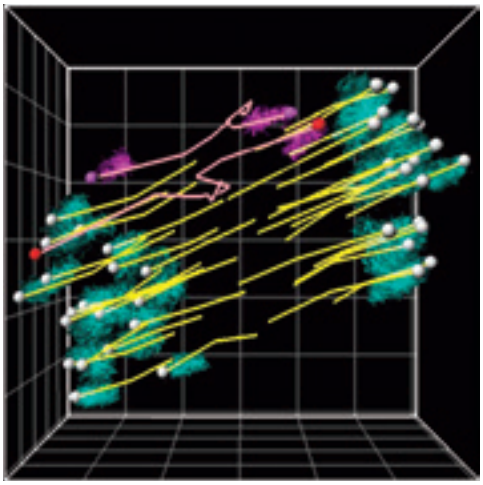
Kyogoku H et al. Nucleolus Precursor Body (NPB): A Distinct Structure in Mammalian Oocytes and Zygotes. *Nucleus* 5(6).493-498 (2014).

Kitajima TS et al. Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. *Cell* 146.568-81 (2011)

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



Kinetochore-microtubule attachments



Chromosome segregation error

Histogenetic Dynamics

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

Team Leader

Erina KURANAGA Ph.D.

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

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

Staff

Research Scientist
Emi MAEKAWA
Hiroyuki UECHI
Daiki UMETSU

Technical Staff
Ayako ISOMURA

Student Trainee
Yuka HAYASHI
Yuhei KAWAMOTO

Part-Time Staff
Arata KURANAGA
Yoko UMEGAKI

Recent Publications

Sato K, et al. Left-right asymmetric cell intercalation drives directional collective cell movement in epithelial morphogenesis. *Nat Commun* 6.10074 (2015)

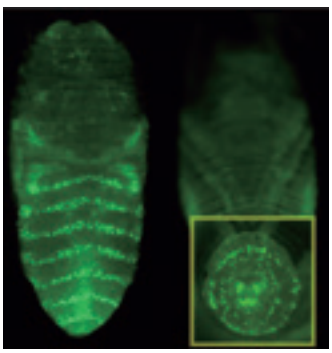
Obata F, et al. Necrosis-driven systemic immune response alters SAM metabolism through the FOXO-GNMT axis. *Cell Rep* 7.821-833 (2014)

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

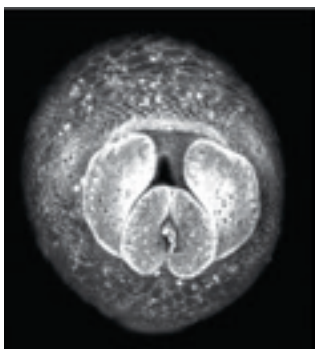
Kuranaga E, et al. Apoptosis controls the speed of looping morphogenesis in Drosophila male terminalia. *Development* 138.1493-9 (2011)

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. Yellow square indicates location of male genitalia.



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

Sensory Development

Senior Investigator

Raj LADHER Ph.D.

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. He is now a member of the faculty at the National Centre for Biogocial Sciences (NCBS) in Bangalore, India.

The Sensory Development lab closed in March 2015.
Dr. Ladher in now affiliated with NCBS in Bangalore, India.

Staff

Research Scientist
Akira HONDA

Visiting Researcher
Tomoko KITA

Part-Time Staff
Yoshiko KONDO

Assistant
Noriko HIROI

Recent Publications

Mak S-S, et al. Characterization of the finch embryo supports evolutionary conservation of the naive stage of development in amniotes. *Elife*. doi:10.7554/eLife.07178 (2015)

Freeman S., et al. Expression of the heparan sulfate 6-O-endosulfatases, Sulf1 and Sulf2, in the avian and mammalian inner ear suggests a role for sulfation in inner ear development. *Dev Dyn.* 244.168-180 (2015)

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

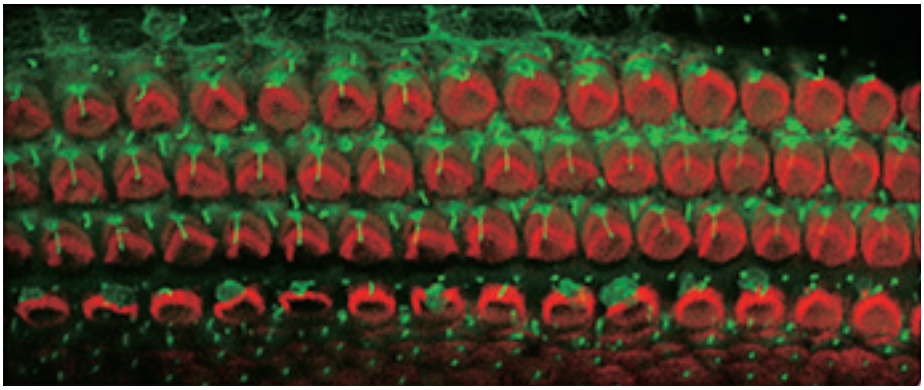
Sai X, et al. Junctionally restricted RhoA activity is necessary for apical constriction during phase 2 inner ear placode invagination *Dev. Biol.* 394.206-16 (2014)

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* 137.1777-1785 (2010)

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

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



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

Cell Asymmetry

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

Team Leader

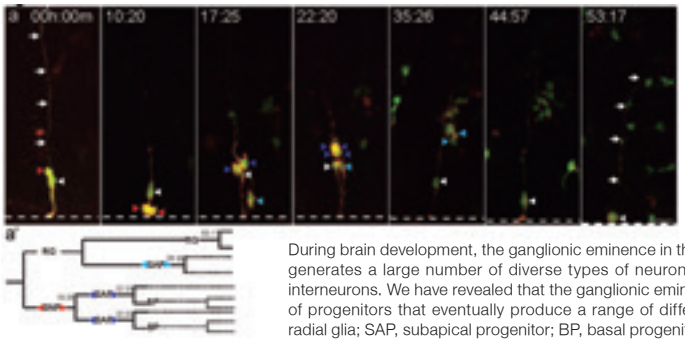
Fumio MATSUZAKI Ph.D.

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

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

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

The vertebrate brain evolved rapidly, resulting in an expansion of the size of the brain, which comprises a larger number of neurons arranged in a vastly more complex functional network than that in invertebrates. Neural stem cells typically adopt three states—proliferative (symmetrically dividing), neurogenic (asymmetrically dividing), and resting—and undergo transitions among the states, on which the basic organization of the brain depend. We are investigating mechanisms that determine the individual states of neural stem cells, and control transitions between states in mouse as well as mechanisms for generating neural progenitor cell diversity (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. We have revealed that the ganglionic eminence generate a variety of progenitors that eventually produce a range of different cell lineages. RG, radial glia; SAP, subapical progenitor; BP, basal progenitor.

Staff

Research Specialist
Keiko MUGURUMA
Atsunori SHITAMUKAI

Special Postdoctoral Researcher
Shigeki YOSHIURA

Research Scientist
Ikumi FUJITA
Daijiro KONNO
Yuji TSUNEKAWA

Visiting Researcher
Wu QUAN

Visiting Scientist
Yoshihito ISHIDA
Taisuke KADOSHIMA
Ayano KAWAGUCHI
Tomoko KITA
Yoichi KOSODO

Technical Staff
Chiaki KISHIDA
Ayaka NISHIYAMA
Taeko SUETSUGU
Yuko TAKEUCHI
Ryo YOSHIDA

Junior Research Associate
Shun MASE
Atsushi SHIRAISHI

Student Trainee
Kalyn KAWAMOTO
Yuki KOBAYASHI
Raymond TERHUNE-KUNIKANE
Fumiya KUSUMOTO
Yoko WATANABE

Part-Time Staff
Misato IWASHITA
Yoko OTSUKA

Assistant
Junko ISHIGAI

Recent Publications

Okamoto et al. Cell cycle–independent transitions in temporal identity of mammalian neural progenitor cells. *Nat Commun*. In press.

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

Klotz L, et al. Cardiac lymphatics are heterogeneous in origin and respond to injury. *Nature*. 522. 62-67 (2015)

Kasahara K, et al. Ubiquitin-proteasome system controls ciliogenesis at the initial step of axoneme extension. *Nat Commun* 5.5081 (2014)

Mora-Bermúdez F, et al. Specific polar subpopulations of astral microtubules control spindle orientation and symmetric neural stem cell division. *Elife*. doi: 10.7554/eLife.02875 (2014)

Pilz GA, et al. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. *Nat Commun* 4.2125 (2013)

Lung Development

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

Team Leader

Mitsuru MORIMOTO Ph.D.

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

Staff

Research Scientist
Keishi KISHIMOTO
Masafumi NOGUCHI

Student Trainee
Yuki KIKUCHI

Technical Staff
Chisa MATSUOKA

Part-Time Staff
Akira YAMAOKA

Assistant
Yuka NODA

Recent Publications

Noguchi M, et al. Directed migration of pulmonary neuroendocrine cells toward airway branches organizes the stereotypic location of neuroepithelial bodies. *Cell Rep* 13.2679-2686 (2015)

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

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

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. rtTA toxicity limits the usefulness of the SP-C-rtTA transgenic mouse. *Dev Biol* 325.171-8 (2009)

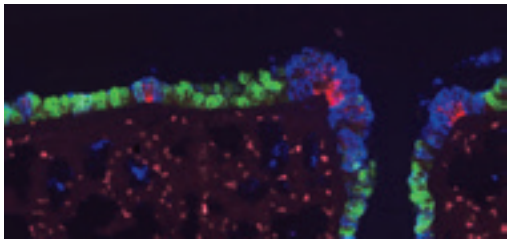
Morimoto M, et al. The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity. *Nature* 435.354-9 (2005)

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

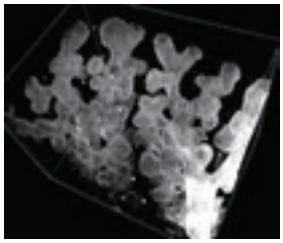
Respiratory organs in higher mammals are characterized by their efficient gas exchange, enabled by the functions of specialized cells. The development of such organs relies on the coordinated activities of both epithelial and mesenchymal tissue types, which arise from tissue-specific populations of stem cells in the developing embryo. The epithelial tissues of the conducting airways serve as the channel for the intake and exhalation of gases in the respiratory cycle, and are composed mainly of 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.

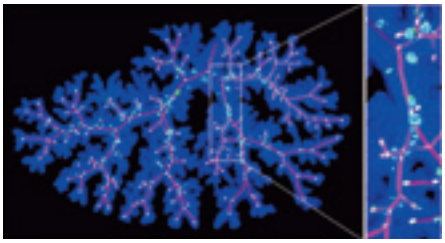
We focus on issues of how tissue morphology influences the formation of stem cell/niche tissue complexes in the development, repair and regeneration of respiratory organs, as well as mechanisms regulating cell proliferation and differentiation in developmental and regenerative processes.



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



Reconstructed 3D-branching bronchiole on PC



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

Growth Control Signaling

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

Team Leader

Takashi NISHIMURA Ph.D.

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

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

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

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

To better understand the interface between nutrient availability and growth regulation, we are focusing on how nutrition controls systemic growth through *Drosophila* insulin-like peptides (Dilps). Members of the insulin family of peptides have conserved roles in the regulation of growth and metabolism in a wide variety of metazoans. We are now analyzing the molecular mechanism underlying the nutrient-dependent expression of Dilp genes. We have also conducted in vivo RNAi screening to identify new players regulating growth and developmental timing at the organismal level. We described the first demonstration of the glia-derived endocrine factor regulating systemic body growth. The identification of SDR protein in *Drosophila* provides a new concept for the regulation of insulin/IGF signaling.

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

Staff

Research Scientist
Naoki OKAMOTO
Tetsuo YASUGI

Research Associate
Kota BANZAI

Visiting Scientist
Ken-ichi HIRONAKA

Technical Staff
Takayuki YAMADA

Part-Time Staff
Okiko HABARA
Kanako HIGUCHI
Hitomi KUBO
Noriko NISHIMURA
Tomomi OKUGOUCHI
Junko SHINNO

Recent Publications

Okamoto N, et al. Signaling from glia and cholinergic neurons controls nutrient-dependent production of an insulin-like peptide for *Drosophila* body growth. *Dev Cell* 35.295-310 (2015)

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

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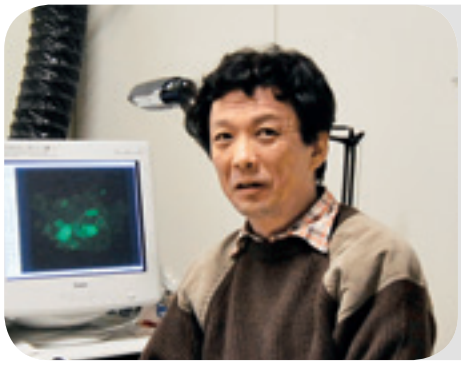
Wirtz-Peitz F, et al. Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. *Cell* 135.161-73 (2008)

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

Pluripotent Stem Cell Studies

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



Team Leader

Hitoshi NIWA M.D., Ph.D.

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

Research Scientist
Yoko NAKAI-FUTATSUGI
Satomi NISHIKAWA-TORIKAI
Satoshi OHTSUKA
Robert Odell STEPHENSON

Research Associate
Mariko YAMANE

Visiting Scientist
Hiroki URA

Technical Staff
Kumi MATSUURA
Azusa NOMA

Part-Time Staff
Sachiko HASHIMOTO
Yayoi NAKAI

Assistant
Miho SAKURAI

Recent Publications

Yamane, M., Fujii, S., Ohtsuka, S. and Niwa, H.: Zscan10 is dispensable for maintenance of pluripotency in mouse embryonic stem cells. *Biochem Biophys Res Commun* 468.26-831 (2015)

Ohtsuka, S. and Niwa, H. The differential activation of intracellular signaling pathways confers the permissiveness of embryonic stem cell derivation from different mouse strains. *Development* 142.431-437 (2015)

Fujii S. et al. Nr0b1 is a negative regulator of Zscan4c in mouse embryonic stem cells. *Sci Rep,* 16;5;9146 (2015)

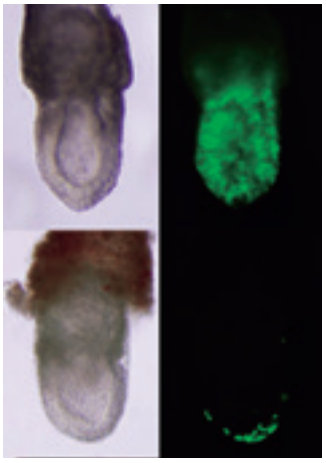
Adachi K, et al. Context-dependent wiring of sox2 regulatory networks for self-renewal of embryonic and trophoblast stem cells. *Mol Cell* 52.380-92 (2013)

Niwa H, et al. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* 460.118-22 (2009)

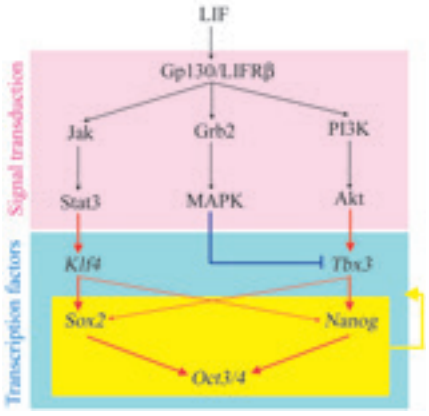
Niwa H. How is pluripotency determined and maintained? *Development* 134.635-46 (2007)

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.



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



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

Early Embryogenesis

Senior Investigator
Guojun SHENG Ph.D.

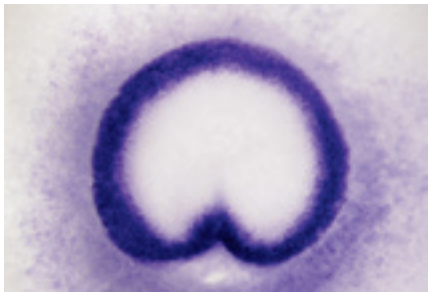
Guojun Sheng received his B.S. in microbiology from Fudan University in Shanghai, China in 1990. He entered the Rockefeller University the next year, attending the summer course in embryology at the Woods Hole Marine Biological Laboratory in 1996 and receiving his Ph.D. under Claude Desplan in 1997.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

The Early Embryogenesis lab closed in September 2015.
Dr. Sheng is now affiliated with Kumamoto University.

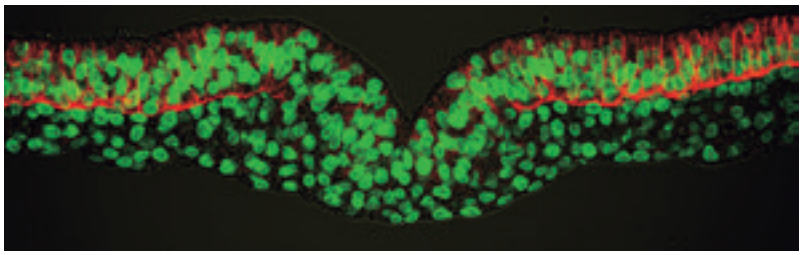
The lab studies how mesoderm cells form and differentiate during early vertebrate development, using the chick as the main model organism. Our research currently focuses on two aspects: 1) the formation and early regionalization of mesoderm cells during gastrulation; 2) differentiation of the ventral-most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



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



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

Staff

Research Scientist
Cantas ALEV
Yukiko NAKAYA
Wei WENG

Technical Staff
Hiroki NAGAI
Erike Widyasari SUKOWATI
YuPing WU

Part-Time Staff
Anna Barbara WRABEL

Assistant
Noriko HIROI
Fumie NAKAZAWA

Recent Publications

Mak S-S, et al. Characterization of the finch embryo supports evolutionary conservation of the naive stage of development in amniotes. *Elife*. doi: 10.7554/elife.07178 (2015)

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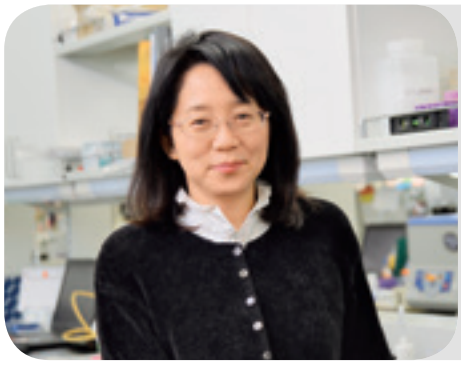
Retinal Regeneration

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



Project Leader

Masayo TAKAHASHI M.D., Ph.D.

Masayo Takahashi received her M.D. in 1986, and her Ph.D. in 1992 from Kyoto University. After an assistant professorship in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1995, where she discovered the potential of stem cells as a tool for retinal therapy. She returned to Kyoto University Hospital in 1997, and was appointed associate professor at the Translational Research Center in the same hospital in 2001. She joined the CDB as a team leader of the Lab for Retinal Regeneration in 2006. In 2013, her team launched a pilot clinical study of autologous iPS cell-derived RPE cell sheets for exudative aged-related macular degeneration (AMD), and performed the first RPE cell sheet graft transplantation in Sept. 2014. Her clinical specialty is retinal disease–specifically, macular diseases and retinal hereditary diseases. Her aim is to understand these diseases at a fundamental level and develop retinal regeneration therapies.

Staff

Deputy Project Leader
Michiko MANDAI
Sunao SUGITA

Research Scientist
Jun KANEKO
Hiroyuki KITAJIMA
Naoshi KOIDE
Takesi Hoyos MATSUYAMA
Chikako MORINAGA
Akishi ONISHI
Genshiro SUNAGAWA

Research Associate
Yuuki ARAI
Akihiro TACHIBANA

Technical Staff
Momo FUJII
Kanako KAWAI
Tomoyo HASHIGUCHI
Naoko HAYASHI
Yukako HIRAO
Ayumi HONO
Kyoko ISEKI
Hiromi ITO
Michiru MATSUMURA
Noriko SAKAI
Yumiko SHIBATA
Junki SHO
Motoki TERADA
Kazuko TSUJIMOTO
Chikako YAMADA

Part-Time Staff
Shoko FUJINO

Recent Publications

Shirai H, et al. Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc Natl Acad Sci U S A* 113. E81-E90 (2016)

Sugita S, et al. Inhibition of T-cell activation by retinal pigment epithelial cells derived from induced pluripotent stem cells. *Invest Ophthalmol Vis Sci*. 56(2).1051-62 (2015)

Assawachananont J, et al. Transplantation of Embryonic and Induced Pluripotent Stem Cell-Derived 3D Retinal Sheets into Retinal Degenerative Mice. *Stem Cell Reports* 2.662-74 (2014)

Kamao H, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Reports* 2.205-18 (2014)

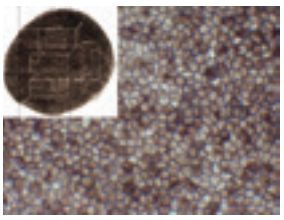
Jin Z. B, et al. Modeling retinal degeneration using patient-specific induced pluripotent stem cells. *PLoS One* 6.e17084 (2011)

Osakada F, et al. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nat Biotechnol* 26.215-24 (2008)

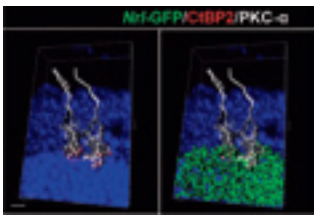
The retina has been called the “approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that in adult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

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

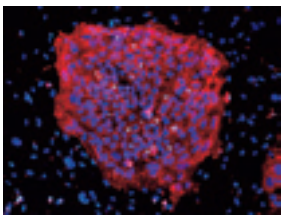
Therapeutic applications cannot be developed from basic research alone; the clinical approach a thorough understanding of the medical condition to be treated is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily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



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



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



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

Cell Adhesion and Tissue Patterning

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

Team Leader

Masatoshi TAKEICHI Ph.D.

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

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

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

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

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

Staff

Research Scientist
Shuichi HAYASHI
Shoko ITO
Toshiya KIMURA
Anna PLATEK
Varisa PONGRAKHANANON
Takuji TANOUE
Mika TOYA
Vassil VASSILEV

Visiting Scientist
Tamako NISHIMURA

Technical Staff
Sylvain HIVER
Yoko INOUE
Miwa KAWASAKI
Hiroko SAITO

Assistant
Mutsuko AISO-WATANABE

Recent Publications

Toya M., et al. CAMSAP3 orients the apical-to-basal polarity of microtubule arrays in epithelial cells. *Proc Natl Acad Sci U S A* 113.332-337 (2016)

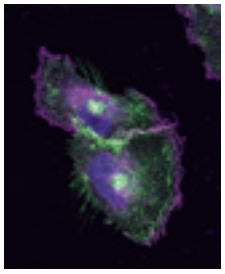
Tsukasaki Y, et al. Giant cadherins Fat and Dachsous self-bend to organize properly spaced intercellular junctions. *Proc Natl Acad Sci U S A* 111.1601-6 (2014)

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

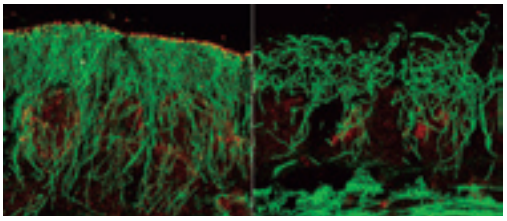
Takeichi M. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. *Nat Rev Mol Cell Biol* 15.397-410 (2014)

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

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



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



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

Organogenesis and Neurogenesis

Team Leader
Masatoshi TAKEICHI Ph.D.

This laboratory was led by Yoshiki Sasai M.D. Ph.D. until August 2014, after which Masatoshi Takeichi served as interim leader. The laboratory closed in March 2015.

Recent Publications

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

Ohgushi M, et al. Rho-signaling-directed YAP/TAZ activity underlies the long-term survival and expansion of human embryonic stem cells. *Cell Stem Cell* 17, 448-461 (2015)

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

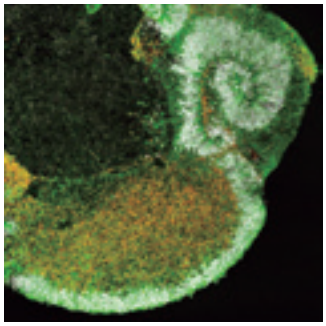
Muguruma K, et al. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep* 10. 537-550 (2015)

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

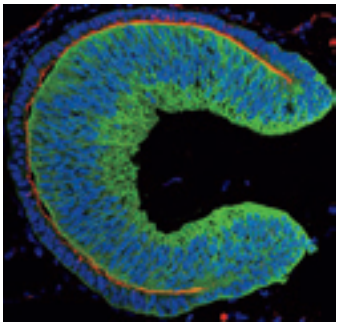
Using the African clawed frog, *Xenopus laevis*, as a model in molecular embryological studies, the group was engaged in clarifying the structure and extent of the signaling networks involved in setting up the dorsal-ventral axis and determining neural fate in the ectoderm. These studies focused on molecules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

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

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



Self-formation of layered cortical tissue from human ES cells



Self-organized formation of optic cup from human ES cells

Organ Regeneration

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

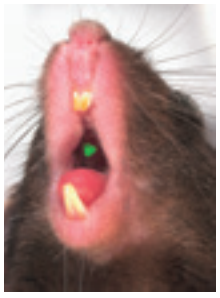
Team Leader

Takashi TSUJI Ph.D.

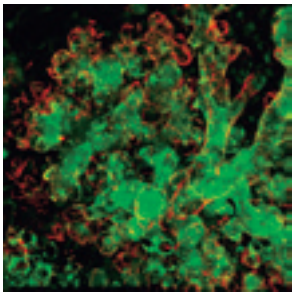
Takashi Tsuji received his Master's degree from Niigata University in 1986, and after working in the pharmaceutical industry for three years, returned to complete his doctorate at Kyushu University, and he received his doctorate in 1992 from Niigata University. He conducted research at Niigata University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

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

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



Bioengineered tooth



Bioengineered salivary gland

Bioengineered hair follicle

Staff

Collaborative Scientist
Miho OGAWA
Koh-ei TOYOSHIMA

Research Scientist
Kyosuke ASAKAWA
Mamoru ISHII
Jun ISHIKWA
Ryohei MINAMIDE
Chikafumi OZONE

Technical Staff
Yukiko MORIOKA
Yumi NISHIMURA
Azusa NOMA
Hiroko SASAKI
Aya SHIOKAWA
Miki TAKASE

Student
Kei SAKAKIBARA

Assistant
Asako ANDO
Mayumi MUROFUSHI

Recent Publications

Chikafumi Ozone, et al. Functional anterior pituitary generated in self-organizing culture of human embryonic stem cells, *Nat Commun* 7.10351 (2015)

Ogawa M, et al. Functional salivary gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 4.2498 (2013)

Hirayama M, et al. Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 4.2497 (2013)

Toyoshima K. E, et al. Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. *Nat Commun* 3.784 (2012)

Ikeda E, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci U S A* 106.13475-80 (2009)

Nakao K, et al. The development of a bioengineered organ germ method. *Nat Methods* 4.227-30 (2007)

Epithelial Morphogenesis

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

Team Leader

Yu-Chiun WANG Ph.D.

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

Staff

Research Scientist
Niraj K. NIRALA

Technical Staff
Mustafa SAMI
Michiko TAKEDA

International Program Associate
Anthony ERITANO

Assistant
Yuko FUJIYAMA

Recent Publications

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

Gavin-Smyth J, et al. A genetic network conferring canalization to a bistable patterning system in *Drosophila*. *Curr Biol* 23.2296-302 (2013)

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

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

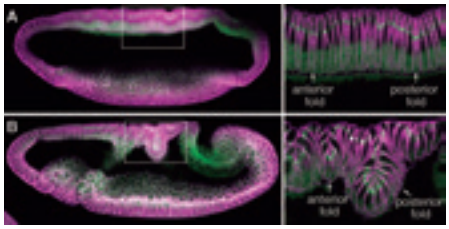
Wang Y. C. and Ferguson E. L. Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. *Nature* 434.229-34 (2005)

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

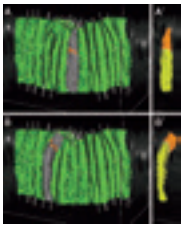
The central question in developmental biology is how cells, tissues and organs acquire their specific functions and shapes. A large body of work over the past several decades has yielded a broad understanding of how functional specialization is achieved through differential gene expression. In contrast,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

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

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



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



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

2015 Events

The RIKEN Center for Developmental Biology strives to engage with the public through a variety of media, including its website and printed materials, media coverage, and direct interactions such as guided tours, open house events, and other public outreach activities. In addition to this work in public engagement, the CDB also organizes events for bringing scientists together outside of the laboratory environment.

The 26th CDB Meeting: Mechanistic Perspectives of Multicellular Organization

The 26th CDB Meeting, "Mechanical Perspectives of Multicellular Organization," was held September 8 to 9. The meeting was organized by a group of young researchers primarily from the CDB, focusing on the theme of physics and mechanics involved in tissue formation of multicellular organisms. Over 130 scientists and students with diverse backgrounds ranging from biology, medicine, physics, and engineering convened at the CDB to take part in the two days of talks and discussions. Talks were given on the themes of morphological dynamics, quantitative measurements of active passive cell behaviors, in vitro constructions and controls of multicellular structures, and computational simulations of multicellular systems. Key note lectures were given by Pierre-François Lenne, Frank Jülicher and Hiroshi Hamada, three leading scientists in this interdisciplinary research field.

CDB Retreat in Sasayama

The annual CDB Retreat was held September 29 and 30 in the mountains of Sasayama, located in northern Hyogo Prefecture. Laboratory heads, research scientists, student trainees, technical staff and members of the administration participated in this closed meeting to share and discuss the latest developments in their work in addition to getting better acquainted with each other outside normal lab settings. The two-day program featured talks by recent appointees, research scientists and students, as well as special lectures by Chikara Furusawa from RIKEN QBiC and CDB alumnus Yoshiko Takahashi (Kyoto University).

Open House 2015

The CDB opened its doors to the public as part of the RIKEN Kobe Open House on Saturday, October 24. Over 1,600 people visited the CDB to see and learn firsthand about the latest research in development and regenerative biology. All of the laboratories within the CDB took part in the Open House in some capacity. Six CDB laboratories welcomed visitors to take a peek inside their laboratories, and see sample specimens related to the research carried out in the respective laboratories. Another popular event was the model organism exhibit where a variety of organisms from chicken and frogs, to flies and fish used in developmental biology research were on display. New this year was a talk show featuring a panel of CDB and QBiC scientists, as well as a booth where visitors could try carrying out actual experimental techniques used in the lab. For those looking for a quiet spot, there was a book lounge showcasing books that were recommended by CDB scientists. This is the largest annual event held at the CDB that gives an opportunity for scientists and the public to engage face-to-face.

2015 Courses

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

CDB internship for undergraduate students

The CDB invited 29 undergraduate students from universities around Japan to spend the first week of August as an intern working alongside scientists in various labs on the CDB campus. The busy week included talks given by CDB scientists, and opportunities to visit different labs, in addition to carrying out small-scale projects in the host labs. The internship wrapped up with a presentation session on the last day, where the interns shared their findings and their interpretation of the results with the others. This is the fourth year the internship has been organized.

Summer school for high school students

The ninth annual one-day summer school for high school students was held at the CDB on August 4 and 6. This popular summer program includes a talk by a CDB scientist, a laboratory visit and scientific experiments. This year's program was organized around the topic of genes, with the students learning about how genes regulate development and shape our bodies, as well as how individual differences arise from even small changes to a gene. The lab experiments were designed so the students could predict their alcohol tolerance levels by analyzing their own DNA, extracted from cells collected from their mouths.

Intensive lecture program for graduate students

The CDB maintains close ties with a number of graduate and medical schools in the Kansai area, and hosts a two-day lecture program every year for graduate students enrolled in partnering institutions. Lectures are given by laboratory heads and scientists at the CDB, introducing the students to the research carried out at the Center. Close to 190 students took part in the event held on August 26 and 27. The program includes exhibits of model organisms, and tours of labs and other facilities such as the research aquarium, electron microscopy room, and gene sequencing unit.

High school teacher workshop

A weekend workshop for high school biology teachers, held on October 3 and 4, was co-organized by the CDB and the Japanese Society for Developmental Biologists (JSDB). In its eighth year, the workshop aims to help enrich biology education in the high school classroom by giving teachers a refresher course in developmental biology basics, as well as giving them an opportunity to learn actual experimental techniques used in the field. The theme this year was the 'organizer', a region of early stage embryos that influences development of other parts of the embryo, and the program included a talk by Hidehiko Inomata, head of Laboratory for Axial Pattern Dynamics, on the recent developments in organizer-related research, a tour of his laboratory and a practical session with experiments using embryos of chicken and African clawed frogs (*Xenopus*). The program also introduced a few experiments that could be adapted for a classroom setting.

As an extension of this workshop, several teachers who took part in this program had the opportunity to bring their own students to a special tutorial organized at the CDB in December and teach them to carry out similar experiments.

2015 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
Masayo Takahashi	Project Leader	Retinal Regeneration	The Johnson & Johnson Innovation Award	The Japanese Society for Regenerative Medicine
Masayo Takahashi Michiko Mandai Chikako Morinaga Noriko Sakai Chikako Yamada	Project Leader Deputy Project Leader Researcher Technical Staff Technical Staff	Retinal Regeneration	MEXT Prize (Science and Technology Prize)	The Ministry of Education, Culture, Sports, Science and Technology (MEXT)
Takeshi Imai	Team Leader	Sensory Circuit Formation	MEXT Prize (The Young Scientist's Prize)	The Ministry of Education, Culture, Sports, Science and Technology (MEXT)
Masayo Takahashi	Project Leader	Retinal Regeneration	The Award for Academic Startups 2015	Japan Science and Technology Agency
Masayo Takahashi	Project Leader	Retinal Regeneration	The Ogawa-Yamanaka Stem Cell Prize	Gladstone Institutes, USA
Hirohisa Kyogoku	Visiting Scientist	Chromosome Segregation	The 108th SRD Meeting, Outstanding Presentation Award (Oral Presentation)	The Society for Reproduction and Development
Masayo Takahashi	Project Leader	Retinal Regeneration	Hyogo Prefecture Science Prize	Hyogo Prefecture
Masayo Takahashi	Project Leader	Retinal Regeneration	The Soroptimist Japan Foundation's Dream Award	The Soroptimist Japan Foundation
Masayo Takahashi	Project Leader	Retinal Regeneration	Kyoto Akebono Prize	Kyoto Prefecture
Hirohisa Kyogoku	Visiting Scientist	Chromosome Segregation	ARBS 12th Annual Conference Excellent Poster Presentation Award	Asian Reproductive Biotechnology Society
Masayo Takahashi	Project Leader	Retinal Regeneration	Nikkei Woman: Woman of the Year 2016	Nikkei WOMAN, Nikkei BP
Hirohisa Kyogoku	Visiting Scientist	Chromosome Segregation	BMB2015 Award for Outstanding Presentation by Young Scientist	Biochemistry and Molecular Biology

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

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

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

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

Kobe at sunset. Photography: Shigekazu SHINYA

RIKEN in Kobe

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

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

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

Administrative support at RIKEN Kobe and CDB

Kobe Branch

- RIKEN Kobe Administrative Division
  - General Affairs
  - Human Resources
  - Finance
- Information Networks Office – Kobe Branch
- RIKEN Kobe Safety Center
- Developmental Biology Planning Office
  The Planning Office coordinates funding and external review activities, as well as providing support for international communications, scientific meetings, and the Center's non-Japanese staff.
- Life Science Technologies Planning Office
- Quantitative Biology Planning 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 (QBiC)

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

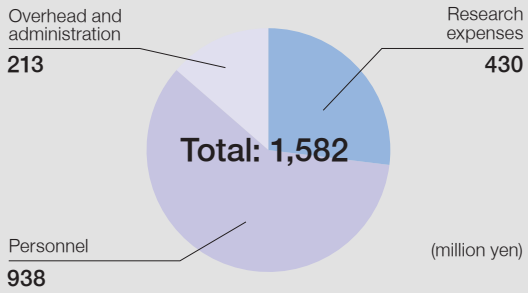
Kobe Administrative Services

Since the reform of the RIKEN organization in early 2013, the Kobe Administrative Division has been dedicated to providing core administrative services to the entire RIKEN Kobe Branch. Its main areas of responsibility are general affairs, facilities management, human resources, contracts, finances and accounting. The Information Networks Office maintains both network access and multiple intranet services of the Kobe Branch. Within the CDB, the Developmental Biology Planning Office coordinates important activities including budget and funding management, and administrative support for laboratory performance reviews and contract renewals. The Library Office manages the CDB research literature collections and interlibrary loans.

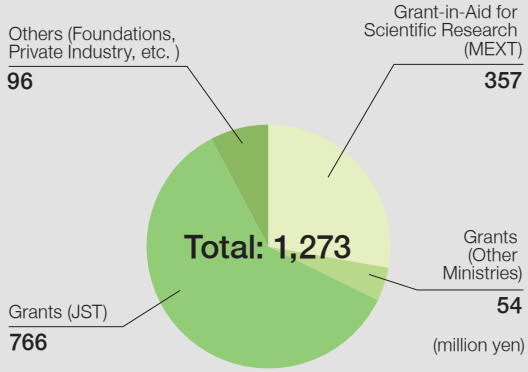
Safety Center

The Kobe Safety Center provides training and supervision for lab and workplace safety, implements and monitors disaster prevention measures, and educates staff on and ensures compliances with national and institutional regulations governing laboratory practice and technology. The office also handles all administrative affairs of the Institutional Review Board, which meets regularly to review and discuss investigations with potential ethical, legal, social or public health and safety implications prior to their implementation.

2015 CDB Budget



In addition to the dedicated funds outlined above, individual labs and investigators are encourages 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.



2015 CDB Staff

Laboratory heads	18
Deputy leaders	2
Research scientists	49
Research associates	7
Technical staff	59
Assistants	14
Visiting scientists	100
Student trainees	22
Part-time staff	33
Other	7
Total	311
Kobe Branch Administration	71

2015 CDB Symposium

Time in Development

March 23-25, 2015

The RIKEN CDB hosted its thirteenth annual symposium on March 23 to 25 in the CDB auditorium. This year's symposium was themed, "Time in Development," and drew an audience of 144 scientists and students from over 10 different countries to take part in a program of talks and discussions exploring the different contexts of time in relation to development.

The three-day program featured over 30 invited and selected talks and more than 50 poster presentations that covered a range of topics including temporal cell fate, oscillation and patterning, developmental timing, life span, and evolutionary time. The co-organizers of this year's symposium were Carina Hanashima and Takashi Nishimura from the CDB, Shigeru Kuratani from RIKEN and Claudio Stern (University College London, UK).

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

Session 1: Temporal Cell Fate

Yukiko Gotoh (The University of Tokyo, Japan)
Carina Hanashima
(RIKEN Center for Developmental Biology, Japan)
Fengzhu Xiong (Harvard Medical School, USA)
Claude Desplan (New York University, USA)
Stefan Thor (Linköing University, Sweden)
Connie Cepko
(Harvard Medical School and Howard Hughes Medical Institute, USA)

Session 2: Oscillation and Patterning

Olivier Pourquié
(Harvard Medical School and Brigham and Woman's Hospital, USA)
Erik Clark (University of Cambridge, UK)
Taijiro Yabe (National Institute for Basic Biology, Japan)
Ryoichiro Kageyama (Kyoto University, Japan)
Takao Kondo (Nagoya University, Japan)
Berta Verd (Centre for Genomic Regulation (CRG), Spain)
Andrew C. Oates (MRC National Institute for Medical Research, UK)
Koichiro Uriu (RIKEN, Japan)
Claudio D Stern (University College London, UK)









Session 3: Developmental Timing

Patrick H. O'Farrell (University of California, San Francisco, USA)
Takashi Saitou (Ehime University Hospital, Japan)
Chieh Chang (University of Illinois at Chicago, USA)
Victor Ambros (University of Massachusetts Medical School, USA)
Yun-Bo Shi
(Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), NIH, USA)
H. Fred Nijhout (Duke University, USA)
Michael B. O'Connor (University of Minnesota, USA)
Ryusuke Niwa (University of Tsukuba, Japan)
Takashi Nishimura (RIKEN Center for Developmental Biology, Japan)

Session 4: Life Span

Ayumi Nakamura (University of North Carolina at Chapel Hill, USA)
Shigeru Kuratani (RIKEN, Japan)

Session 5: Evolutionary Time

Ralf J. Sommer
(Max Planck Institute for Developmental Biology, Germany)
Tatsuya Hirasawa (RIKEN, Japan)
Shigehiro Kuraku
(RIKEN Center for Life Science Technologies (CLST), Japan)
Naoki Irie (The University of Tokyo, Japan)
Qi Zhou (University of California, Berkeley, USA)
Pavel Tomancak
(Max Planck Institute of Molecular Cell Biology and Genetics, Germany)





2016 CDB Symposium

Size in Development: Growth, Shape and Allometry

March 28-30, 2016

The fourteenth annual symposium "Size in Development: Growth, Shape and Allometry" will be held on March 28-30, 2016 in the CDB Auditorium.

This symposium will focus on the question of organismal size control. The size of animals and plants varies greatly among species, and sometimes within species as well. Despite this variability, individuals maintain remarkably constant shapes through the poorly understood process of scaling, and deviations in shape control often lead to pathological conditions. Furthermore, allometric conversion of organ shapes is a major driver of evolutionary diversification. Recent advances in the study of growth control and morphogen functions, as well as quantitative approaches of tissue shape control have brought us a fresh opportunity to revisit these long-standing questions.

Invited Speakers

Ehab Abouheif
(McGill University, Canada)
Alexander Aulehla
(EMBL Heidelberg, Germany)
Mary Baylies
(Sloan-Kettering Institute for Cancer Research, USA)
Mototsugu Eiraku
(RIKEN CDB, Japan)
Makoto Furutani-Seiki
(University of Bath, UK)
Georg Halder
(VIB Center for the Biology of Disease, Belgium)
Shigeo Hayashi
(RIKEN CDB, Japan)
Iswar Hariharan
(University of California, Berkeley, USA)
Mitsuyasu Hasebe
(National Institute for Basic Biology, Japan)
Rebecca Heald
(University of California, Berkeley, USA)
Hidehiko Inomata
(RIKEN CDB, Japan)
David Kingsley
(Stanford University, USA)
Mitsuru Morimoto
(RIKEN CDB, Japan)
Elaine A. Ostrander
(National Institutes of Health, USA)
Duojia Pan
(Howard Hughes Medical Institute and Johns Hopkins University School of Medicine, USA)
Stefano Piccolo
(University of Padova, Italy)
Nadia Rosenthal
(The Jackson Laboratory, USA)
Hiroshi Sasaki
(Osaka University, Japan)
Richard Schneider
(University of California, San Francisco, USA)
James M. Wells
(Cincinnati Children's Hospital Medical Center, USA)
Yingzi Yang
(Harvard School of Dental Medicine, USA)

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 750 such talks, in addition to numerous meetings, internal forums, and colloquia. The following speakers presented seminars in the period from January to December 2015.

Date	Title	Speaker
01-13	Liquid like compartments in cells: Implications for polarity and disease	Anthony HYMAN
02-05	Mechanism and Reconstitution In Vitro of Mammalian Germ Cell Development	Mitinori SAITOU
02-23	Dynamic programming and reprogramming of the stomatal lineage of plants	Dominique BERGMANN
03-02	Autophagy: an intracellular degradation system	Noboru MIZUSHIMA
03-12	How to screen out liars	Yoshihiro TAGUCHI
04-13	Transition zone: The dramatic change of lung progenitors to terminally differentiated cells	Munemasa MORI
04-17	Practical application of Platanus genome assembler	Rei KAJITANI
04-20	Making and patterning the spinal cord: cells, morphogens and transcriptional networks	James BRISCOE
05-25	Cell shape and morphogenesis: sub cellular and supracellular mechanisms	Maria LEPTIN
06-01	Symmetry breaking in mouse development	Takashi HIIRAGI
06-08	Reverse genetics in *Xenopus tropicalis* as a model for understanding human genetic diseases involved in eye and brain formation	Takuya NAKAYAMA
06-26	Modeling Electrodiffusion and Osmosis in Physiological Systems	Yoichiro MORI
07-22	Spatially asymmetric neuronal connectivity in motion-sensitive circuits	Keisuke YONEHARA
07-24	Dissecting alternative pathways and functions of the microRNA biogenesis machinery in mammalian neurogenesis and neurodevelopmental disorders	Davide DE PIETRI TONELLI
07-29	Degeneration and regeneration of circuits in the vertebrate retina	Rachel WONG
08-18	Epigenetic reprogramming in cancer and energy metabolism	Mitsuyoshi NAKAO
08-20	In toto imaging reveals multi-scale mechanisms for robustness in patterning and morphogenesis	Sean MEGASON
08-21	Engineering hematopoietic stem cells from human pluripotent stem cells	Ryohichi SUGIMURA

Date	Title	Speaker
08-25	Forming and transforming tubes in the mouse lung	Jichao CHEN
10-19	Stem cell dynamics in the mouse testis	Shosei YOSHIDA
10-28	The Making of Blood Vessels through Sprouting Angiogenesis	Li-Kun PHNG
11-20	Gene and genome duplications, and vertebrate evolution	Yukuto SATO
11-30	Genome-wide remodeling of sperm epigenome upon fertilization	Azusa INOUE
11-30	Endogenous stem cells in lung repair and regeneration	Brigid HOGAN
12-03	Regulating mesoderm regionalization generates kidney organoids from human pluripotent stem cells	Minoru TAKASATO
12-10	Noise, Regulation, and Cell-Cell Heterogeneity	Namiko MITARAI
12-15	Nail stem cell and its unique role in digit bone homeostasis and regeneration	Makoto TAKEO
12-16	Two-photon imaging of subthreshold membrane potential dynamics at cellular resolution *in vivo*	Yuki BANDO
12-17	Extrinsic Regulation of Muscle Stem Cell Function	C. Florian BENTZINGER

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Sendai

Center for Advanced Photonics
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
Wako Administrative Headquarters

Center for Emergent Matter Science
Center for Advanced Photonics
Center for Sustainable Resource Science
Brain Science Institute
Nishina Center for Accelerator-Based Science
Advanced Center for Computing and Communication
Cluster for Industry Partnerships
Chief Scientist Laboratories
Associate Chief Scientist Laboratories
Distinguished Senior Scientist Laboratories
Initiative Research Units
Special Research Units
Research Groups
Global Research Cluster
2-1, Hirosawa, Wako, Saitama 351-0198
Tel: +81-48-462-1111 Fax: +81-48-462-1554

Tokyo Liaison Office
Fukoku Seimei Building, 23rd floor (Room2311)
2-2-2, Uchisaiwaicho, 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
Center for Life Science Technologies
1-7-22 Suehiro, Tsurumi-ku, Yokohama, Kanagawa 230-0045
Tel: +81-45-503-9111 Fax: +81-45-503-9113

RIKEN

http://www.riken.jp/

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Nagoya

2271-130, Anagahora, Shimoshidami, Moriyama-ku,Nagoya, Aichi 463-0003
Tel: +81-48-462-1111 Fax: +81-48-462-1554

Osaka

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

Kobe

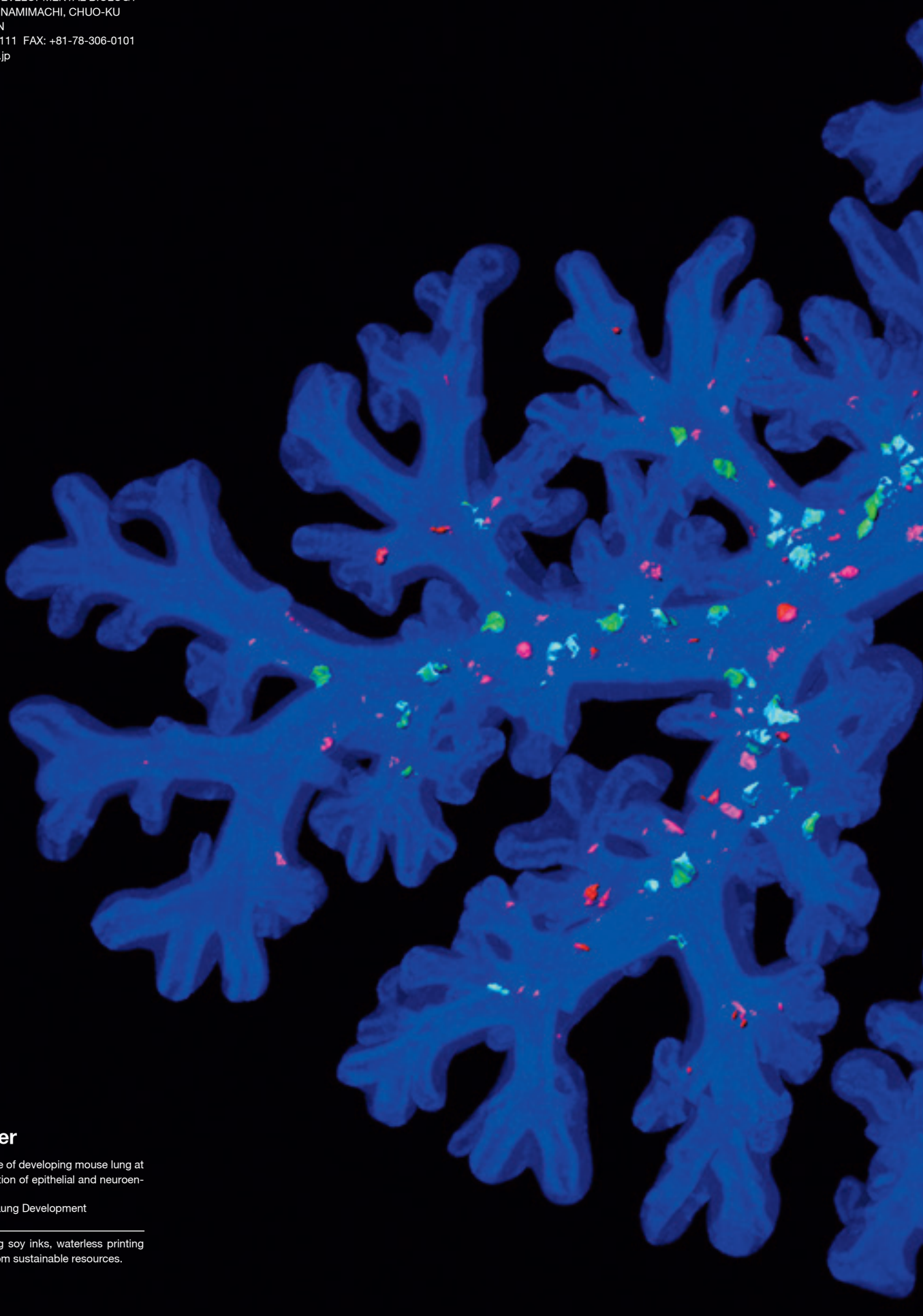
Center for Developmental Biology
2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047
Tel: +81-78-306-0111 Fax: +81-78-306-0101
Center for Life Science Technologies
6-7-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047
Tel: +81-78-304-7111 Fax: +81-78-304-7112
Advanced Institute for Computational Science
7-1-26 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047
Tel: +81-78-940-5555 Fax: +81-78-304-4955
HPCI Program for Computational Life Sciences
7-1-26 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047
Tel: +81-78-940-5835 Fax: +81-78-304-8785

Harima

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

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

RIKEN 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 image of developing mouse lung at E16.5 showing localization of epithelial and neuroendocrine cells.
Image: Laboratory for Lung Development

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