RIKEN Center for Developmental Biology

2012 Annual Report

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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 aging society were among the issues addressed, and developmental biology was included within this remit as a scientific field with the potential to contribute to the development of regenerative medicine.

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

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


CDB
Origin and Development of the Vertebrate Traits

Message from the Director

December 2012 marked the tenth anniversary of the completion of our second main research building at the RIKEN Center for Developmental Biology, a date which also in a way represents the unofficial start of full-scale research activities at the Center. Over the course of the past decade, the CDB has grown and matured as an institute committed to a deeper understanding of fundamental processes and mechanisms that underlie animal development, organogenesis, regeneration, and stem cell biology, with an eye to supporting the development of regenerative medicine by providing a solid scientific foundation for translational studies.

The past year has seen a great many changes in the CDB's laboratory programs, with several labs moving on after two successful five-year terms at the Center, and six new teams and units joining our institute since January. These many new faces bring a new injection of talent, energy, and ideas to the research staff, in line with our philosophy of balancing continuity with change, and diversity of perspectives and approaches with a common cause of pushing back the frontiers of knowledge and contributing to the greater good.

Even as these transitions have played out in recent years, our scientists have remained consistently innovative and productive. In 2012, our labs published studies in such diverse areas as somatic cell nuclear transfer, the control of cell polarity, orientation and differentiation, cytoskeleton dynamics, and the phylogenetic origins of vertebrates. In addition to these advances in fundamental biology, we are proud to support efforts to translate stem cell biology into clinical applications in full compliance with the national regulatory framework for stem cell clinical research. The award of the 2012 Nobel Prize in Physiology or Medicine to Sir John Gurdon for his work on cloning by SCNT, and Shinya Yamanaka for his development of induced pluripotent stem cells lent further impetus to the field, and it is gratifying that the Japanese government has redoubled its support for the development of regenerative medicine and other applications of stem cells.

This past year, the Center expanded its programs for engaging with students at all levels of higher education. We now hold summer and winter courses for local high school students, as well as practical workshops for high school science teachers in the fall. In August, we established a lab internship program for undergrads, complementing our annual intensive lecture program for graduate and medical school students.

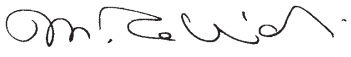
The upcoming year will be a transformative one for RIKEN, as it reorganizes its research system at the start of a new 5-year plan with greater focus on multidisciplinary collaboration and the development of applications to address challenges in energy, environment, health, and human welfare. The Center for Developmental Biology will continue to focus on its core strengths of developmental biology, regeneration and stem cell research, but with a keener eye to develop new quantitative approaches, and closer cooperation with labs throughout the RIKEN system, enabling us to take the fullest advantage of the extraordinary resources and facilities this institution provides.

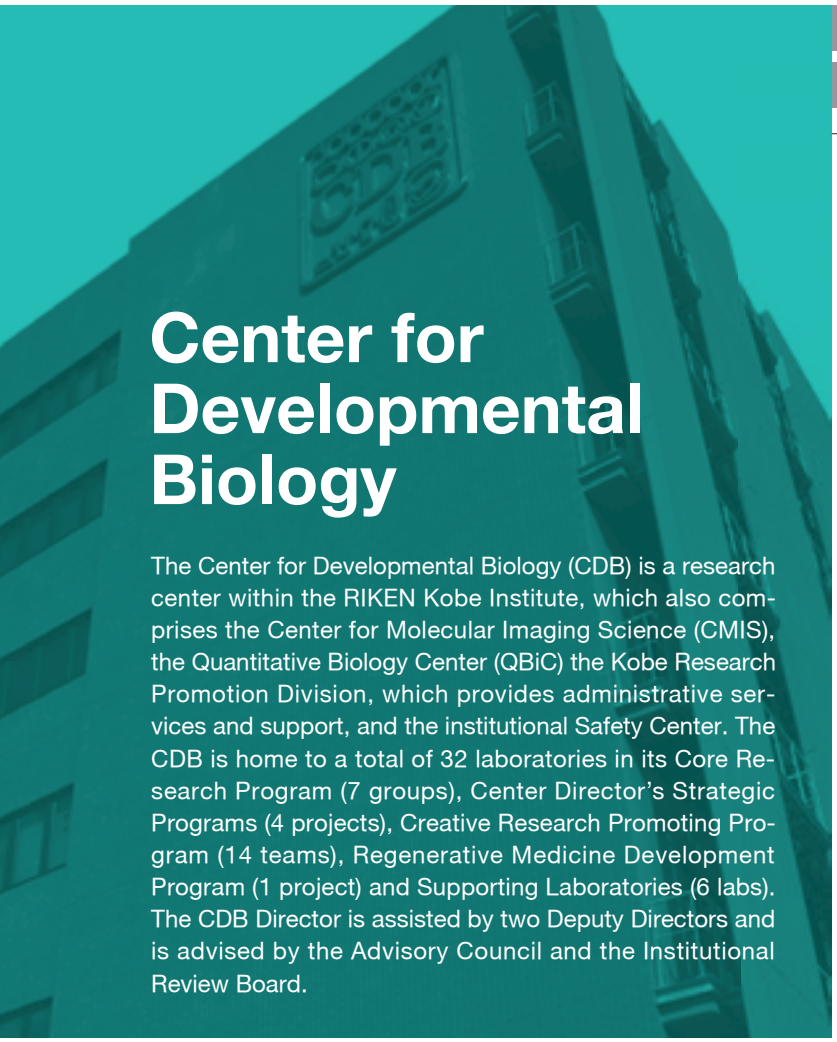
We will also look to strengthen even further our ties with colleagues around the world, as evidenced by the new cooperative research agreements the CDN entered into with three institutes in Barcelona, Spain in the past year. Our research staff is increasingly diverse, with more than one in ten of our scientists coming from overseas, and our very successful annual symposium marked its tenth anniversary as well. We look forward to the continued collaboration of the international community in our shared endeavors, and we thank our colleagues from around the world for their continued support.

The upcoming year promises to be an exciting one, for the CDB, for Japan, and for the world. We hope to welcome many more new faces here in Kobe, and to see the start of important new undertakings in line with our research mission and the nation's plan to contribute to human society through scientific progress and technology development. It is with a combined sense of profound responsibility and wonderful opportunity that we have undertaken these challenges, and I would like to thank all of our many colleagues and supporters for their cooperation and encouragement in these efforts.

Masatoshi Takeichi

Director, RIKEN Center for Developmental Biology





Center for Developmental Biology

The Center for Developmental Biology (CDB) is a research center within the RIKEN Kobe Institute, which also comprises the Center for Molecular Imaging Science (CMIS), the Quantitative Biology Center (QBiC) the Kobe Research Promotion Division, which provides administrative services and support, and the institutional Safety Center. The CDB is home to a total of 32 laboratories in its Core Research Program (7 groups), Center Director's Strategic Programs (4 projects), Creative Research Promoting Program (14 teams), Regenerative Medicine Development Program (1 project) and Supporting Laboratories (6 labs). The CDB Director is assisted by two Deputy Directors and is advised by the Advisory Council and the Institutional Review Board.

Center for Molecular Imaging Science

Quantitative Biology Center

HPCI Program for Computational Life Sciences

Research Promotion Division

Safety Center

Institutional Review Board

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

Core Program

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

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

Center Director's Strategic Program

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

- Pluripotent Stem Cell Studies
  Hitoshi NIWA M.D., Ph.D.
- Systems Biology
  Hiroki R. UEDA M.D., Ph.D.
- Physical Biology
  Tatsuo SHIBATA Ph.D.
- Developmental Morphogeometry
  Yoshihiro MORISHITA Ph.D.

Advisory Council

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

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

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

Director Masatoshi TAKEICHI

Deputy Directors Shin-Ichi NISHIKAWA, Shinichi AIZAWA

Creative Research Promoting Program

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

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

Regenerative Medicine Development Program

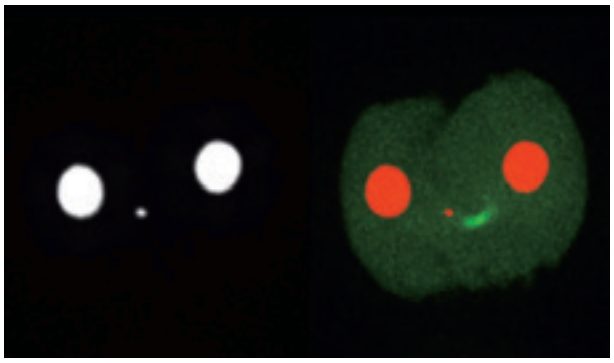
The Regenerative Medicine Development Program provides support for labs engaged in the clinical translation of research relating to regenerative medicine, such as cell transplantation and tissue engineering.

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

Supporting Laboratories

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

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

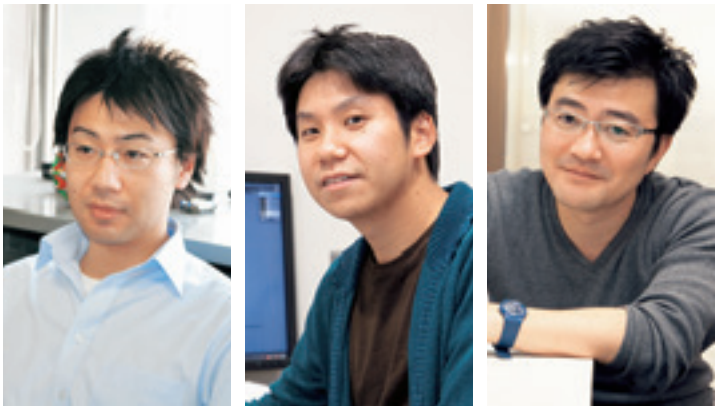


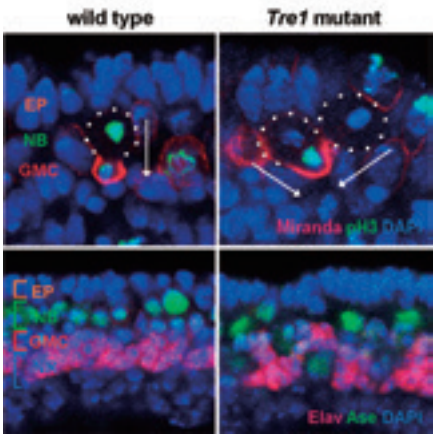
Secrets of cloning efficiency unlocked

The cloning of mammals by somatic cell nuclear transfer has been haunted by remarkably low efficiency – only a few percent of SCNT attempts are successful, even after more than a decade of research in the field. The Lab for Genomic reprogramming found that early defects in chromosome segregation may be the reason behind cloning failure.

Three new research leaders

The RIKEN CDB appointed three new leaders to its research programs in the first three months of 2012: Yoshihiro Morishita was named Unit Leader of the Laboratory for Developmental Morphogeometry, Tomoya Kitajima was appointed Team Leader of the Laboratory for Chromosomal Segregation, and Shigehiro Kuraku returned to the CDB as head of the Genome Resource and Analysis Unit.





Tre1 orients stem cell alignment

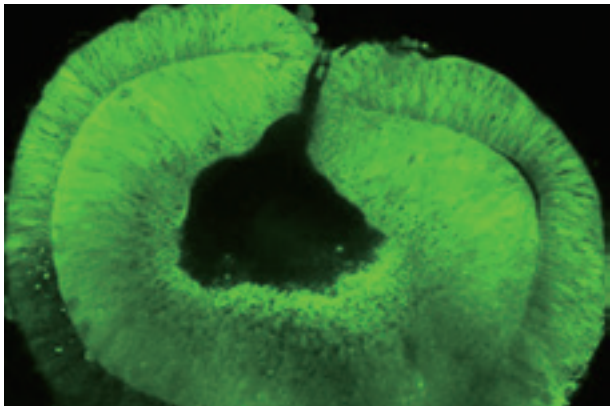
The Laboratory for Cell Asymmetry reported how the G Protein-coupled receptor Tre1 controls the orientation of neuroblast in the fruit fly. This stem cell alignment is critical to the establishment of cell polarity and tissue patterning in the fly brain, and may represent a more widely conserved feature of asymmetric cell division in general.

CDB Symposium on Quantitative Developmental Biology

The CDB held its tenth annual symposium, “Quantitative Developmental Biology” on March 26-28. Around 160 participants from around the world gathered to discuss computational, and theoretical approaches to the study of development.

Human ES cells give rise to retinal tissue

Building on technqiues first developed in mouse embryonic stem cells, the Laboratory for Organogenesis and Neurogenesis showed that human ES cells can also be induced to self-organize into complex, three-dimensional tissue-like structures in vitro. In this study, the Sasai group derived an hESC-derived optic cup, which is the embryonic tissue that gives rise to the retina.



Summer lectures for grad school affiliates

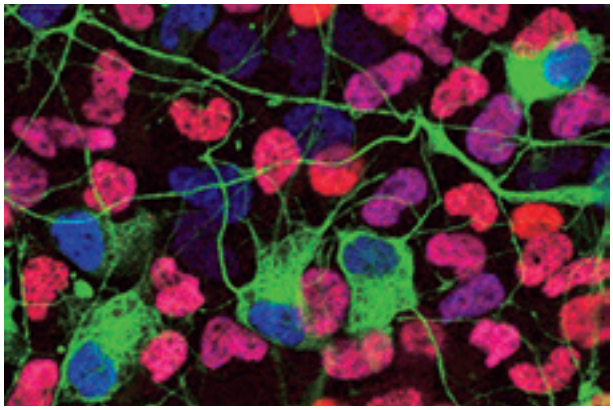
As part of its commitment to contribute to the development of future research leaders, the CDB held a two-day lecture program for graduate and medical school students. More than 150 aspiring scientists joined the event, which included talks by CDB scientists and visits to many of the Center’s labs.

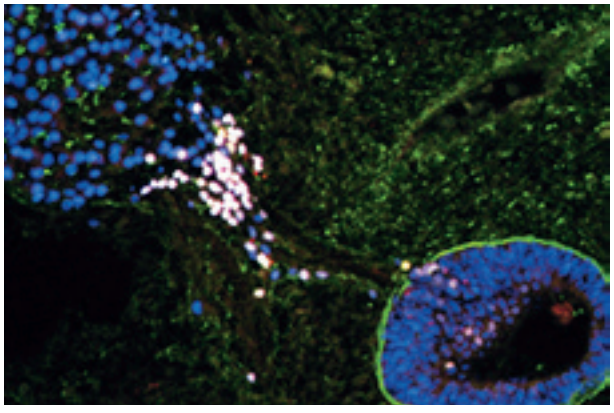
Summer school at the CDB

The Office for Research Communications organized two one-day courses developed in cooperation with the Laboratory for Early Embryogenesis (Guojun Sheng, Team Leader). The curriculum focused on basic facts about blood biology, and the development of the blood system in chick, as well as an introduction to globin switching, and how defects in this mechanism are linked to disease.

Enteric neural crest cells cut corners

New work by the Lab for Neuronal Differentiation and Regeneration revealed a surprising feature of the development of the enteric nervous system, showing how subset of enteric neural crest cells in mouse takes a short cut across the mesentery on the way to innervating the large intestine.





Roots of the paratympanic organ resolved

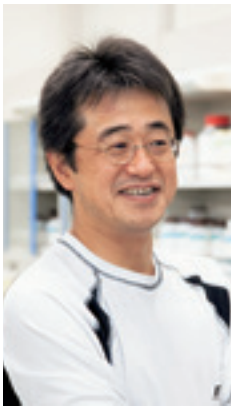
Birds possess a little-studied sensory structure called the paratympanic organ, which is thought to be used in monitoring air pressure or altitude. The Laboratory for Sensory Development has now shown that this organ traces its origin back to a related structure in fish.

High school teachers come to learn

The CDB's annual course in developmental biology for high school teachers welcomed over 20 biology instructors to the center. Instruction focused on how transcription factors establish cell identity, and included a practical component on the culture and observation of chick embryos.

More new faces

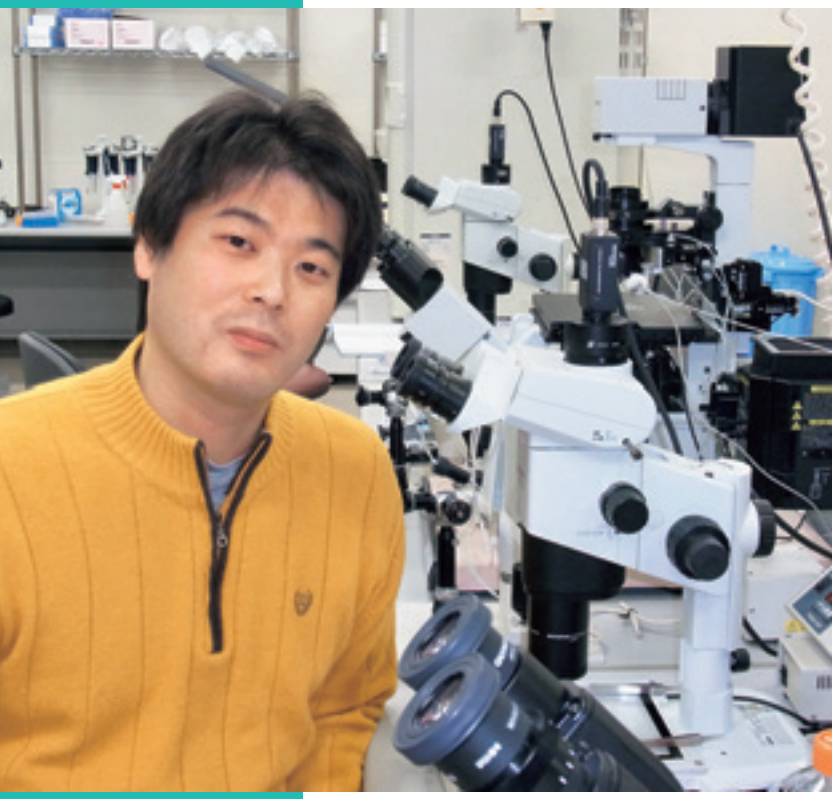
The RIKEN CDB welcomed three new laboratory heads in the closing months of 2012: Yasuhide Furuta was appointed head of the Laboratory for Animal Resources and Genetic Engineering, Hironobu Fujiwara was named Team Leader of the Laboratory for Tissue Microenvironment, and Mitsuru Morimoto joined as Team Leader of the Laboratory for Lung Development.

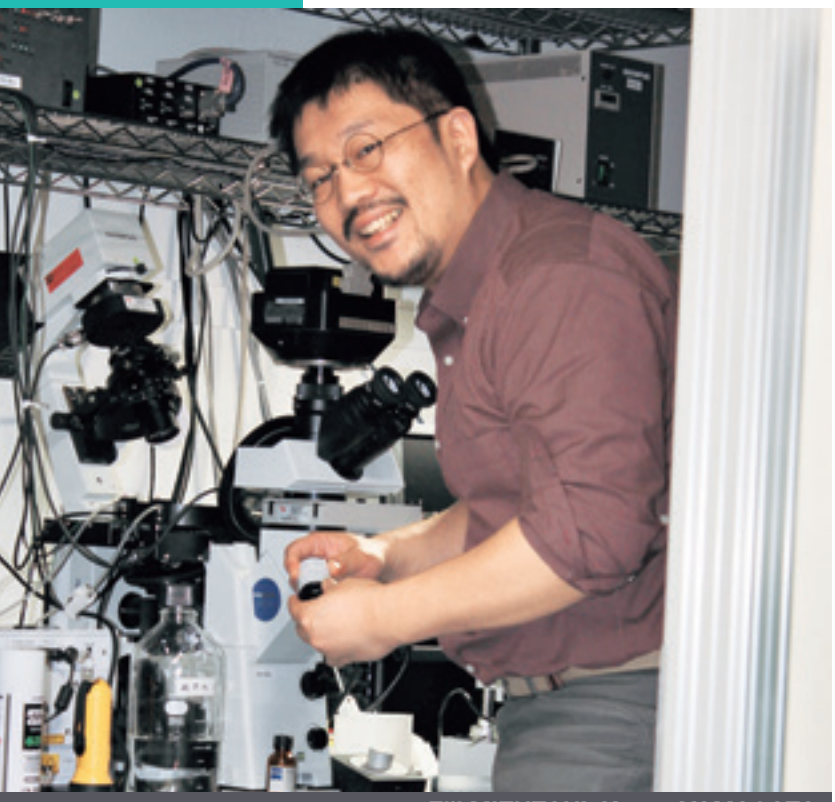


CDB Group Director Yoshiki Sasai awarded science prizes

In 2012, Yoshiki Sasai, group director of the Lab for Organogenesis and Neurogenesis, was honored with a trio of prestigious science prizes – the Inoue Science Prize, the Yamazaki-Teiichi Prize, and the Takeda Medical Prize – in recognition of his work in the self-organization of embryonic stem cells (ESCs) into various tissue types.

Chromosome segregation underlies cloning efficiency





Eiji MIZUTANI, Kazuo YAMAGATA

The announcement of the birth of Dolly the sheep in 1997 represented the first cloning by somatic cell nuclear transfer (SCNT) of a mammal, and sparked a global fascination with the possibilities of creating genetic copies of individual organisms. This technology has been adopted for use in animal husbandry and conservation, but in the 15 years since that first achievement, SCNT remains plagued by an extraordinarily low efficiency in giving rise to viable cloned offspring, which has never risen above a few percent of attempts. Many groups have tackled this challenge through studies of gene expression and epigenetic signatures in cloned embryos, but to date the problem of low efficiency remains unsolved.

A new report by Eiji Mizutani, Kazuo Yamagata, and others in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) may shed new light on this murky question, showing that defects in chromosome segregation during early developmental stages may lie at its root. Published in *Developmental Biology*, this study takes advantage of live imaging technologies that enable the microscopic observation of living embryos. Mizutani has since moved to the RIKEN BioResources Center in Tsukuba, and Yamagata to Osaka University.

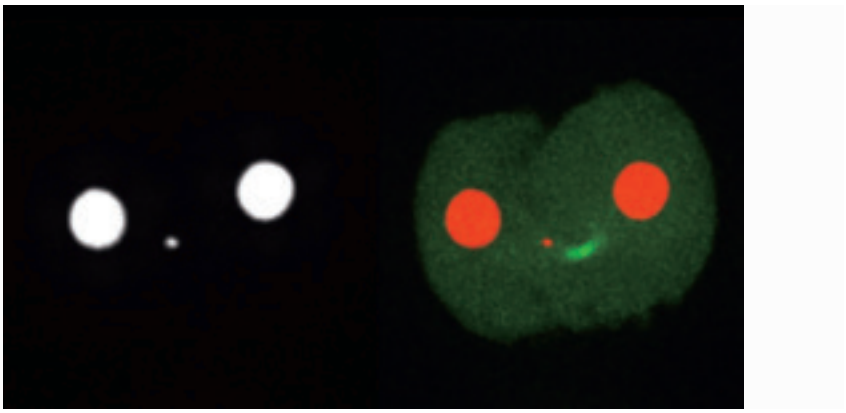
Yamagata and Mizutani began by focusing on the possibility that chromosomal abnormalities, rather than transcriptional or epigenetic differences, might account for the lack of progress in SCNT efficiency. To do so in live embryos, however, meant that they needed to find non-invasive approaches as an alternative to other traditional technologies such as immunolabeling and in situ hybridization, which require embryos to be fixed in advance. This led them to begin to develop a live imaging system used in this study, which allows users to observe chromosome distribution in very early embryos without disturbing their development.

They used mRNAs to introduce fluorescent probes for histone H2B and α-tubulin to label the nucleus and mitotic spindle, and tweaked the doses to optimal levels using their microscopy setup to minimize stress to the embryos. They next watched cloned embryos develop for around 60 hours to the morula-blastocyst stage using their 3D fluorescence imaging system, before returning the embryos to surrogate mothers, and confirming their developmental viability.

This meticulous observation yielded a surprising finding: of 330 embryos studied, around 80% showed some

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

aberrant chromosomal segregation by the 8-cell stage. All such embryos failed to develop to term. Interestingly, however, the rate of chromosomal abnormality between the 8- and 16-cell stages was only 2.5%, and by the end of this early embryonic period, only 7.1% of the cloned embryos had not experienced a chromosome segregation error. Clearly, defects in chromosomal segregation in the first few embryonic cell divisions set the stage for developmental failure.

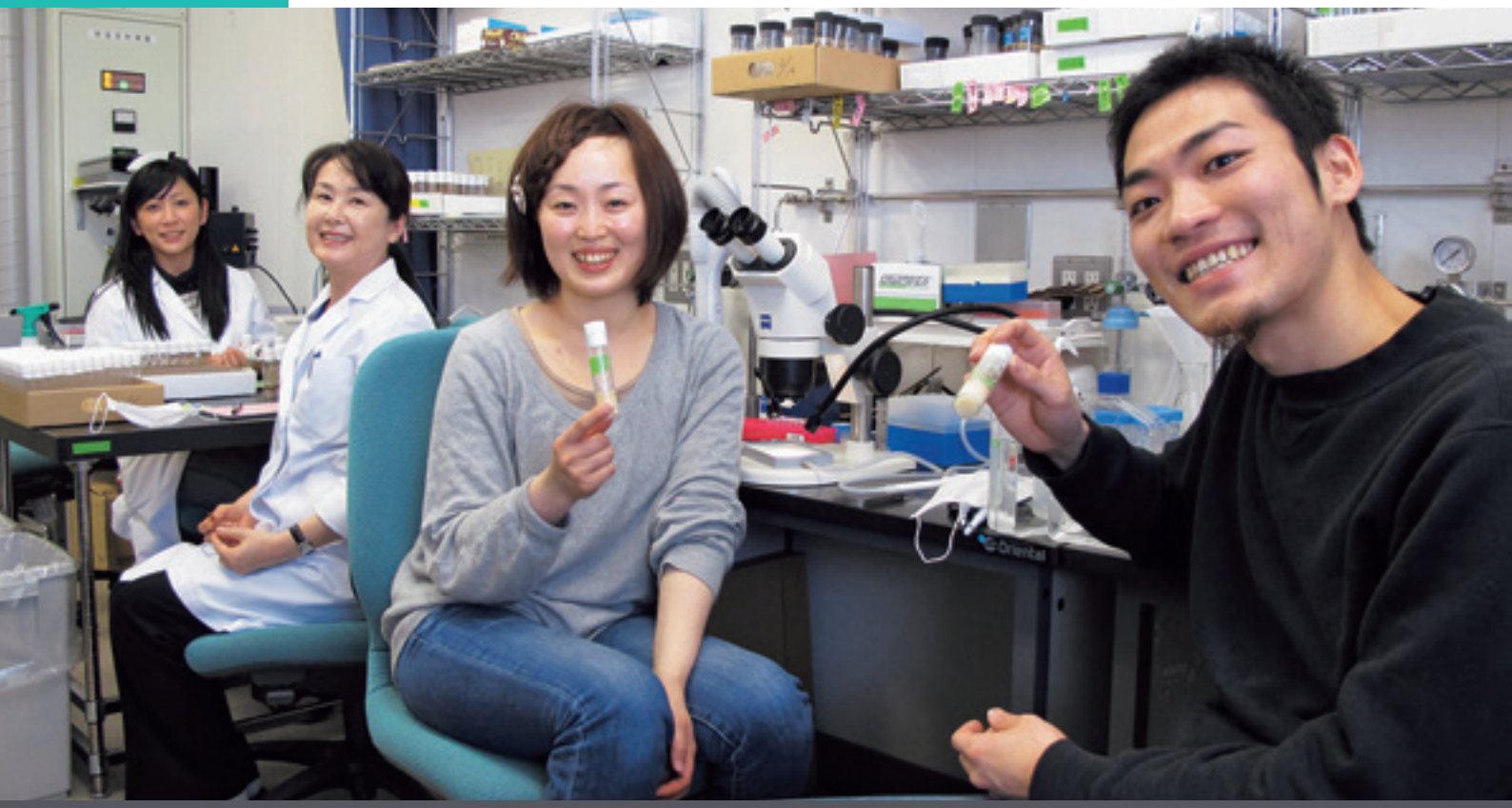


Video showing chromosome segregation (image on right labeled for H2B (red) and tubulin (green).

Mizutani and Yamagata next selected 72 cloned embryos at random and implanted them in surrogate mothers one-by-one to check their developmental potential. Of these, only three resulted in live births, and on reviewing the fluorescence imaging of their early development in vitro, they found that all three embryos were free of chromosomal defects by the 8-cell stage.

“We believe that the technology described in this study can be used to improve the screening of cloned embryos for the absence or presence of chromosomal segregation errors, which appear to be strongly connected to their developmental viability,” says Wakayama. “We also recognize however that there are likely to be other factors than epigenetics and chromosomal segregation behind SCNT efficiency rates, and we hope to continue in our search for those in future work.”

New conserved mechanism for insulin regulation



Kanako HIGUCHI, Junko SHINNO, Yuka NISHIMORI, Naoki OKAMOTO

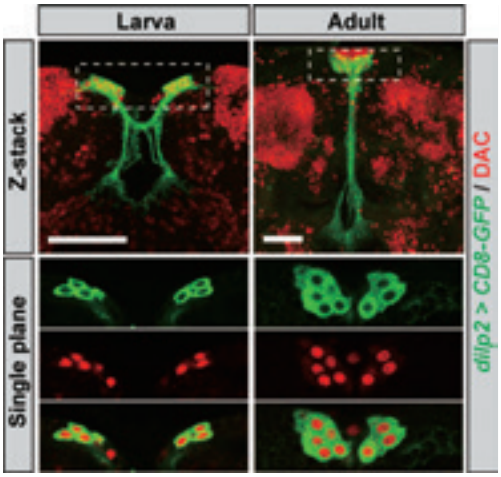
Peptides of the insulin family are found across a broad range of taxa spanning both vertebrates and invertebrates, in which they play roles in the regulation of processes such as metabolism, growth, reproduction, and longevity. The genome of the fruit fly *Drosophila melanogaster* includes seven genes encoding such peptides, known as *dilps* (*Drosophila* insulin-like peptides), which are primarily expressed in secretory insulin-producing cells (IPCs) within the brain. The expression of each *dilp* is independently regulated, but just how this level of coordination is achieved remains poorly understood.

Now, Naoki Okamoto and others in the Laboratory for Growth Control Signaling (Takashi Nishimura, Team Leader) have identified an important clue to this puzzle, showing that a highly conserved nuclear protein Dachshund (Dac) acts as a transcriptional regulator specific to *dilp5*. Published in the *Proceedings of the National Academy of Science*, these findings may open new avenues of understanding into the means by which diverse insulin-family peptides are kept working in harmony.

The IPCs of the fruit fly brain express several genes known to be involved in the development of the eye. The team knocked down the function of several of these genes in a tissue-specific manner to check for possible effects on *dilp* expression. They found that the knockdown of *dac* was accompanied by a dramatic down-regulation of *dilp5*, suggesting that Dac might function in Dilp5 regulation in IPCs.

Okamoto N, et al. Conserved role for the Dachshund protein with Drosophila Pax6 homolog Eyeless in insulin expression. *Proc Natl Acad Sci U S A* 109.2406-11 (2012)

An examination of Dac expression in IPCs showed the protein is expressed continuously throughout development. When Okamoto next analyzed homozygous mutants for *dac*, he found that while IPCs formed normally, the expression of *dilp5* in these cells was markedly lower in young larvae, but intriguingly its expression was normal in later larval stages, suggesting stage-dependent differential mechanisms for its regulation. A subsequent analysis using heterozygous *dac* mutants revealed that expression levels of that gene correlated closely with the expression of *dilp5*. A second protein called Eyeless (Ey) is known to control *dilp5* expression, and he found that *ey* mutants revealed similar phenotypes as found in the *dac* studies.

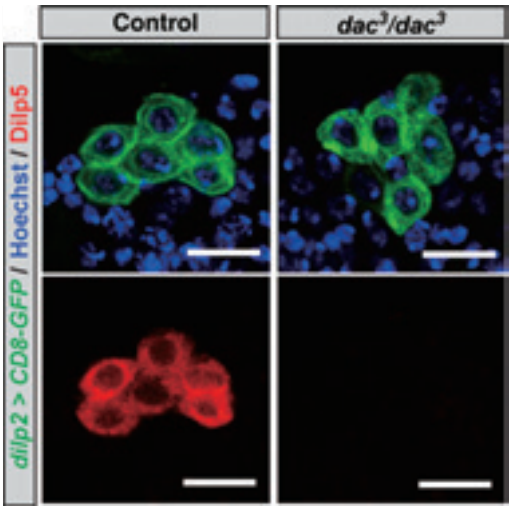


Expression of Dac (red) in the IPCs (green) of larval (left) and adult (right) flies. Upper panels show IPCs and axons (green) with Dac labeled (red). Lower panels show the areas indicated by white dotted lines above at higher magnification.

The team next focused on possible interactions between Dac and Ey, but found that the loss of function of one had no discernible effect on the other, suggesting that their expressions are independently regulated. Their effects on *dilp5* were synergistic, however, as shown by tests in which the expression of both was perturbed. Looking next for an interaction between Dac, Ey and the *dilp5* promoter, they found that while Dac alone did not interact, the binding of Ey to the *dilp5* promoter was accelerated in the presence of Dac. Further experiments revealed Dac forms a physical complex with Ey, and with itself, through specific protein domains.

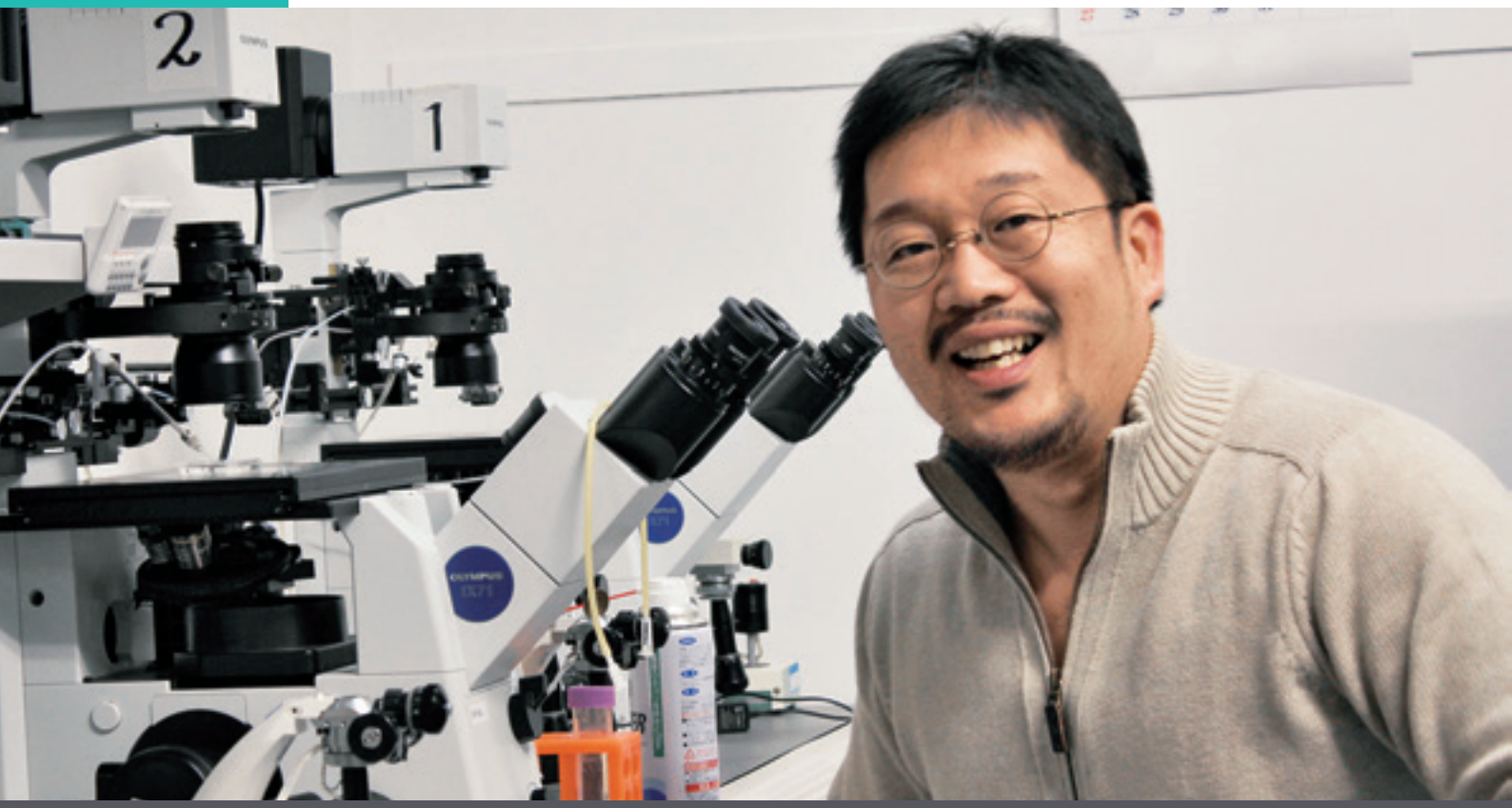
Knowing that Dac is evolutionarily conserved in mammals, Okamoto next turned to the insulin-secreting β-cells in the islets of Langerhans of the pancreas, in which Dach and Pax6, homologs of the *Drosophila* Dac and Ey, play important developmental roles. Importantly, Pax6 is also involved in the transcription of genes encoding critical pancreatic hormones, such as insulin and glucagon. Using cultured rat β-cells-derived cell line, they examined the functions of these two regulatory proteins and found that, as in the fruit fly, Pax6 and Dach had similar combinatorial effects on the activation of insulin expression.

"The expression of *dilp5* can change in response to nutritional status, and we still don't know how this gene is regulated in later stage larvae," says Nishimura, "which suggests that the regulatory situation is even more complex than we expected. We are looking forward to tackling the link between nutrition and *dilp5* expression in future work."



In wildtype young larvae (left) IPCs (green) express Dilp5 (red), while in *dac* mutants (right) Dilp5 expression is dramatically reduced.

New, lower-cost approach to fluorescence imaging



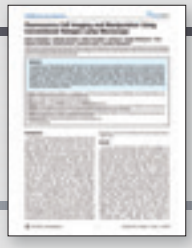
Kazuo YAMAGATA

Fluorescence imaging is a foundational technology in modern cell and molecular biology, making it possible to label specific molecules and analyze their structural and functional properties. This imaging technique is not, however, without its disadvantages. The fluorescent light used to obtain images, for example, can cause significant damage to living cells, and many of the dyes used lose their color quickly after exposure. Cost can also be an issue, as expensive equipment such as mercury lamps and lasers are need to generate the light needed to excite fluorescent molecules into their light-emitting state.

Now, Kazuo Yamagata and others in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) have showed that halogen lamps can serve as a cheaper, lower-intensity light source for use in fluorescence imaging studies in living cells. The report of this new technological development is published in *PLoS ONE*. Yamagata has since moved to take a position at Osaka University.

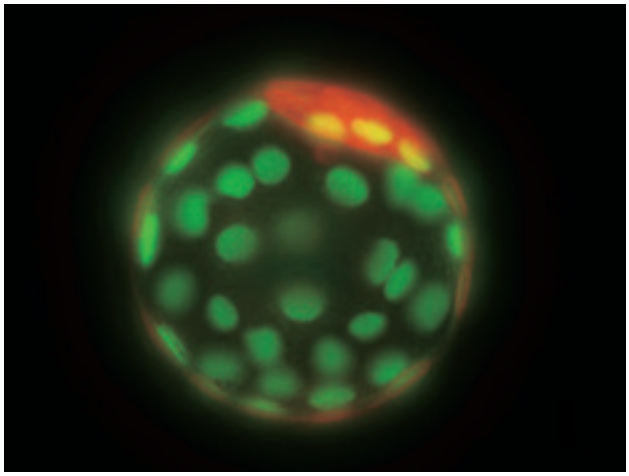
The demand for an alternative light source was first brought home to the team through their frequent interactions with labs in developing countries in Asia, for whom mercury lamps and lasers can be prohibitively expensive. “We knew that the motivation levels are very high in these labs, but their limited financial resources make it difficult to acquire advanced microscopes, which inevitably deepens the ‘research divide’ between developed and developing nations,” says Wakayama. “Fluorescence imaging traditionally requires powerful light sources, but with the advent of newer molecules, we thought it might be possible to achieve similar results even with weaker lamps.”

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



Imaging of this sort requires the use of excitation filters, which exclude light of wavelengths other than those needed to charge target fluorescent molecules into an excited, light-emitting state. In collaboration with Olympus, Yamagata et al. developed an adaptor to allow ordinary upright and inverted microscopes using only halogen lamps to be equipped with such filters, as well as filters to absorb light other than that emitted from a fluorescing sample. When they tested this new equipment on fluorescence-labeled mouse embryos, they found the results to be remarkably vivid, comparable to those typical of a traditional fluorescence microscope.

Fluorescence imaging normally produces images on a dark field, due to the filtering of light at visible wavelengths, but this can prove disadvantageous when scientists desire to observe cellular features in a brighter context. By modifying their adaptors, however, the team was able to create filters smaller than the optical path with "leaky" diaphragms that can be opened to allow light around the periphery of the image area to escape filtration, thereby making it possible to obtain bright field and fluorescence images simultaneously.



Mouse embryo imaged by fluorescence microscopy using an upright optical microscope equipped with halogen lamps and an excitation filter. Inner cell mass (red) and trophectoderm cells (green) are shown.

Length of exposure to intense light is a critical factor in determining cellular damage during imaging. To compare the efficiency of their halogen lamp system with that of traditional mercury lamps, the team injected oocytes with phycoerythrin-labeled antibody against histone (or fluorescent dye for the nucleus), and observed them using each approach. Those observed using a conventional fluorescence microscope faded within around 30 seconds, but the same sample tested using the halogen light system remained continuously visible for over 10 min. This lower-power approach may have real biological consequences - early embryos imaged using conventional technology show reduced developmental viability on implant in surrogate mothers, but those visualized using the new system showed no such effect.

"One of the biggest advantages of this approach is its low cost, which should put it within reach for many labs in economically developing parts of the world," says Wakayama. "Of course, there are many different types of halogen lamp, so we will need to keep studying the optimum intensity, but we are hopeful that the adaptors we have developed will make it possible to attach fluorescence filters to ordinary optic microscopes at much lower cost."

Triple-jointed: Notch signaling a key to insect joint diversity

ReikoTAJIRI

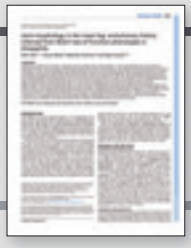
Insects show a remarkable variety of joint structures in their limbs, which despite their tiny size and simplicity and the rigid, external nature of their skeletons, rival our own in diversity of function. The basis for this articular range, however, remains something of a mystery.

Now, Reiko Tajiri and others in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) have shown that, diversity of appearance notwithstanding, insect limb joint structures can be classed into one of three general forms, and that the developmental decisions that guide their morphogenesis are under the control of Notch signaling. Published in *Development*, this work goes a long way to building a framework for understanding how insect joints are formed.

This new study built in previous work from the Hayashi lab, which showed that cell differentiation and movement are key processes in the sculpting of a specific type of joint – the ball-and-socket form - in the adult *Drosophila* leg, known as the tarsus. Taking this as a starting point, Tajiri began comparing tarsal joint morphologies in other insect species to gain a clearer understanding of the extent to which this model is conserved. The ball-and-socket type joint is not found in the tarsal limbs of primitive insects (Apterygota, Paleoptera), in which the joint cavity is covered entirely by cuticle with no underlying ball-socket distinction, which suggests that the cell differentiation needed to form this form of joint does not take place.

In other joints in the tarsus of several primitive species, however, the joint is formed by two cuticular components positioned side-by-side, without the encirclement seen in a true ball-and-socket joint.

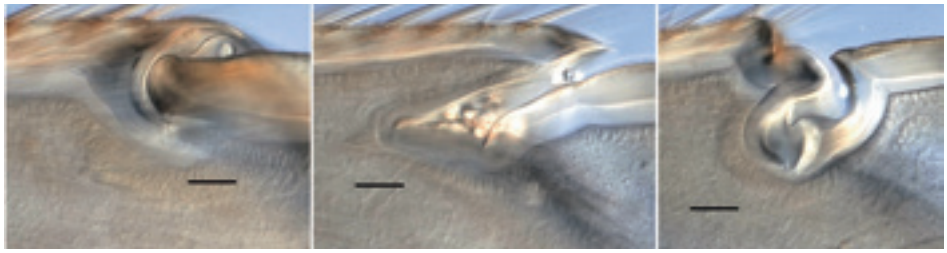
Tajiri R, et al. Joint morphology in the insect leg: evolutionary history inferred from Notch loss-of-function phenotypes in Drosophila. *Development* 138.4621-6 (2011)



In other, more highly diverged insect lineages, such as Polyneoptera, Paraneoptera, and Holometabola, ball-and socket-joints are common, in contrast to their basal relations. From these observations, the group realized that only three main morphologies account for all tarsal limb joint forms in insects: uniform, side-by-side, and ball-and-socket.

These findings additionally suggested that the formation of the most complex ball-and-socket joint relies generally on the two essential modular processes of cell differentiation and cell movement, and that gains or losses in either of these results in the failure of ball-and-socket formation. The evident diversity of joint forms in insect limbs presumably has evolutionary significance; each joint type may confer advantages under specific environmental conditions, and all may have evolved multiple times independently. But what Tajiri wished to know was, how might such diversity arise at the genetic level?

Previous reports have demonstrated the importance of the Notch signaling pathway in *Drosophila* limb development, so the group conditionally knocked down Notch at various stages in pupal development to watch for possible changes in joint structure. By altering the absence, presence and timing of Notch expression using a temperature-sensitive mutant, Tajiri was able to shift ordinarily ball-and-socket joints to take on the simpler uniform conformation, or a range of incomplete variations on its usual form. Labeling of a subset of cells in the developing joint showed that Notch knockdown affected cell motility, resulting in the failure of the cell movement step of joint formation.



Ball-and-socket, uniform, and side-by-side joint types in insect limb (scale bar = 10 µm)

But what of differentiation? Using a constitutively active form of its gene and an RNAi construct to interfere with its expression, Tajiri tested the role of differing levels of Notch expression role in determining the fate of cuticle cells. The model that developed was clear: higher levels of Notch signaling drive “ball” differentiation, while lower levels promote “socket.”

“What was fascinating about this study was that it showed that Notch signaling is not simply a switch driving differentiation, but a subtle regulator of the balance between differentiation and cell movement,” says Tajiri, who has since moved to the University of Tokyo. “It will be interesting to find out what specific factors Notch interacts with at each step in joint morphogenesis, which may lead to a better understanding of the links between development and evolution in this system.”

Stem cells show sense of direction

Shigeki YOSHIURA

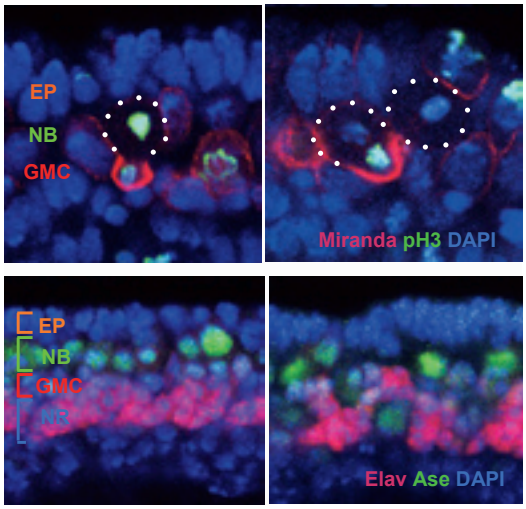
Brains, whether they belong to flies, mice, or humans, are built of numerous types of neurons and supporting cells. This great diversity is the result of asymmetric cell divisions, in which daughter cells often inherit different sets of protein components from their progenitors, and consequently develop different properties. Such activity is highly coordinated, to ensure that the appropriate number and type of cells are produced at just the right times and locations. The *Drosophila* neuroblast, a type of stem cell, provides a good example of this, dividing perpendicular to its overlying epithelium to give rise to a pair of daughter cells of which one is a more highly differentiated intermediate neural progenitor and the other is a neuroblast like its parent cell. The means by which this division is oriented, however, are not well understood. Previous work has indicated that centrosomes and microtubules play a crucial role in larval brains, but other studies suggest that other factors may be important as well in embryonic central nervous systems.

Shigeki Yoshiura and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) have now found that the key may lie in molecular signals across the cell membrane that tell neuroblasts how to align. Published in *Developmental Cell*, the group shows that the G protein-coupled receptor (GPCR) Tre1 is a key factor in a molecular interaction that orients these cells with respect to the epithelium.

The group's study began with a genetic screen for non-cell-autonomous factors involved in orientation, by monitoring the orientation of neuroblasts in embryos lacking genes coding for candidate membrane proteins. They found that mutation of the rhodopsin family orphan GPCR gene *trapped in endoderm 1* (*tre1*) was associated with orientation defects, raising the possibility of a causal relationship. Deletion mutants for *tre1* showed significant perturbations of neuroblast orientation with respect to the epithelial ectoderm, resulting in disorganized

Yoshiura S, et al. Tre1 GPCR signaling orients stem cell divisions in the Drosophila central nervous system. *Dev Cell* 22.79-91 (2012)

stratification of the developing tissue in central nervous system (CNS). This phenotype could be rescued by introducing a genomic fragment of the gene, strongly suggesting that it was responsible for the neuroblasts' disorientation.



In wild type embryos (left), neuroblasts (NB) asymmetrically divide perpendicular to the epithelial layer (EP) to bud off the daughter ganglion mother cell (GMC) inward, giving rise to a layered organization from the epithelium to neurons (NR). In contrast, in tre1 mutants, the orientation of the polarity and division of neuroblasts is randomized (right upper), and the developing nervous tissue is disarranged (bottom right).

Interestingly, however, cell polarity was unaffected by *tre1* deletion, and the early-stage orientation defects in such mutants were rectified over the course of development by some unknown compensatory mechanism. Yoshiura et al. speculated that this correction might be due to interactions between neurons and other neighboring cells, such as glia. And indeed when they tested flies in which both *tre1* and *repo*, a critical glial cell gene, were deleted, even later-stage neuronal organization was aberrant, suggesting that neuron-glia interactions are required for the development of the CNS.

Using flies in which the distribution of Tre1 could be visualized, the group next sought to determine its mode of function. Yoshiura found that this protein was distributed widely in neuroblasts across the cell cortex, but to a much weaker degree in ganglion mother cells and neurons, indicating that it might function in a neuroblast-autonomous fashion. Disruption of microtubule activity had no effect on orientation in neuroblasts in the embryonic CNS in which Tre1 was normally expressed, suggesting that this protein functions independent of microtubules at embryonic stages.

To exclude the possibility that Tre1 helps orient neuroblasts via signals from a tissue other than epithelium (such as mesoderm), the group examined a brain region in which only epithelium, neuroblasts and their progeny are present, and found that the cells remained oriented perpendicular to the epithelium, as expected.

They next used co-immunoprecipitation to find binding partners of Tre1, and found that it interacts with Goα, a neuroblast protein of unknown function. Using a toxin that specifically interferes with Goα, they found that loss of this protein's function results in a phenotype mirroring that of *tre1* deletion, suggesting that Tre1 may orient neuroblasts in response to signals from the epithelium, by facilitating GDP-GTP hydrolysis by Goα.

These findings brought another factor into the picture, as the protein Pins is known to interact with Goα in vitro. Pins is known for its role in mitotic spindle orientation, via binding with another Gα protein, Gαi. In vivo tests using a different assay, however, indicated that only the Goα-Pins interaction depends on Tre1, suggesting that only the GTP form (but not the GDP form) of Goα binds to Pins. Additional testing using Gαi, in addition to Goα and Pins, in tissue cultures, showed that to be the case - the GTP form of Goα showed much higher interactivity with Pins than the GDP form of Goα or Gαi. Constructing a cell culture assay system, the group also found that Tre1 could recruit Pins in the presence of Goα.

The scenario that emerges is a sort of "handoff" mechanism; binding with the GDP form of Gαi is necessary and sufficient for Pins to be recruited to the cell membrane. At the membrane, the GTP form of Goα, in turn, is generated from its GDP form only on the apical side proximal to the adjacent epithelium, from which an unidentified signal presumably activates Tre1. Gαi is then replaced by the GTP form of Goα, due to its more stable binding to Pins, which serves to recruit Pins to the apical membrane. In neuroblasts, Pins associates with the polarity-organizing aPKC-Par complex via interaction with Inscuteable, and consequently recruits this complex to the apical side of neuroblasts. As the aPKC-Par complex determines the polarity and orientation of division in neuroblasts, the Tre1-Pins mechanism ultimately directs the orientation of neuroblasts with respect to the epithelium.

Pair of factors directs heterochromatin assembly via RNAi

Aki HAYASHI

The enormous information set encoded in a cell's genome is bundled into tightly wound coils of DNA and proteins known as chromatin. The prevailing view has been that this structure takes two main forms: loosely packed stretches of euchromatin which tend to permit active transcription, and more securely locked down heterochromatin, where gene expression is typically silenced, and which provides the structural basis for regions such as the centromere and telomeres. Heterochromatin is the end product a cascade of events involving multiple molecular complexes that interact to form this critical chromosomal component, which is characterized in part by its coating of the protein HP1. Some studies have suggested that HP1 plays a role upstream in the cascade as well, but the details of this have resisted explication.

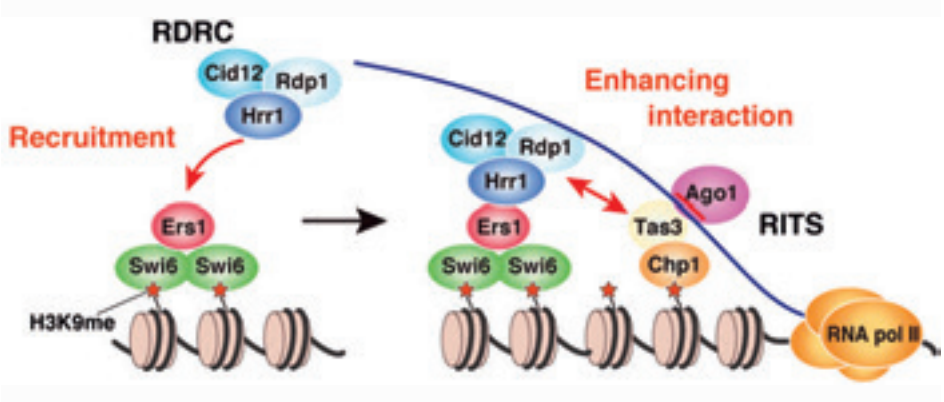
Now, Aki Hayashi and others in the Laboratory for Chromatin Dynamics (Jun-ichi Nakayama, Team Leader) have shown that the co-factors Ers1 and the HP1 homolog Swi6 work together to recruit the RNAi machinery to sites of heterochromatin assembly in the fission yeast, *Schizosaccharomyces pombe*. Published in the *Proceedings of the National Academy of Sciences*, this work refines our understanding of how this crucial basis of chromosome structure is formed.

Heterochromatin assembly is regulated by Histone H3 Lysine 9 (H3K9me) methylation. In fission yeast, the histone methyltransferase Clr4 marks specific sites on chromosomes, serving as a binding site for chromodomain proteins such as Swi6, which in turn induces heterochromatin compaction. Interestingly, the molecular machinery behind RNAi, best known as an epigenetic form of post-transcriptional regulation, also plays a part. In *S. pombe*, the RNA-induced transcriptional silencing (RITS) complex and RNA-dependent RNA polymerase complex (RDRC) both function in the RNAi process, but recent work has shown that these are, surprisingly, also essential to heterochromatin assembly in the centromeres, as loss of function of either results in aberrant centromeres and defective gene silencing. This growing body of work has shown that both RITS and RDRC localize at centromere heterochromatic regions, highlighting the possibility that this RNAi machinery is involved in heterochromatin assembly.

Hayashi approached this question using a genetic screen for mutations affecting gene silencing at the centromeres, which yielded a number of hits associated with the RNAi machinery, including a factor called Ers1, which had previously been necessary to RNAi, but remained

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

poorly understood. The team found that *ers1* mutants showed not only defective silencing, but also low production of siRNAs (short interfering RNAs) and reduced H3K9 methylation. On testing the ability of various genes involved in heterochromatin assembly or RNAi to rescue these phenotypes, they found that overexpression of the RNA helicase Hrr1 and the methyltransferase Clr4 had a partially compensatory effect, pointing to the possibility of a functional connection. Yeast two-hybrid assays showed that Ers1 and Hrr1 bind directly, but did not show any physical association between either Ers1 or Hrr1 with Clr4.



Functional relationships between Ers1 and other factors linking RNAi and heterochromatin assembly

Given these findings, questions remained regarding the molecular mechanisms behind the functional association. Hayashi et al. next used EGFP to label Ers1 in wildtype cells, allowing them to observe its punctate localization in the nucleus, consistent with co-localization with heterochromatin. In Clr4 and Swi6 mutants, however, the localization was more diffuse, indicating their importance to the localization of Ers1 to heterochromatic regions. A subsequent yeast two-hybrid screen confirmed that Ers1 binds directly with Swi6, fitting a piece into a puzzle that had begun clarifying into a scenario in which the molecular complex of Ers1 and Swi6 is recruited to heterochromatin regions methylated by Clr4.

The bigger picture reveals that Ers1 interacts with both Hrr1 and Swi6. Hrr1 is a necessary component of RDRC, which the team found was recruited to centromeres by Ers1 and Swi6. Thus, Swi6 acts as a kind of platform to localize this complex to these highly methylated heterochromatic regions in order to proliferate further siRNA generation.

“Recent work has revealed the direct association between the RITS and RDRC complexes,” says Nakayama. “It seems that Ers1 is playing a critical role in bridging the RNAi machinery with heterochromatin assembly. It is will be very interesting to see whether this bridging mechanism, as well as the mechanism behind RNAi mediated heterochromatin formation, are conserved in other organisms. ”

2012 Events

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

Kobe-Barcelona joint meeting

The Center hosted a joint meeting with colleagues from research centers and graduate school programs in Barcelona, including the Center for Genomic Regulation, Universitat Pompeu-Fabra, and the Institute for Research in Biomedicine. The CDB subsequently entered into programs of cooperation with all three organizations.

RNA Sciences in Cell and Developmental Biology II

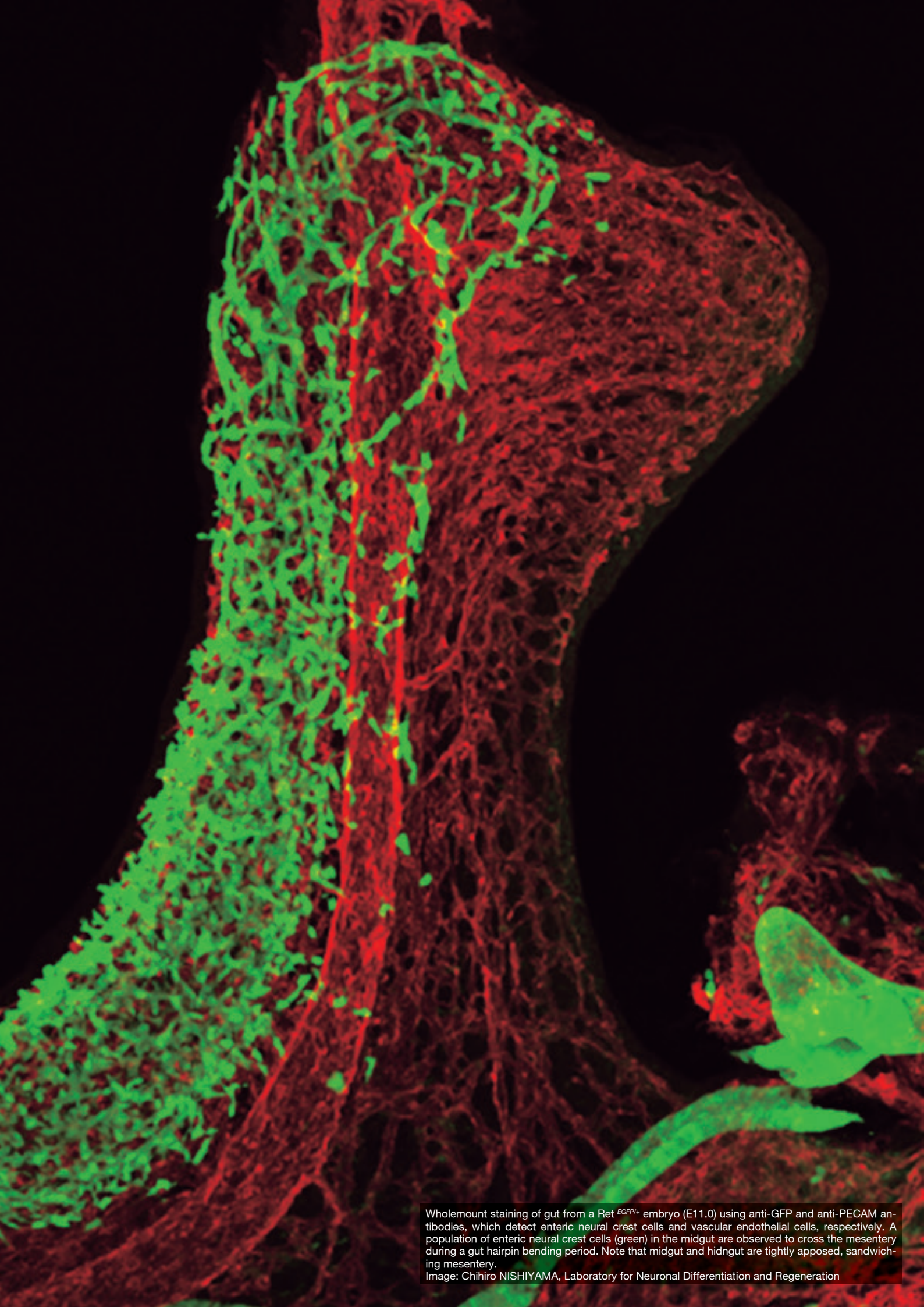
This two-day program in June brought together scientists from around the world to discuss new developments and insights into the diverse function of RNAs in the developing embryo. This represents the second CDB meeting with this focus, following a successful start in 2010.

Kobe Institute Open House

The RIKEN Kobe Institute held its annual Open House on October 20, welcoming more than 1,500 visitors to learn more about the research being done at its research centers, including the Center for Developmental Biology.

2012 Retreat

The CDB held its annual retreat in the mountains of Sasayama on September 25 to 26. This overnight stay is designed to encourage research scientists and students share their most recent work in an relaxed, informal setting. This year's retreat additionally featured talks by CDB alumnus Kinya G. Ota and Advisory Council member Christopher Wylie.



Wholemount staining of gut from a Ret $^{EGFP/+}$ embryo (E11.0) using anti-GFP and anti-PECAM antibodies, which detect enteric neural crest cells and vascular endothelial cells, respectively. A population of enteric neural crest cells (green) in the midgut are observed to cross the mesentery during a gut hairpin bending period. Note that midgut and hidngut are tightly apposed, sandwiching mesentery.
Image: Chihiro NISHIYAMA, Laboratory for Neuronal Differentiation and Regeneration

Vertebrate Body Plan

Shinichi AIZAWA Ph.D.

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

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

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Recent Publications

Inoue F, al at. Gbx2 expression to forebrain and midbrain, competing with class Ⅲ POU factors. *Mol Cel Biol* 32. 2618-27 (2012)

Kurokawa D, et al. A lineage specific enhancer drives Otx2 expression in teleost organizer tissues. *Mech Dev* 128. 653-61 (2012)

Shibata M, et al. MicroRNA-9 regulates neurogenesis in mouse telencephalon by targeting multiple transcription factors. *J Neurosci.* 31. 3407-22 (2011)

Kurokawa D, et al. Evolutionary origin of the Otx2 enhancer for its expression in visceral endoderm. *Dev. Biol*. 342. 110-20 (2010)

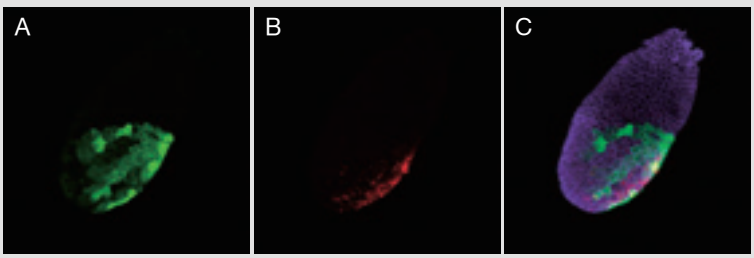
Suda Y, et al. The same enhancer regulates the earliest Emx2 expression in caudal forebrain primordium, subsequent expression in dorsal telencephalon and later expression in the cortical ventricular zone. *Development* 137. 2939-49 (2010)

Takeuchi M, et al. Germ layer patterning in bichir and lamprey; an insight into its evolution in vertebrates. *Dev Biol* 332. 90-102 (2009)

All vertebrate species share certain features in the development of the brain, in that all vertebrate brains comprise four regions - telencephalon, diencephalon, mesencephalon and metencephalon (or cerebellum) - an organizational trait that is thought to have appeared with the advent of the vertebrate lineage. However, dramatic changes in this organization took place during the divergence of jawed (gnathostome) fishes from their jawless (agnathan) ancestors. Evolutionary modifications of the telencephalon first manifested in the reptiles, becoming even more pronounced with the development of a neocortex stratified into six layers regionalized by distinct physiological functions. However, the mechanisms that instruct the brain's laminar regions to conform to anterior-posterior and dorsal-ventral axis patterning programs, and the means by which each individual region is formed, remain unknown.

The head is the most highly layered of the body's structures and its development begins with anterior-posterior axis formation. This A-P axis patterning is one of the most primary phenomena in animal development, and the emergence of the vertebrate lineage has been accompanied by widespread adaptations to this fundamental rule, as evidenced by the diversity of mechanisms by which vertebrate taxa achieve the common ends of axis formation and jaw induction.

A long road lies ahead in the search for the origins of the *Bauplan* of the vertebrate head, but application of powerful new molecular biological techniques to the study of multiple vertebrate model species has now made it possible to study the question more comprehensively and in greater detail than ever before. Our laboratory is focusing on the ontogenetic and phylogenetic roles played by the *Otx/Emx* family of head gap genes as a route toward a better understanding of brain development. The question of what molecular mechanisms underlie the development of neocortical regions is central to that pursuit.



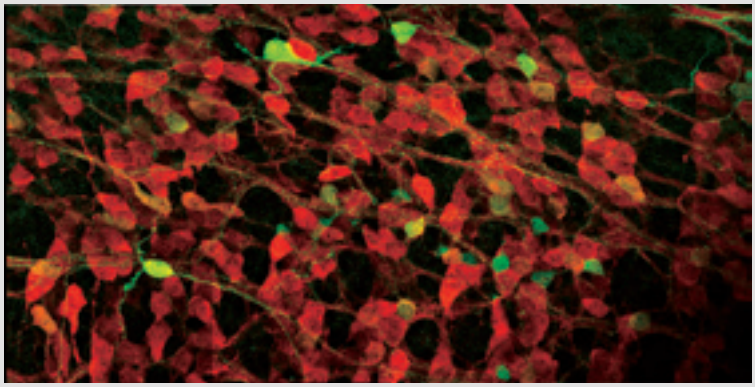
(A) shows descendants of distal visceral endoderm cells that expressed a head organizer gene (*hog*), (B) the descendants retaining *hog* expression, and (C) their merged view.

Neuronal Differentiation and Regeneration

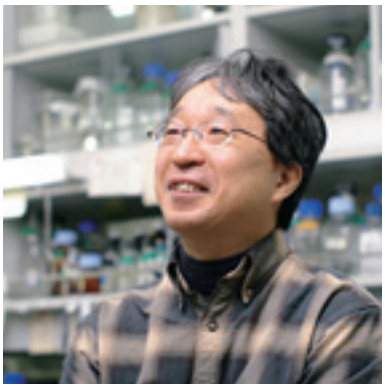
The architecture of the nervous system, which underlies much of the dynamism of animal life, is frequently described as complex, but closer examination reveals great beauty as well. The construction of this exquisite and elaborate structure involves the birth, growth and death of countless individual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

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

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



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



Hideki ENOMOTO M.D., Ph.D.

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

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

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Assistant
Kaori HAMADA
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Recent Publications

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

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Tissue Microenvironment

Hironobu FUJIWARA Ph.D.

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

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

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Recent Publications

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

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

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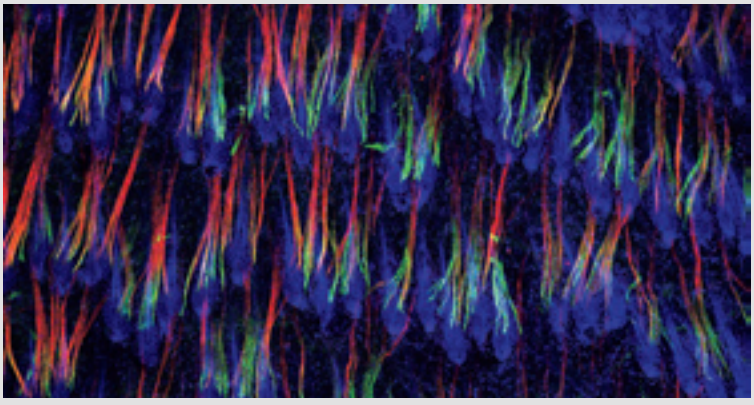
Fujiwara H, et al. Regulation of mesodermal differentiation of mouse embryonic stem cells by basement membranes. *J Biol Chem* 282.29701-11 (2007)

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

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

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

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

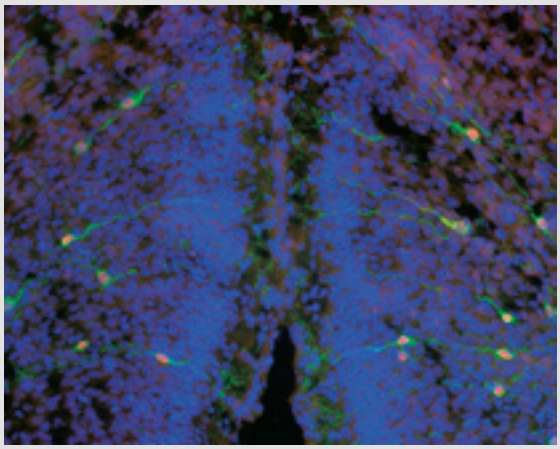


The image shows arrector pili muscles anchored to the bulge of hair follicles in a whole-mount preparation of dorsal skin in mouse, viewed from the dermal side. Arrector pili muscles are visualized by staining for α-smooth muscle actin (green) and SM22α (red). The whole-mount is labelled with a nuclear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

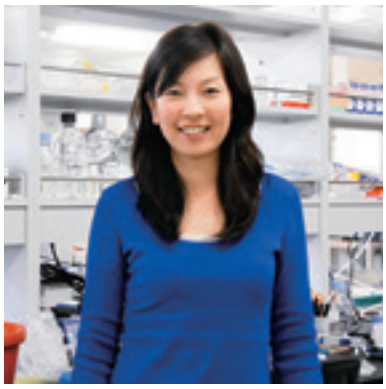
Neocortical Development

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

Previous work has shown that the fate of neocortical neurons is at least in part pre-determined within the progenitor cells. We have found that specification of deep-layer projection neurons is dependent on transcriptional repressor that function cell-autonomously to prevent neurons from acquiring an earlier neuronal phenotype. These results imply cortical intrinsic programs in which neuron fate is established by temporal changes in gene expression may be co-opted. However, in the mature cortex, cortical areas differ in their types and numbers of specific layer neurons along both the anteriorposterior (AP) and medial-lateral (ML) axes. We are exploring the extent to which intrinsic determinants control the specification of neuronal subtypes within discrete regions of the neocortex, as well as the extrinsic influences that refine the boundaries between functional areas of neocortex. To further these studies, we employ genetic manipulations in mice that will enable conditional loss of gene and cellular functions, recombination mediated cell-lineage tracing, and systematic approaches to identify novel molecules responsible for precise areal specification. Through these studies we wish to understand the mechanistic basis by which unique sensory perceptions and functional circuitries develop in the human neocortex.



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



Carina HANASHIMA Ph.D.

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

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Recent Publications

Gonda Y, et al. Robo1 Regulates the Migration and Laminar Distribution of Upper-Layer Pyramidal Neurons of the Cerebral Cortex. *Cereb Cortex* (2012)

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

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

Morphogenetic Signaling

Shigeo HAYASHI Ph.D.

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

Shigeo Hayashi received his B. Sc. in Biology from Kyoto University in 1982, and his Ph.D. in Biophysics from the same institution in 1987, for his work on lens-specific regulation of the chicken *delta crystallin* gene. Inspired by the discovery of the homeobox, he changed his research focus to the developmental genetics of Drosophila and spent three years as a post-doctoral 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*.

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Noriko MORIMITU

Assistant
Ryoko ARAKI

Recent Publications

Otani T, et al. IKKε regulates cell elongation through recycling endosome shuttling. *Dev Cell* 20. 219-32 (2011)

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

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

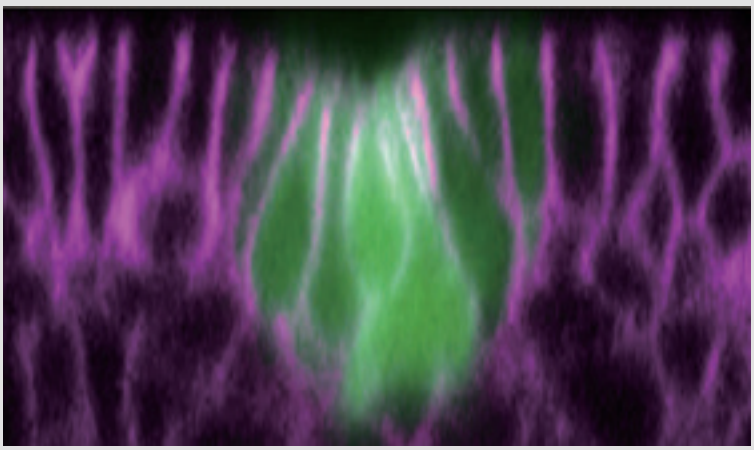
Tajiri R, et al. Dynamic shape changes of ECM-producing cells drive morphogenesis of ball-and-socket joints in the fly leg. *Development* 137.2055-63 (2010)

Niwa N, et al. Evolutionary origin of the insect wing via integration of two developmental modules. *Evol Dev* 12.168-76 (2010)

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

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

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



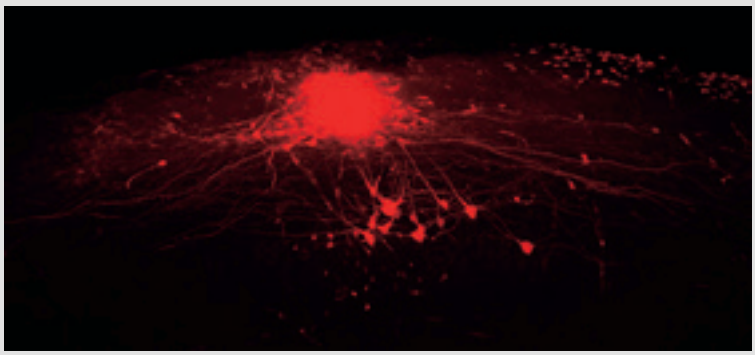
Formation of new organ primodia often involves segregation from the epithelial placode by invagination. This picture shows a cross section of the *Drosophila* tracheal placode. At the center of the placode, tracheal primordial cells (green) constrict apical region facing outside of the epithelia and invaginate inwardly. This process involves a complex interplay of cell boundary tension in the plane of epithelia orchestrated by EGF receptor signaling and inward (basal) movement of cells driving invagination. Cell boundaries are marked with magenta.

Sensory Circuit Formation

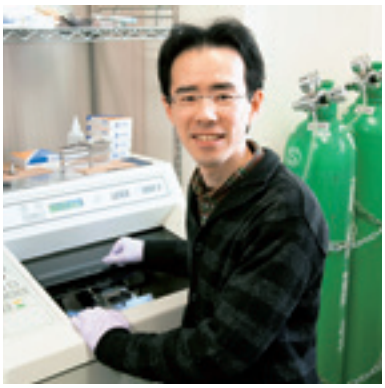
The mammalian nervous system is composed of enormous numbers of neurons, but how do these cells take on diverse fates and organize and array themselves during development? In recent years, it has become clear that the mouse olfactory system provides an excellent platform for addressing these questions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

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

Our lab will seek to develop a better understanding of odorant receptor-dependent axon projection of olfactory sensory neurons, and the formation of neuronal circuitry in the olfactory bulb dependent on inputs from these neurons. We will also seek to develop next-generation genetic tools and imaging techniques to aid in our developmental and functional analyses of specific neuronal circuits in the brain.



Mitral and tufted cells in the mouse olfactory bulb labeled with TMR-dextran. Each mitral/tufted cell extends a single primary dendrite to a single glomerulus, where it receives inputs from a single specific type of olfactory sensory neurons.



Takeshi IMAI Ph.D.

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

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

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Meng-Tsen KE

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Recent Publications

Imai T. Positional information in neural map development: Lessons from the olfactory system. Dev Growth Differ 54.358-365 (2012)

Imai T. and Sakano H. Axon-axon interactions in neuronal circuit assembly: lessons from olfactory map formation. *Eur J Neurosci* 34.1647-54 (2011)

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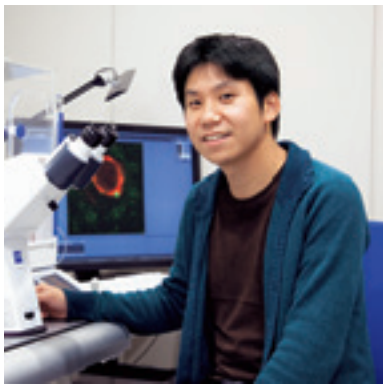
Chromosome Segregation



Tomoya KITAJIMA Ph.D.

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

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Kaori HAMADA

Recent Publications

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

Lee J, et al. Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. *Nat Cell Biol* 10.42-52 (2008)

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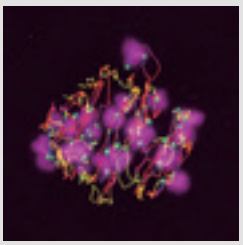
Kitajima T. S, et al. The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. *Nature* 427.510-7 (2004)

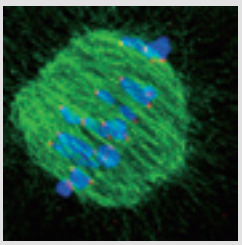
Kitajima T. S, et al. Distinct cohesin complexes organize meiotic chromosome domains. *Science* 300.1152-5 (2003)

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

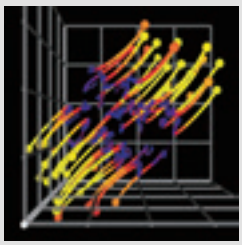
Using the mouse as a model, we will seek to conduct detailed and comprehensive analyses of the dynamics of chromosomes and the molecular machinery that underlies chromosome segregation during cell division. We plan to take advantage of the latest live imaging technologies to study the chromosome dynamics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



Tracking of kinetochores (green) and chromosomes



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

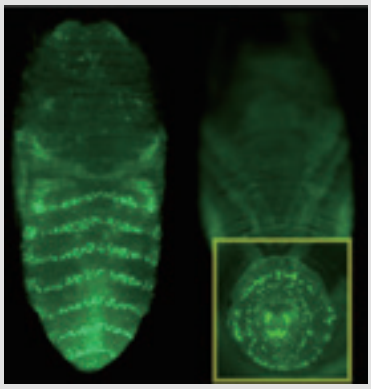


Kinetochore tracking during anaphase of meiosis I.

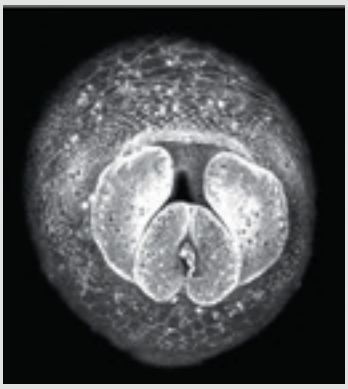
Histogenetic Dynamics

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

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



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



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

Erina KURANAGA Ph.D.

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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.

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Arata KURANAGA

Recent Publications

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

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

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Evolutionary Morphology

Shigeru KURATANI Ph.D.

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

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Recent Publications

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

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

Irie N. and Kuratani S. Comparative transcriptome analysis reveals vertebrate phylotypic period during organogenesis. *Nat Commun* 2.248 (2011)

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

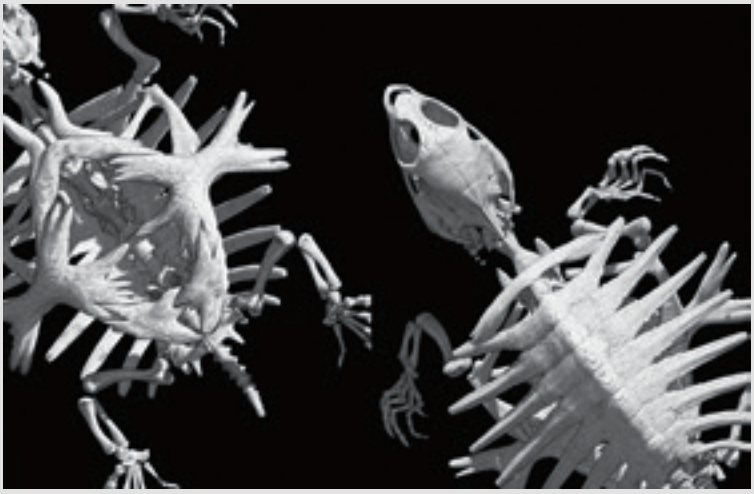
Ota K G, et al. Hagfish embryology with reference to the evolution of the neural crest. *Nature* 446.672-5 (2007)

Takio Y, et al. Evolutionary biology: lamprey Hox genes and the evolution of jaws. *Nature* 429.1 p following 262 (2004)

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

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

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



CT-scanned skeleton of *Pelodiscus sinensis* juvenile

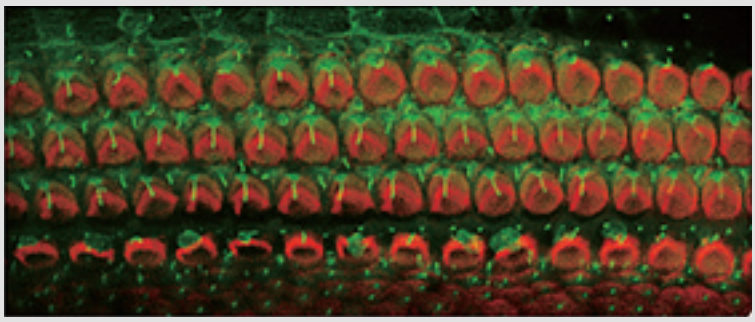
Sensory Development

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

The ear provides a particularly rich system for study. The inner ear, which converts mechanical stimulation to electrical impulses, is formed from a piece of tissue originally destined to become skin; a fate that is altered by instructive signals in a process known as induction. In our research to date, we have identified the mechanism and the signals that set this early tissue on its path toward an inner ear fate as well as those that determine its position. We next hope to learn how these signals are themselves established. We are also investigating the role these signals play in the regulated differentiation of the otic placode, and in better understanding the cellular, molecular and embryological process responsible for this elegantly engineered organ of hearing.

The inner ear cannot function alone. In higher vertebrates, it requires the middle ear to transduce sound that has been channeled through the external ear, as well as specialized regions within the brain to interpret the information it sends. But how is this intricate integration achieved? Using a combination of molecular and embryological techniques, we are characterizing the systems the embryo uses in the coordinated construction of the auditory apparatus.

We believe that an understanding of the embryological systems that control the development of the inner ear can be applied to the development of other organs, sensory organs in particular. By working to comprehend the induction and development of other sense organs, such as the olfactory system and the eye, we are now taking steps toward achieving insights into common mechanisms underpinning the development of organ systems.



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).

Raj LADHER Ph.D.

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

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

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Recent Publications

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

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

Zou Y, et al. Induction of the chick columella and its integration with the inner ear. *Dev Dyn* 241.1104-10 (2012)

Ladher R. K. Squeezing into differentiation. *Dev Cell* 21.607-8 (2011)

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

Freter S, et al. Progressive restriction of otic fate: the role of FGF and Wnt in resolving inner ear potential. *Development* 135.3415-24 (2008)

Sai X. and Ladher R. K. FGF signaling regulates cytoskeletal remodeling during epithelial morphogenesis. *Curr Biol* 18.976-81 (2008)

Tissue interactions behind the middle ear

YiHui ZOU, Siu Shan MAK, Raj LADHER

The vertebrate ear forms in three parts – inner, middle, and outer – that are derived independently from different embryonic tissues. Despite these distinct origins, however, the adult ear manifests as a functionally integrated whole, suggesting that these regions must be closely coordinated during embryonic development. The precise manner in which this transpires, however, remains something of a mystery. In the case of the inner and middle ear, for example, the inner ear forms from a neuroectodermal primordium called the otic placode, followed by the middle ear, which arises from a migratory cell population derived from the neural crest. While the induction of the inner ear is fairly well understood, that of the middle ear, as well the integration between these neighboring tissue regions, is less clear.

Now, YiHui Zou, Siu-Shan Mak, and colleagues in the Laboratory for Sensory Development (Raj Ladher, Team Leader) have shed new light on this process, showing that an underlying strip of mesoderm is needed to induce the bony middle ear in chick. Published in *Developmental Dynamics*, this report opens up avenues toward an improved understanding of the ways in which organ systems involving multiple tissue types can be integrated during development.

In mammals, the middle ear is famously made up of three bones, known familiarly as the hammer, anvil, and stirrup, while in birds it comprises only a single bone, the columella. This structure arises from a neural crest-derived population of precursor cells, which at the relevant stage of development are surrounded by the inner ear, the pharyngeal endoderm, and the cephalic paraxial mesoderm (CPM). The first contact between the nascent columella and these tissues is with the CPM,

Zou Y, et al. Induction of the chick columella and its integration with the inner ear.
Dev Dyn 241.1104-10 (2012)

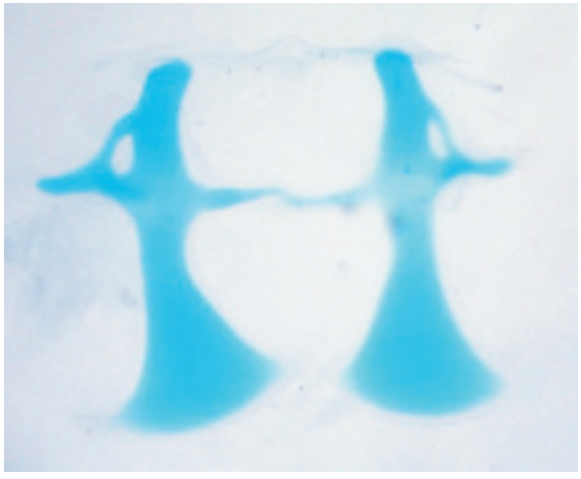
at Hamburger-Hamilton stages 10–11, around 40 hours after fertilization. Zou and Mak decided to test whether this tissue would show inductive activity by transplanting mesoderm taken from the same site and developmental stage of quail embryos. Quail CPM from HH10 had no effect on chick columella, but when they did the same experiment using CPM from quail HH8 embryos, they found morphological changes in the recipient columella, which was interesting as this stage precedes that at which direct contact between these tissues occurs in normal development.

Chick middle ear: the middle ear ossicle of the chicken, the columella, is the cross-shaped bone in this skeletal stain of the 14-day-old chick head. Mineralized bone (red) ; cartilage (blue).

The team speculated that the cephalic paraxial mesoderm might work indirectly by exerting an effect on another neighboring tissue that subsequently induces middle ear. Previous work had shown that CPM induces the otic placode, the tissue that gives rise to the inner ear, so Mak and Zou first tried ablating the inner ear and watching for effects on columella development. Although some anomalies were detected in the fine structure of the ossicle, the effects were modest and suggested that the tissue mediating the CPM's inductive action is not inner ear.

They turned next to the pharyngeal endoderm (PE), which is known to act in the formation of other skeletal elements. Again using a classical explant approach, Zou and Mak transferred corresponding quail tissue into chick embryos and watched for changes in columella formation. When tissue from the second branchial arch (the site of origin for the middle ear) was transplanted from a HH10 quail embryos into chick embryos at the same stage, they observed a range of severe defects in middle ear development; transplants of other endodermal tissue had no such effect, suggesting that pharyngeal endoderm works specifically in this context.

Given that both the CPM and the pharyngeal endoderm affect columella tissue patterning, the team conjectured that CPM might be triggering inductive activity in the PE, which is known to express *Fgf19*, the signaling molecule primarily responsible for inner ear induction. Chick endoderm cultured in isolation did not show expression of this gene, but when this tissue was recombined with quail CPM, it was switched on, suggesting that endodermal Fgf19 is regulated by one or more signals from the cephalic paraxial mesoderm.



A pair of dissected columellae, stained for cartilage, from the left and right side of a chick at day 12 of development.

"How organ systems with components derived from different lineages develop in a coordinated and integrated fashion has not really been investigated," says Ladher. "The auditory system gives us some insight into one way that integration could happen."

Celsr1 bridges key processes in formation of neural tube

Masatoshi TAKEICHI, Tamako (and Tomoki) NISHIMURA

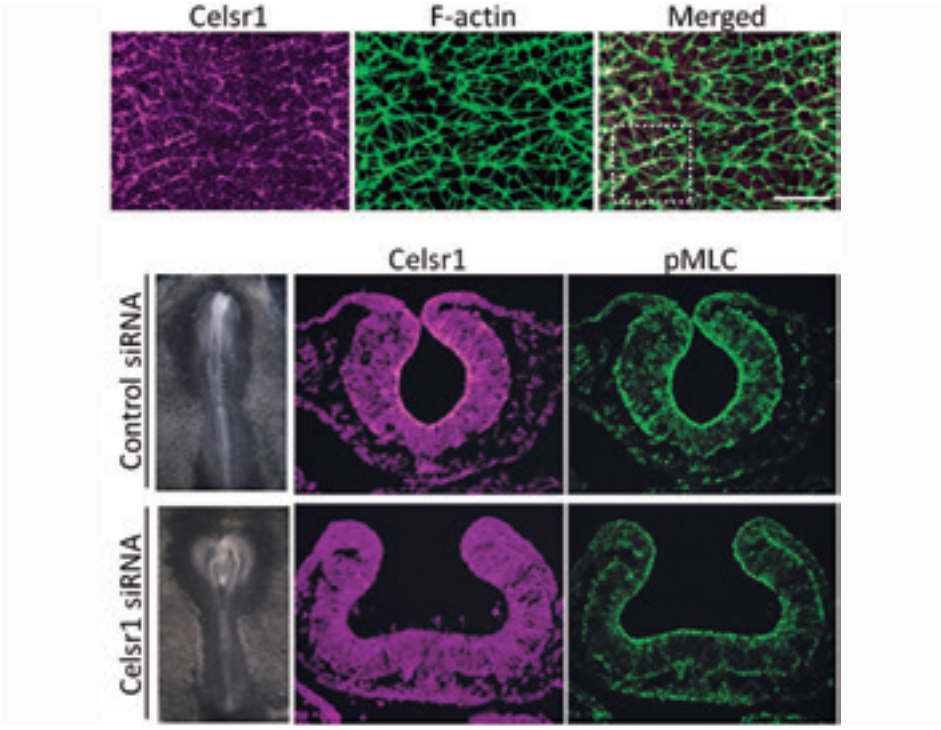
Topology plays a central role in development. Sheets of cells held together by adhesion molecules fold or curl into functional barriers separating regions of the body or tubes that serve as tightly sealed channels. The neural tube is one such structure, which forms from an epithelial sheet called the neural plate that bends in, rolls up, and pinches off to form an enclosed cylinder that subsequently serves as the source of an extremely wide range of cells types; indeed, this transient structure is sometimes referred to as the "fourth germ layer." The early steps of neural tube formation involve a process of cell rearrangement known as convergent extension, in which sheets of cells spread toward each other and merge by intercalation, as well as a signaling pathway known as planar cell polarity (PCP) that reorients the alignment of intracellular components in the merging cells. How PCP and convergent extension work together to form the neural tube, however, is unknown.

Using embryonic chick as a model, Tamako Nishimura and others in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) have shown that the PCP signaling molecule Celsr1 is concentrated in the apical junctions of polarized cells in the merging neural plates. Published in *Cell*, this work shows how this single molecule coordinates both apical constriction and convergence of neuroepithelial sheets in the nascent neural tube.

The inward bending of the neural plate relies on the purse string-like constriction of the apical surfaces of certain key cells in the region to generate a "hinge" effect, a process that is known to involve the activation of actomyosin in the region by the Rho kinase, ROCK. The resulting depression resembles a furrow, rather than a bowl, suggesting that this apical constriction is oriented along the anterior-posterior (A-P) axis of the neural plate.

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

To gain a better understanding of how this occurs, Nishimura watched adherens junctions in the neural plate in chick embryos as the component cells remodeled the tissue by apical constriction. She found that activated actomyosin (as identified by the molecule pMLC) accumulated at junctions perpendicular to the A-P axis, and that cells tended to contract uniformly in the direction of the midline. Live imaging analyses of the region further revealed changes in both the cell morphology of the apically constricted cells and rearrangements of spatial relationships between cells along this axis, pointing to the possibility that this oriented constriction is a driver of convergent extension.



Celsr1 co-localizes with contracting actin filaments (F-actin) in cell junctions perpendicular to the A-P axis (top). Interference with Celsr1 function results in defects in neural tube closure (bottom).

This next raised the question of how this orientation is generated. Previous work from the Takeichi lab identified the *Drosophila* protein Flamingo as a member of the cadherin molecular superfamily involved in the regulation of planar cell polarity. Interestingly, mice lacking Celsr1, a vertebrate homolog of Flamingo, exhibit defects in neural tube closure – a common form of congenital defect in humans. The group speculated that Celsr1 might play a role in determining cell polarity and thus the orientation of apical constriction. Similar to pMLC, Celsr1 localized in junctions perpendicular to the neural plate A-P axis, and knockdown of the gene function by siRNA produced neural tube defects reminiscent of those seen in Celsr1 knockout mice, and while pMLC activity was maintained, its localization to the cell-cell junctions was reduced. Cellular rearrangements underlying convergent extension were also diminished, strongly suggesting that Celsr1 plays a critical role in orienting apical constriction in this context.

“Studies in *Drosophila* have highlighted the potential for local constriction of cell adhesion sites to exert significant effects on tissue morphology,” says Takeichi. “In this study, we have shown how this can occur in vertebrates as well. We next hope to focus on what drives the localization of Celsr1 and, more generally, whether this system is conserved in other tissues, and other taxa.”

Role for Robo in cortical neuron distribution

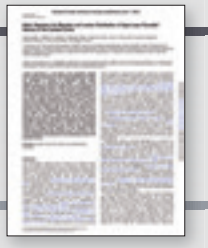
Yuko GONDA

The cerebral cortex is the thin, but structurally complex, surface of the mammalian forebrain and serves as the locus for important functions of the nervous such as the processing of sensory inputs, decision-making, and memory. This “gray matter” shows a neatly stratified organization into six layers, each characterized by a distinct type of neuron, all of which connect and interact with other parts of the cortex and the rest of the brain. Positioning of cells in their appropriate locations is therefore key to brain function. The mechanisms by which cortical neurons arrive at and establish themselves in their specific destinations, however, remain poorly understood.

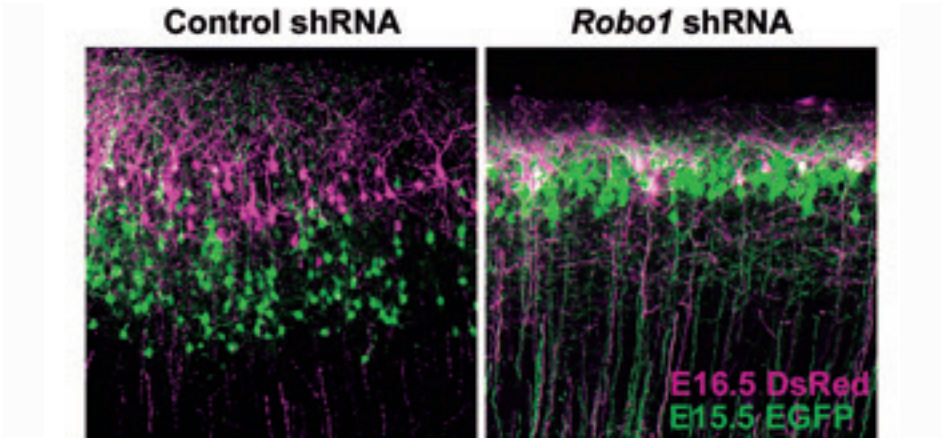
Yuko Gonda in the Laboratory for Neocortical Development (Carina Hanashima, Team Leader), working with colleagues at the National Institute of Neuroscience, have now shown how the receptor protein Robo1 helps determine the positioning of certain neurons in the neocortex. Published in the journal *Cerebral Cortex*, this work reveals an important new molecular basis underlying neuronal arrangement in the cortex.

The Robo proteins were first identified as receptors for chemorepulsive signals in *Drosophila*, and were later found to operate in axon guidance in the mammalian central nervous system as well. In a screen to identify genes expressed in subset of neocortical neurons, Gonda first found that *Robo1* mRNA exhibit layer-specific expression. Further analyses revealed the Robo1^{+} cells to be pyramidal neurons, and that the protein localized mainly in dendrites and axons.

Gonda Y, et al. Robo1 regulates the migration and laminar distribution of upper-layer pyramidal neurons of the cerebral cortex. *Cereb Cortex* (2012)



To better understand its function, the team first examined mice in which *Robo1* had been deleted from the genome. They found higher densities of pyramidal cells in cortical layers II/III in these knockout mice, resulting in a thinning of these layers and consequent shift of layer IV toward the pial surface of the cortex. To further determine the cell-autonomous requirement for Robo1 in these cells, Gonda introduced shRNA constructs against *Robo1* in discrete population of layers II/III pyramidal neurons by electroporation, and found that greater numbers of these cells were retained in the intermediate zone as opposed to entering the cortical plate as in controls, suggesting that loss of Robo1 function affects early neuronal migration in the embryonic cortex.



Suppression of Robo1 affects inside-out layer formation of neocortical layers II/III neurons. In control shRNA transfected neurons, late-born pyramidal cells electroporated with DsRed at E16.5 (magenta) are able to migrate past earlier-born cells (green), resulting in an inside-out neuronal distribution. In Robo1 suppressed cells, neurons fail to undergo birthdate-dependent segregation of these neurons.

The laminar organization of cortical neurons follows an inside-out pattern, in which the early-born neurons migrate to deeper layers of the cortex, while later-born generations make their way successively to more superficial layers. In *Robo1* shRNA transfected layers II/III pyramidal neurons, although the cell movement into the cortical plate was initially delayed, these neurons eventually migrated to the surface of the cortical plate. However, after arrival, these neurons remained in the most superficial part of layers II/III. As the molecular mechanism by which the relative inside-out positioning of cortical neurons is established remains largely unknown, Gonda next assessed the distribution pattern of temporal cohorts of *Robo1* suppressed neurons by performing sequential electroporation analysis. The experiment revealed that Robo1 suppression leads to abnormal laminar distribution of neurons in which the inside-out pattern was compromised. Interference with Robo1 function also caused defects in the growth and branching of neurites, while leaving neuronal identity intact. The picture that emerges is one in which Robo1 is required for determining the layer-specific final positioning of pyramidal neurons, and fine-tuning of axon projections in distinct cortical layers.

“The laminar organization of the mammalian cortex is its most distinguishing feature, but we only have a limited understanding of the molecular factors that give rise to this structure,” says Hanashima. “What we have shown here is how a layer-specific axonal guidance receptor regulates the arrangement of neurons in upper layers of the cortex, which suggests that other molecular control systems may be at work in determining neuronal location in other layers as well.”

Self-organized retinal tissue from human embryonic stem cells

Tokushige NAKANO, Satoshi ANDO

Pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are valued for their ability to give rise to all the many cell types in the adult body. Since the first derivation of human ESCs in 1998, scientists have developed a variety of techniques for inducing these cells to differentiate into specific cell populations at reasonably high efficiencies. More recently, a number of reports have shown that mouse ESC can be prompted not only to give rise to formless cell aggregates, but remarkably well-organized tissue-like structures in vitro, a feat that has been likened to the recapitulation of developmental phenomena in a culture dish.

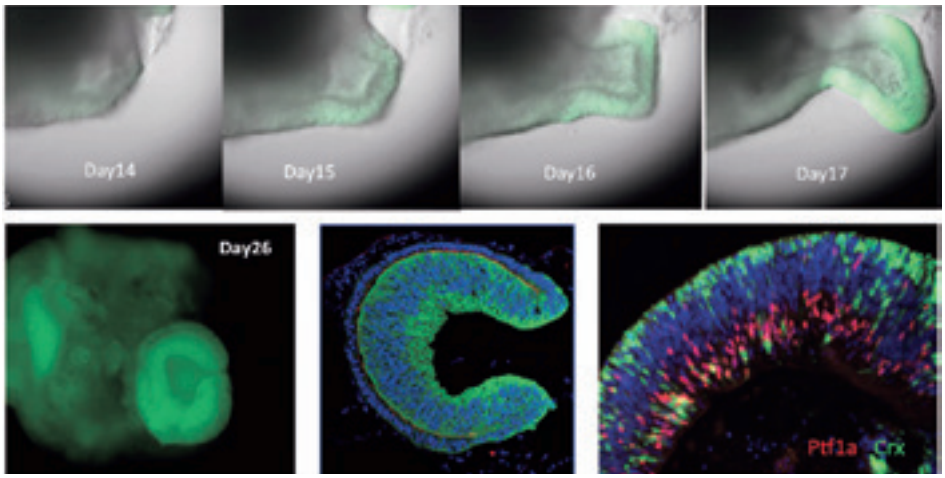
Now, Tokushige Nakano and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director) in collaboration with Sumitomo Chemical, have shown that human ESCs exhibit that same capacity for generating self-organized, complex tissue structures with their report of an hESC-derived optic cup. Published in *Cell Stem Cell*, the report also describes the group's development of a cryopreservation technology capable of freezing these retinal tissues intact.

This represents the latest in a series of achievement in induced differentiation from ESCs by the Sasai group. Using a cell culture system (SFEBq) developed in the same lab, Sasai has previously shown controlled differentiation of a range of neuronal cell types including dopaminergic neurons, cerebellar Purkinje cells, as well as cortical, sensory and motor neurons. In recent years, the group has shown that mouse ESCs can be steered to self-organize into complex tissue-like structures involving multiple cell types, including cerebral cortex, optic cup, and pituitary hypophysis, revealing

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

an intrinsic developmental capacity in these pluripotent cells independent of signals from the surrounding microenvironment.

Their most recent achievement built on the lab's previous demonstration of optic cup formation from mouse ESCs, but required modifications to that protocol to account for difference in tissue size, number of cells, culture media, and the time points at which the growth factors that steer the process needed to be added. The optimized 22–26 day process began with a population of around 9000 hESCs, which formed retinal precursors after about two weeks in culture, after which the structure gradually remodeled to form the optic cup. Interestingly, the resulting structure was around twice the diameter of that derived from mouse ESCs, apparently reflecting the size differences in the embryos of the two species.

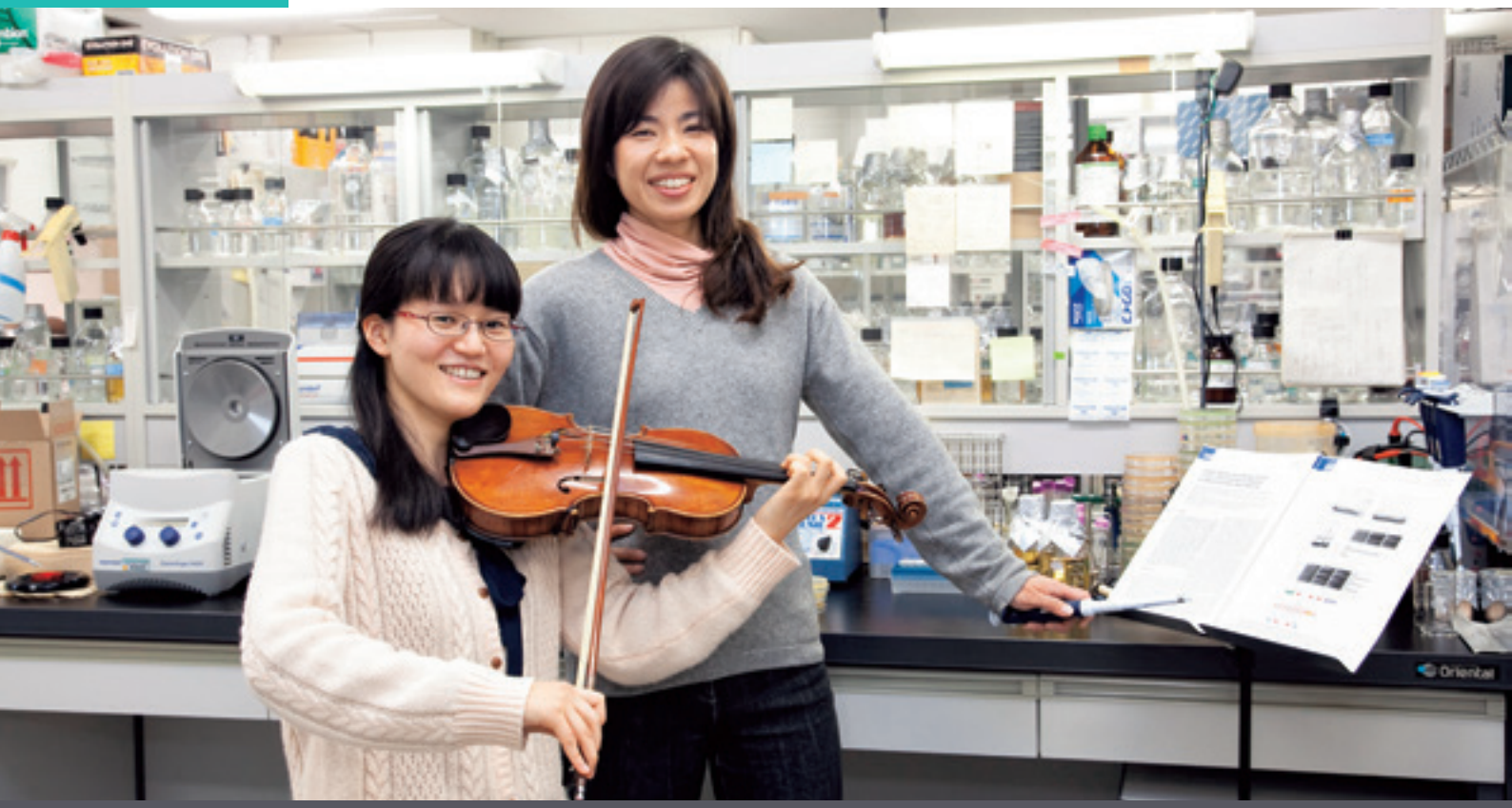


Formation of optic cyst- (top) and optic cup-like structures from human ESCs (bottom left). Staining reveals neural differentiation in inner layer (bottom center), and distinct laminar structure of retina (bottom right).

When the neural retina was cut away from induced optic cups and cultured, it showed even further differentiative capacity, giving rise to ganglion cells, photoreceptors, and other cells types by day 40, and forming a laminar retinal structure resembling that of the adult retina by day 126. Intriguingly, Nakano also observed cone photoreceptors, a cell type not seen in mouse ESC-derived tissue. Recognizing that the long culture times required to give rise to these tissues could represent a pitfall for clinical use, the group also developed a system for pre-treating the optic cups prior to cryopreservation, allowing them to be frozen, stored, and thawed with minimal damage.

"The question of just how complex a structure we can obtain from embryonic stem cells is a fascinating one from the developmental perspective," says Sasai. "We are hopeful that these findings may help to lay a foundation for a regenerative medicine in which intact organized tissues, not just groups of cells, can be developed for use in cell transplantation."

Chromodomain binding to nucleic acids key to heterochromatin assembly and function



Mayumi ISHIDA, Yasuko OHTANI

Chromosomes in eukaryotic cells contain regions of compactly bundled DNA and protein called heterochromatin, which serve as loci for the epigenetic regulation of gene expression, frequently in the form of gene silencing. In a sense, genes in heterochromatic regions are “locked away” from transcription factors that might trigger their expression. Recent studies in the fission yeast, *Schizosaccharomyces pombe*, have shown that the RNAi machinery is involved in heterochromatin assembly in centromeres, but direct mechanistic links between these two processes have remained elusive.

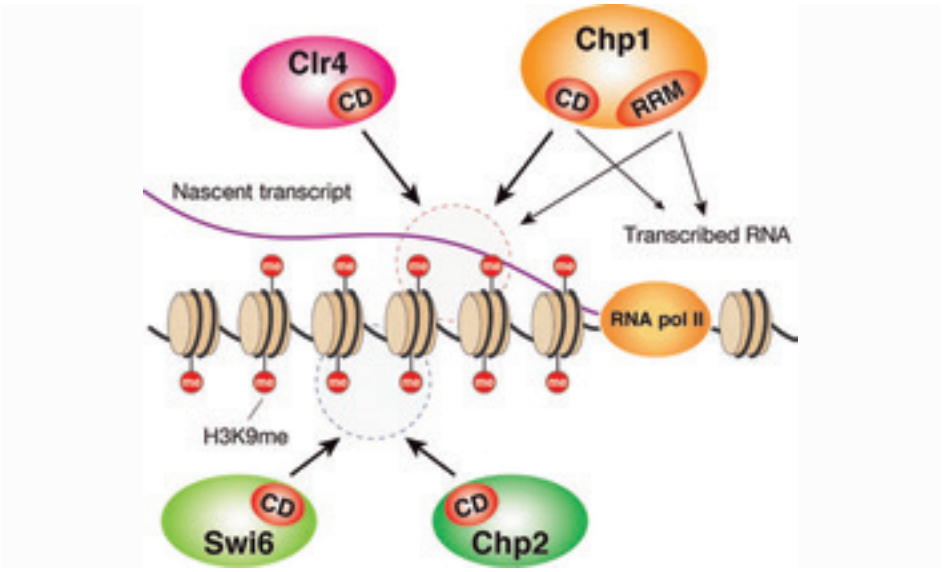
New work by Mayumi Ishida and colleagues in the Laboratory for Chromatin Dynamics (Jun-ichi Nakayama, Team Leader) has now shown how the RNAi factor Chp1 contributes to heterochromatin assembly and gene silencing through direct binding not only to methylated histones but to DNA and RNA as well. Published in *Molecular Cell*, this new report provides new mechanistic insights into this crucial genetic regulatory system.

The construction of heterochromatin regions relies on a process called histone methylation, in which DNA-associated histones are decorated with methyl residues as a kind of identifying label. In fission yeast, a methyltransferase called Clr4 methylates the ninth lysine of histone H3 (called H3K9me), which then binds HP1 proteins (such as Swi6 and Chp2) resulting in the formation of a transcriptionally silent chromatin region. RNAi, in contrast, is a mechanism by which targeted RNAs are degraded post-transcriptionally. The centromeric regions of fission yeast chromosomes express noncoding RNAs at low levels; after their transcription, these are cleaved into double strands by RNAi machineries and enzymatically diced to short sequence fragments known as siRNAs. These ribonu-

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

clear snippets form part of a larger complex called RITS, which itself binds to chromatin by targeting of these non-coding RNAs. This elaborate process is known to recruit Clr4 to the centromere and induce histone methylation.

With this as a background, the Nakayama lab's most recent study focused on Chp1, a component of the RITS complex, which plays a critical role in tethering RITS to the centromere. Notably, the Chp1 protein structure features a region known as a chromodomain (CD), which it shares with other CD proteins, such as Clr4, Swi6, and Chp2. Chp1 also includes a different domain called RRM, which is known as an RNA binding site in other proteins, leading them to ask whether it would have the same function here. On conducting an electrophoretic binding assay to answer this question, they were surprised to find not only that RNA was bound not only by RRM, but by the chromodomain as well. This effect was intensified nearly ten-fold by histone methylation, which also enabled DNA binding by Chp1, suggesting that the binding of this protein to chromatin is stabilized via a CD-mediated interaction with H3K9me.



Four chromodomain proteins affecting heterochromatin formation in the fission yeast. These domains (labeled CD) in Chp1 and Clr4 bind to DNA and RNA, stabilizing chromatin and working to establish heterochromatic structure.

Interesting, but was this discovery important in vivo as well? Ishida generated Chp1 mutants to test for effects on chromatin structure and found that defects in either the methylated histone- or the nucleic acid-binding domains resulted in a dramatic reduction in localization of Chp1 to the centromere, and a concomitant drop in heterochromatin function. The team will be focusing next on whether this activity is unique to Chp1, or more widely shared by its chromodomain protein kin.

"Our next step will be to look into whether the nucleic acid-binding function of Chp1 is at work in other heterochromatic regions, and in other eukaryotes," says Nakayama. "This will be an important part of our broader focus on unraveling the roles of CD proteins in general in heterochromatin assembly and RNAi."

The body's clock from a blood sample

Takeya KASUKAWA

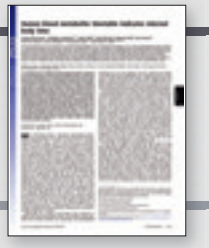
Our bodies have a daily activity cycle that tracks closely with the natural day-night rhythm of the rotating earth, and helps regulate such physiological functions as hormone secretion, blood pressure, and sleep. The importance of these biological clocks can be seen from the many diseases, such as asthma, myocardial infraction, and even some allergies, that show clear tendencies to worsen or occur at specific times of day. Misalignment between internal time and the outside environment is also tied to other problems, such as jetlag, hormone dysregulation, cognitive problems, and affective disorders, all of which make the ability to make clear and accurate measurements of the body's clock an important research goal.

Now, Takeya Kasukawa and others in the Functional Genomics Unit and Laboratory for Systems Biology (both headed by Hiroki R. Ueda) reveal a convenient, non-invasive method for calculating body time in humans. This work, conducted in collaboration with researchers from Keio University, the National Center of Neurology and Psychiatry, and Hokkaido University, and published in the *Proceedings of the National Academy of Sciences*, shows how a timetable of oscillating metabolites provides a readout of the internal clock of potentially great clinical value.

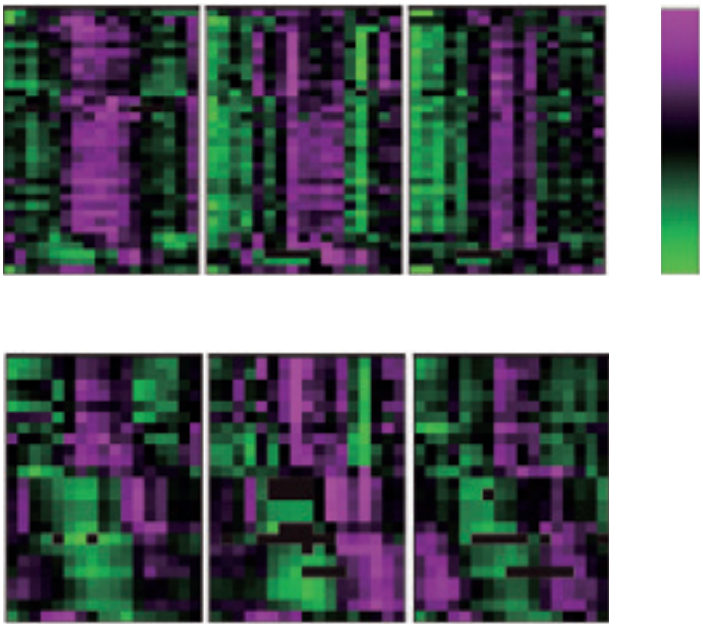
Previously published methods for telling internal time have been based on measurements of known cycling molecules, such as melatonin or cortisol, but these necessitated the isolation and monitoring of the subject and taking multiple samples over an extended period, making them labor-intensive to researchers and burdensome to patients. Taking a hint from an observation made by Carl von Linné (better known as Linnaeus), who noted that, as different flowers bloom at different times of day, it should be possible to tell the time simply by seeing which flowers are in bloom, Kasukawa et al looked for a way to read a molecular version of this "flower clock" in humans. The Ueda lab had previously developed a method for doing just that in mouse, by developing and referring to a 24-hour timetable showing the cycling levels of a large number of hundreds of metabolites.

In this latest study, Kasukawa sought to apply those same techniques in humans, beginning by measuring the rise and fall of metabolite levels over the course of a 24-hour day in three human subjects following predetermined activity routines, in which the subjects stayed in light- and temperature-controlled environments, receiving specific calorie amount every two hours and giving

Kasukawa T, et al. Human blood metabolite timetable indicates internal body time. *PNAS* 109.15036-41 (2012)



blood samples on the hour over a day and a half period. This so-called constant routine minimizes the effects of external influences on internal state making it possible to extract readouts of the unperturbed endogenous levels of metabolites in the blood. These fluctuating abundances were measured by liquid chromatography and mass spectrometry, making it possible to construct a timetable plotting metabolite levels against body time.



Changes in metabolite levels in blood samples from three human subjects over a 24-hour period. Positive ion metabolites shown at top, negative ion metabolites at bottom. (Green indicates lower and magenta higher abundances)

The next step was validation. Blood samples were taken at arbitrary times from three different subjects, analyzed for the same metabolites, and compared to the molecular circadian schedule. Samples were also taken from the six subjects subjected to shift routines, in which external and internal time signals are purposely uncoordinated. Comparing the results from all these groups, Kasukawa et al. found that the readouts matched neatly with the subjects' actual internal time, with a maximum margin of error of only three hours. The success of this method provides an accurate new way to evaluate biological time in humans without requiring multiple blood draws over extended periods.

The Ueda lab's technique has great potential for uses in the study of rhythm disorders and development of effective treatments. "We hope to improve the accuracy and ease of use of this metabolite timetable approach in the future," says Kasukawa, "and to show its potential for clinical application by using it to analyze actual patient blood samples as well."

2012 Courses

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

Learning programs for high school students

The CDB holds regular courses and workshops for area high school students to learn more about embryogenesis and get a feel for research in the lab. This year, the Office for Research Communications organized courses in August and December, giving dozens of local students a chance to study concepts and experimental technqiues in cell and developmental biology.

Internships for undergrads

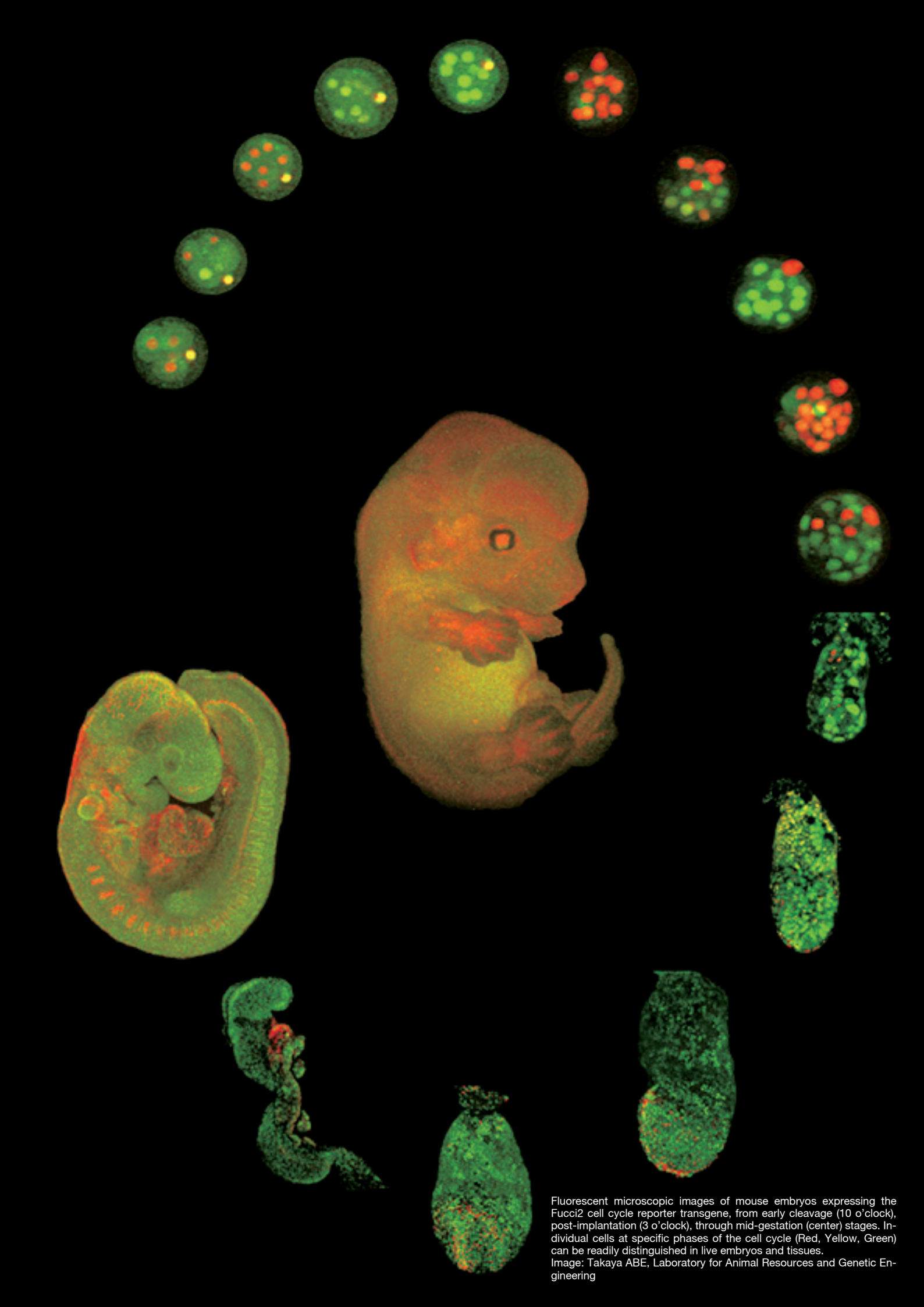
In August, the Center welcomed undergraduate students from local universities to perform one-week internships in various CDB laboratories. Interns selected from among eight research areas, and worked side-by-side with research scientists in host labs.

Graduate school lecture program

Also in August, the CDB hosted its annual intensive lecture program for graduate students potentially interested in pursuing research in developmental biology or related fields. More than 100 students joined the program, which included talks by CDB research leaders and visits to labs.

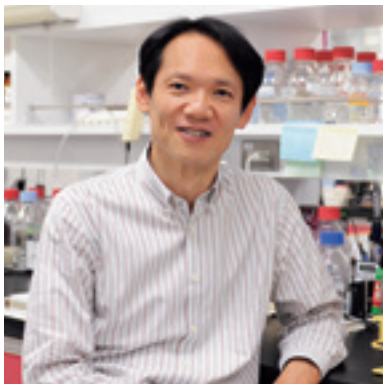
Training workshop for high school teachers

The CDB's annual course in developmental biology for high school teachers was held on October 6–7, with over 20 biology instructors in attendance. The course began with a seminar on how transcription factors establish cell identity followed by a practical course in techniques for culturing and studying chick and quail embryos.



Fluorescent microscopic images of mouse embryos expressing the Fucci2 cell cycle reporter transgene, from early cleavage (10 o'clock), post-implantation (3 o'clock), through mid-gestation (center) stages. Individual cells at specific phases of the cell cycle (Red, Yellow, Green) can be readily distinguished in live embryos and tissues.
Image: Takaya ABE, Laboratory for Animal Resources and Genetic Engineering

Cell Asymmetry



Fumio MATSUZAKI Ph.D.

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

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

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Part-Time Staff
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Recent Publications

Matsuzaki F, and Shitamukai A. Cell division modes and cleavage planes of neural progenitors during mammalian cortical development. *Cold Spring Harb Perspect Biol. Mammalian Develop.* in press.

Iwano T, et al. Prox1 postmitotically defines dentate gyrus cells by specifying granule cell identity over CA3 pyramidal cell fate in the hippocampus. *Development* 139.3051-62 (2012)

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

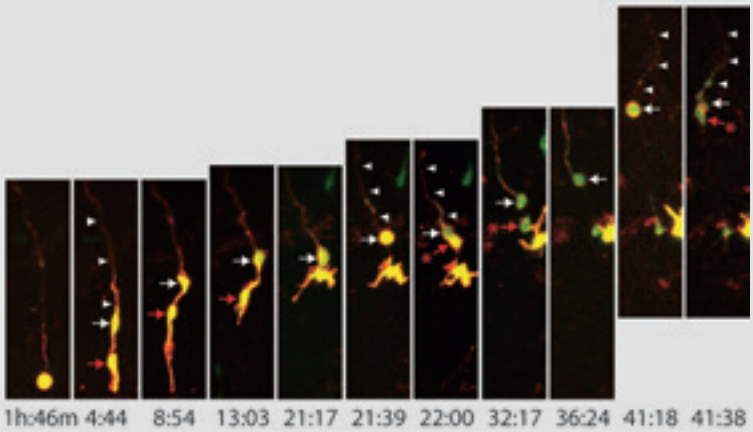
Kosodo Y, et al. Regulation of interkinetic nuclear migration by cell cycle-coupled active and passive mechanisms in the developing brain. *EMBO J* 30.1690-704 (2011)

Shitamukai A, et al. Oblique radial glial divisions in the developing mouse neocortex induce self-renewing progenitors outside the germinal zone that resemble primate outer-subventricular zone progenitors. *J Neurosci* 31.3683-95 (2011)

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

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

The vertebrate brain evolved rapidly, resulting in an expansion of the size of the brain, which comprises a larger number of neurons arranged in a vastly more complex functional network than that in invertebrate. Neural stem cells typically adopt three states - proliferative (symmetrically dividing), neurogenic (asymmetrically dividing), and resting - and undergo transitions among the states, on which the size, complexity and basic organization of the brain depend. We investigate mechanisms that determine the individual states of neural stem cells, and control transitions between states in mouse. 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 another type of neural stem cell, basal radial glia (Shitamukai et al., 2011 and see figure), which is known as 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 this type of stem cells, by developing model mice that produce large numbers of basal radial glia.



In the developing mouse brain, neural stem cells normally maintain epithelial structure during asymmetric division. Oblique cleavages occur stochastically and generate a different type of self-renewing neurogenic progenitors (white arrows) outside of the germinal zone. Divisions occur at 1h:46min, 21:39, and 41:18 in these consecutive images.
(http://www.cdb.riken.jp/en/04_news/articles/11/110704_progenitors.html)

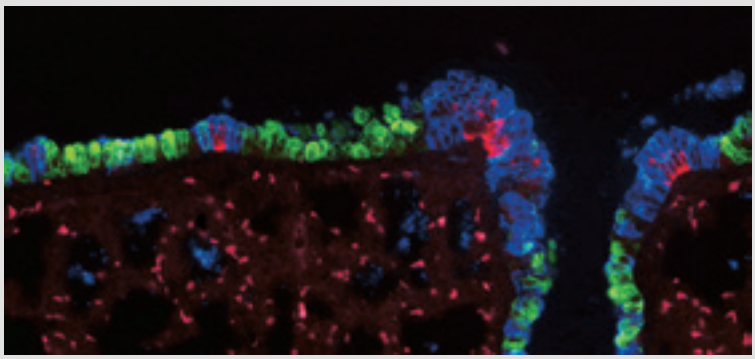
Lung Development

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

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

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

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



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

Mitsuru MORIMOTO Ph.D.

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

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

Staff

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Recent Publications

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

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

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

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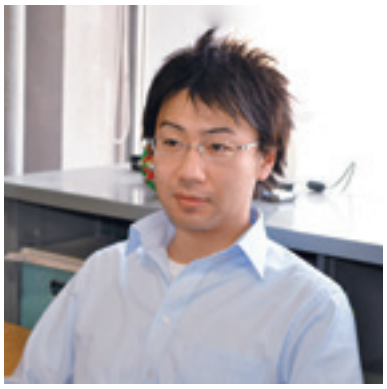
Morimoto M, et al. The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity. *Nature* 435.354-9 (2005)

Developmental Morphogeometry



Yoshihiro MORISHITA Ph.D.

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

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

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Recent Publications

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

Morishita Y. and Iwasa Y. Coding design of positional information for robust morphogenesis. *Biophys J* 101.2324-35 (2011)

Morishita Y. and Iwasa Y. Estimating the spatiotemporal pattern of volumetric growth rate from fate maps in chick limb development. *Dev Dyn* 238.415-22 (2009)

Morishita Y. and Iwasa Y. Growth based morphogenesis of vertebrate limb bud. *Bull Math Biol* 70.1957-78 (2008)

Morishita Y. and Iwasa Y. Optimal placement of multiple morphogen sources. *Phys Rev E Stat Nonlin Soft Matter Phys* 77.041909 (2008)

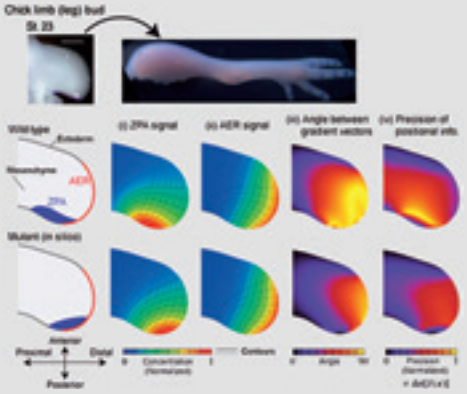
Morishita Y, et al. An optimal number of molecules for signal amplification and discrimination in a chemical cascade. *Biophys J* 91.2072-81 (2006)

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

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

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

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



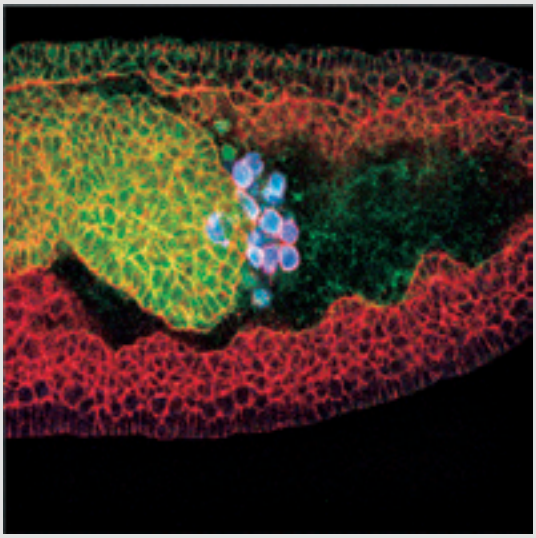
Optimal arrangement of morphogen source (Shh expression region) to maximize the precision of positional information in vertebrate limb bud.

Germline Development

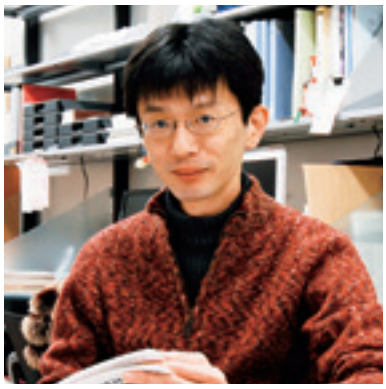
Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the *Drosophila* fruit fly, the formation and differentiation of germ cells is controlled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

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

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



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



Akira NAKAMURA Ph.D.

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

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

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Recent Publications

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

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

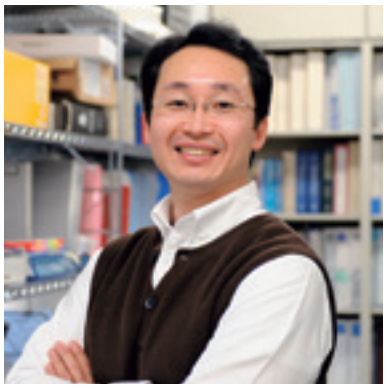
Tanaka T and Nakamura A. The endocytic pathway acts downstream of Oskar in Drosophila germ plasm assembly. *Development* 135. 1107-17 (2008)

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

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

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

Chromatin Dynamics



Jun-ichi NAKAYAMA Ph.D.

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

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

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Recent Publications

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

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

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

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

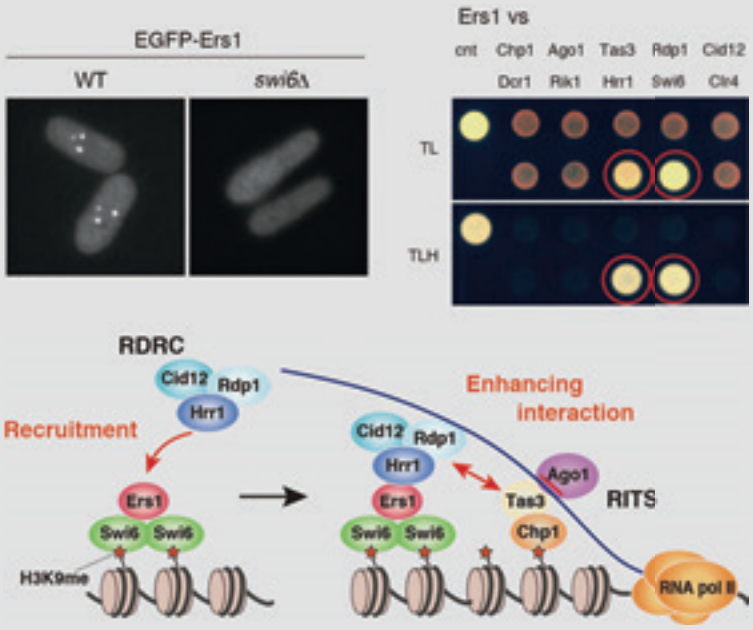
Hiragami-Hamada K, et al. N-terminal phosphorylation of HP1a promotes its chromatin binding. *Mol Cell Biol* 31.1186-200 (2011)

Kitano E, et al. Roles of fission yeast Grc3 protein in ribosomal RNA processing and heterochromatic gene silencing. *J Biol Chem* 286.15391-402 (2011)

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

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

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



Swi6/HP1 recruits RDRC to heterochromatin through Ers1, an RNAi factor intermediate. Heterochromatic localization of Ers1 is dependent on Swi6/HP1 (upper, left). Yeast two-hybrid assay testing interaction between Ers1 and other RNAi factors (upper, right). Model for Swi6-mediated siRNA generation at heterochromatin (lower).

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

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

The Last Supper

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

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

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

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Teruo AKUTA
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Herve Le MOUELLIC
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Yoko OTSUKA
Noriaki SATO
Kazumi YANAI

Assistant
Junko KOYA
Naeko MINAMI

Recent Publications

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

Tanaka Y, et al. The transcriptional programme controlled by Runx1 during early embryonic blood development. *Dev Biol* 366.404-19 (2012)

Tanaka Y, et al. Early ontogenic origin of the hematopoietic stem cell lineage. *Proc Natl Acad Sci USA* 109.4515-20 (2012)

Kobayashi K, et al. Epigenetic regulation of the neuroblastoma genes, Arid3b and Mycn. *Oncogene* (2012)

Hayashi M, et al. Endothelialization and altered hematopoiesis by persistent Etv2 expression in mice. *Exp Hematol* 40.738-50 e11 (2012)

Growth Control Signaling

Takashi NISHIMURA Ph.D.

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

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

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Agency Staff
Aya MASUDA

Part-Time Staff
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Noriko NISHIMURA
Junko SHINNO

Recent Publications

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

Okamoto N, et al. Conserved role for the Dachshund protein with *Drosophila* Pax6 homolog Eyeless in insulin expression. *PNAS* 109. 2406-11 (2012)

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

Nishimura T and Kaibuchi K. Numb controls integrin endocytosis for directional cell migration with aPKC and PAR-3. *Dev Cell* 13. 15-28 (2007)

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

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

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

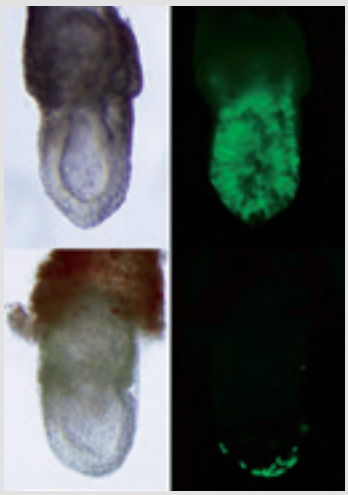
To better understand the interface between nutrient availability and growth regulation, we are now focusing on how nutrition controls systemic growth through *Drosophila* insulin-like peptides (Dilps). Members of the insulin family of peptides have conserved roles in the regulation of growth and metabolism in a wide variety of metazoans. We have also conducted *in vivo* RNAi screening to identify new players regulating growth and developmental timing at the organismal level.

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

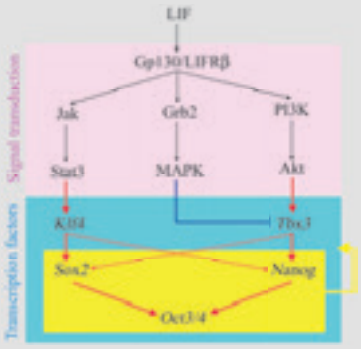
Pluripotent Stem Cell Studies

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

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



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.

Hitoshi NIWA M.D., Ph.D.

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

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

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Recent Publications

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

Martello G, et al. Esrrb is a pivotal target of the gsk3/tcf3 axis regulating embryonic stem cell self-renewal. *Cell Stem Cell* 11.491-504 (2012)

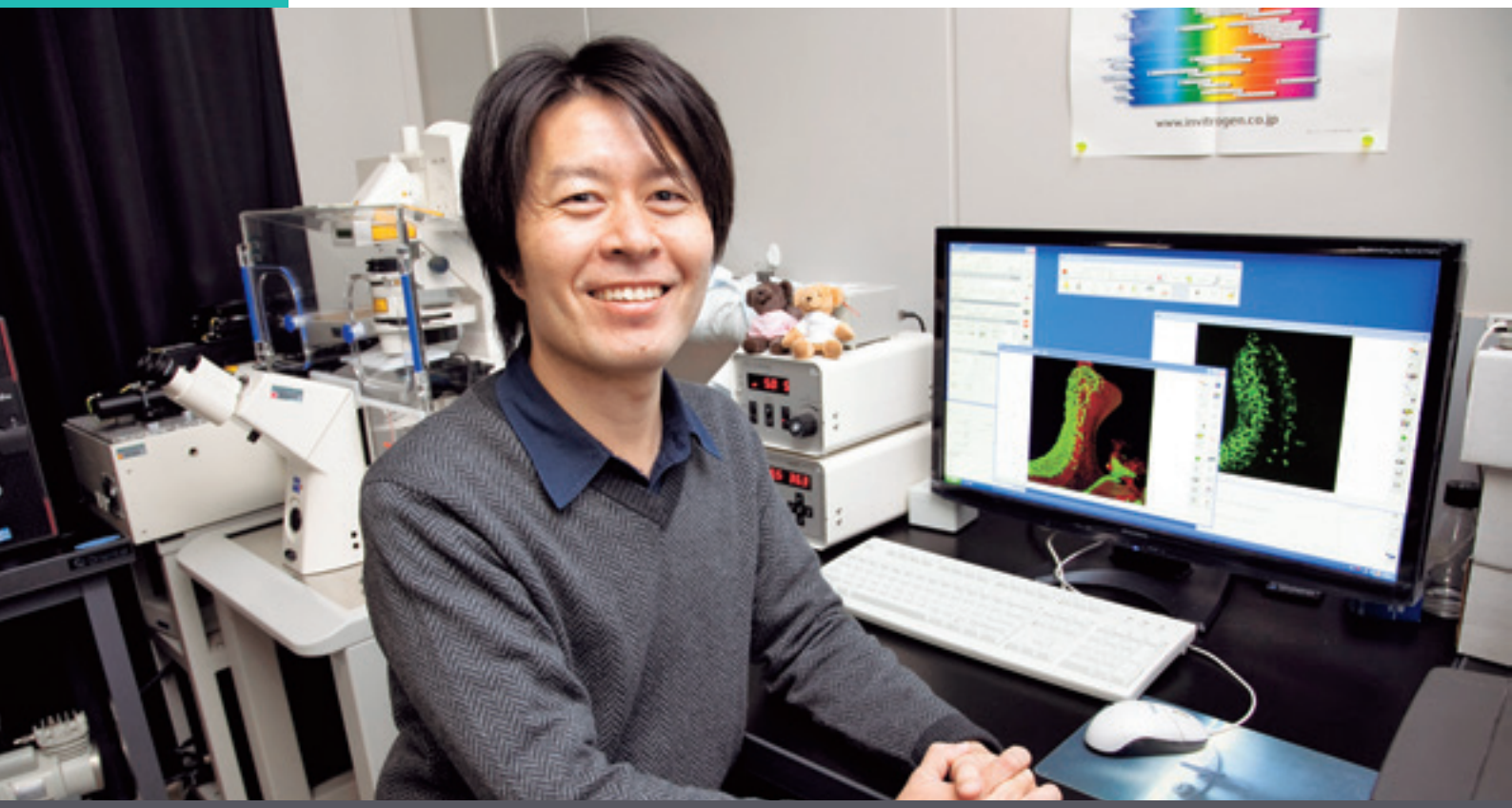
Murakami K., et al. Choice of random rather than imprinted X inactivation in female embryonic stem cell-derived extra-embryonic cells. *Development* 138.197-202 (2011)

Kitajima H. and Niwa H. Clonal expansion of human pluripotent stem cells on gelatin-coated surface. *Biochem Biophys Res Commun* 396.933-8 (2010)

Niwa H. Mouse ES cell culture system as a model of development. *Dev Growth Differ* 52.275-83 (2010)

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

A fast track to gut innervation



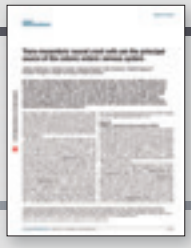
Chihiro NISHIYAMA

The gut is richly innervated with enteric neurons that autonomically control such behaviors as peristaltic movement, secretion and blood flow, earning this long stretch of the nervous system the distinction of being called “the second brain.” During development, the enteric nervous system begins as a migratory population of neural crest cells, wending their way down to populate the entire length of the intestines, which can be several times as long as the entire body. This long journey has always been thought to closely follow the winding course of the intestinal wall – when these neurons fail to complete the trip, it can lead to a complete lack of lower gut innervation, as seen in the congenital condition called Hirschprung’s disease. In some patients, however, aganglionic sections, known as skip areas, in the large intestine are found alternating with normally innervated gut, a phenomenon that the conventional model of enteric innervation cannot explain.

New work by Chihiro Nishiyama and others in the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto, Team Leader) shows how a subset of enteric neural crest cells in mouse takes a short cut across the mesentery on their way to innervate the large intestine. Published in *Nature Neuroscience*, these findings open up new and unexpected insights into the longest migration of neurons in the embryo.

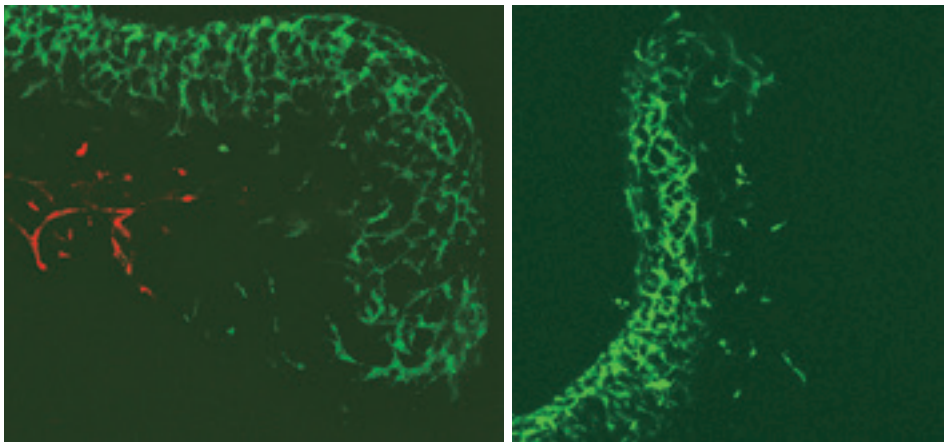
In the mouse, the population of the gut with enteric neurons takes place over the period of embryonic day 9.5 to 14, during which the guts itself undergoes extensive growth and morphological changes. At E10, the gut is still a nearly straight tube, but by the following day, it develops a hairpin loop that gives rise to the mid- and hindgut sections (which form the embryonic bases for the small

Nishiyama C, et al. Trans-mesenteric neural crest cells are the principal source of the colonic enteric nervous system. *Nat Neurosci* 15.1211-8 (2012)



and large intestines). By E11.5, these gut segments begin to pull away from each other. Nishiyama et al. used live imaging to observe the migration of cells in tissue sections from the gut over time, using embryos engineered to express one of two fluorescent proteins – GFP and KikumeGR. Both can be used to label enteric neurons, but KikumeGR has the added advantage of changing color from green to red on exposure to ultraviolet light, making it possible for the team to track the movements of select populations of cells through living gut tissue. Using this technique, they found that in the E12.5–13.5 embryo, precursor cells form bundles that spread in a network throughout the hindgut, with the wavefront region at the distal edge contributing more than 80% of the hindgut neural network.

But how did these precursors arrive at their destination? Looking more closely at the fluorescent-labeled E11.5 gut, Nishiyama found that the population arose around the time that the mid- and hindgut regions are brought into close proximity by the folding action. What the team saw next surprised them; they found that these wavefront cells are derived from a cell population that traveled straight across the mesentery from the mid to the hind region, not from cells taking the long way around the bend. This corner cutting by migrating cells plays a crucial role; when the team blocked this trans-mesenteric movement, they found a dramatic delay in hindgut colonization by precursors.

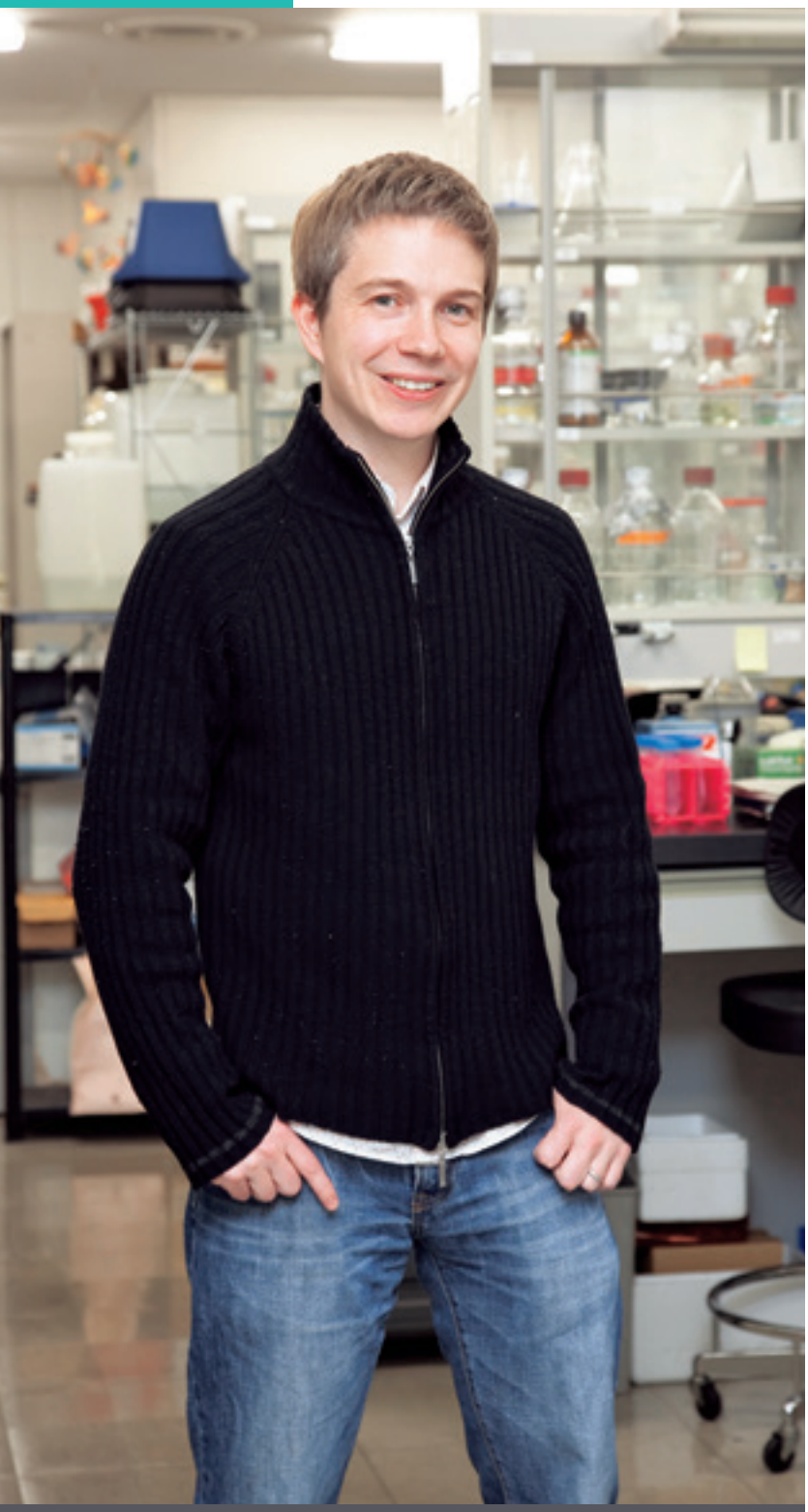


Cells (KikumeGR, red) aligned with mesentery in E11.5 gut proliferate and migrate, giving rise to the neural network of the hindgut. A subpopulation of cells traverses the mesentery to reach the hindgut (right).

Next, using a mouse model of Hirschprung's disease developed by the lab in 2008, they looked for connections between this novel form of migration and disease. They found that in Hirschsprung's mice, the trans-mesenteric migration was markedly reduced, suggesting that a failure in this mechanism leads to the defects in gut innervation as well as the skip areas observed in some patients. Enteric neurons in Hirschsprung's disease are known to show reduced expression of GDNF, an important factor in neuronal development and differentiation, suggesting the intriguing possibility that the defects in trans-mesenteric migration may be tied to aberrant GDNF signaling.

"In mouse, the enteric neurons only have a window of a single day in which the mid- and hindgut are close enough to enable neurons to take this path across the mesentery, so it is really wonderful to see how tightly the timing of cell migration and morphological changes in the tissue are coordinated," says Enomoto. The lab plans to study differences in cell populations that take the trans-mesenteric shortcut and those that take the long way round, as well as to investigate possible applications of these findings in the development of cell therapies for Hirschsprung's disease.

Evolutionary and developmental roots of the paratympanic organ resolved



Paul O'NEILL

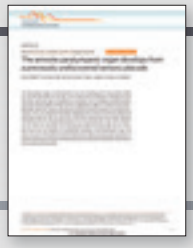
Birds, many species of which experience rapid changes in air pressure during flight, possess a specialized structure called the paratympanic organ (PTO) in the middle ear that appears to function as a kind of barometer or altimeter. This fluid-filled sac is connected to the tympanum (ear drum) by a ligament-like tissue, by which minute pressure-induced changes in the membrane are communicated to the PTO, triggering deformations that stimulate mechanosensory hair cells lining its inner walls. The PTO is for the most part a birds-only structure, and is not present in mammals, amphibians, or most reptiles. The developmental origins of this organ, however, remain poorly understood.

Using the chick embryo as a model, Paul O'Neill and others in the Laboratory for Sensory Development (Raj Ladher, Team Leader) have now found that the PTO arises from a newly discovered tissue primordium known as a placode, and reconfirm its homology to a sensory organ in fish known as the spiracular organ. This work, published in *Nature Communications*, was done in collaboration with Clare Baker from Cambridge University, and resolves a long-standing question over the embryonic and evolutionary roots of this sense organ.

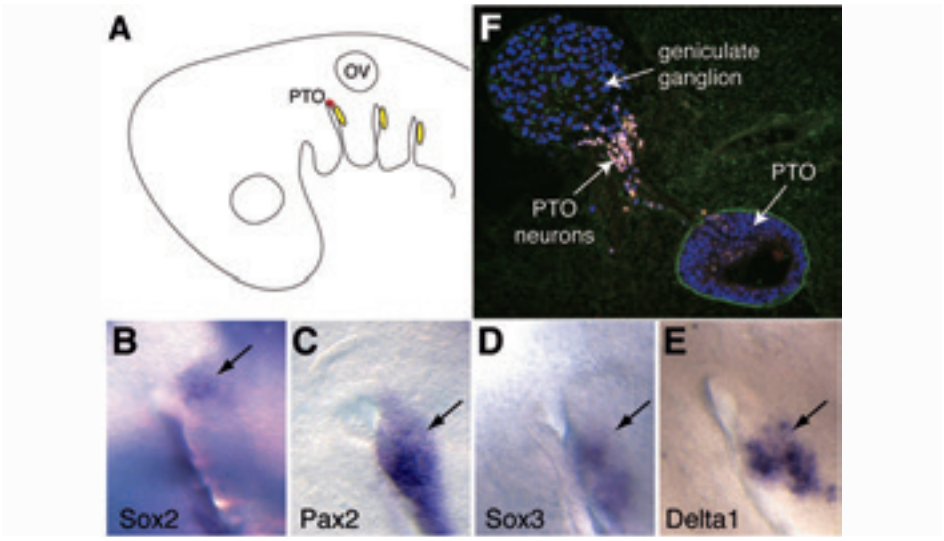
Previous studies have suggested that the PTO and spiracular organ (a sensory pouch found in fish that is thought to detect jaw movements) are homologous structures, based on similarities in tissue morphology, anatomical position, innervation, and the presence of mechanosensory hair cells. The case for this homology was called into question, however, by fate mapping experiments in chick embryos conducted in the 1980s that showed that the geniculate placode, which gives rise to facial neurons innervating the tongue, is the embryonic source tissue for the PTO. As the spiracular organ arises from a different placode, this made the homology between the two organs seem a less likely prospect, and for years the evolutionary relationship between them has been disputed. But O'Neill doubted that a placode that in other species gives rise solely to neurons would also generate the hair cells of the PTO, and so decided to study its development in greater detail to get to the bottom of this question.

The gene *Sox2* is known to play a critical role in hair cell development, so he first examined its expression pattern at embryonic stages when the PTO begins to form, and observed a small patch of *Sox2* expression immediately adjacent to the geniculate placode. Interestingly, the

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



Sox2^{+} region did not express geniculate placode markers, suggesting that it represented a distinct entity. O'Neill next traced the fate of cells derived from the region and found that it gave rise to both the PTO and a population of PTO neurons which migrate to the geniculate ganglion. These findings pointed to a dramatic conclusion – that the PTO is not derived from the geniculate placode, but rather a previously undiscovered separate placode directly alongside it, which the team has christened the PTO placode. Interestingly, although neurons from the geniculate and this newly described placode both reside within the geniculate ganglion, they remain segregated by gene expression and the regions of the brain to which they project.



A: Schematic showing the positions of the PTO placode (red) and epibranchial placodes (yellow).
B: The PTO placode expresses the transcription factor Sox2.
C-E: Expression of epibranchial placode markers. The PTO placode and epibranchial placode are separate entities that are located adjacent to each other.
F: PTO neurons (red) migrate from the PTO to join the geniculate ganglion.

To confirm that the PTO placode and geniculate placode are separate entities, the team next performed experiments in which different regions of head ectoderm were transplanted from quail embryos to the PTO precursor region of chick embryos to evaluate the developmental effects. When equivalent quail tissue was grafted into chick, both the PTO and the geniculate ganglion formed, but when quail tissue from different regions of the head or trunk was transplanted, the PTO failed to form, while the geniculate ganglion developed normally, suggesting that the two structures are formed by different developmental mechanisms.

The discovery of a novel placode is an exciting finding by any standards, and in this case one that helps put a decades-old controversy over the embryonic and evolutionary origins of the PTO to rest. "The next step is to identity the key molecular signals underpinning PTO and spiracular organ formation, and hopefully to understand the evolutionary mechanisms responsible for PTO loss or retention in particular species" commented O'Neill.

Mutations in *Phox2b* tied to common neural crest disorders

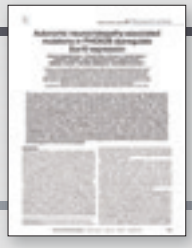
Mayumi NAGASHIMADA

The neural crest is a transient, mobile population of cells that gives rise to a wide array of cell types, including skeletal, neural, endocrine, and pigment cells during development, leading some to refer to it as the fourth germ layer. Given its importance, it is not surprising that alterations in neural crest cell migration and differentiation can lead to serious congenital defects, a group of developmental disorders collectively known as neurocristopathies. Most such diseases, which include such common disorders as neuroblastoma and Hirschsprung's disease, clearly have a genetic basis, but the details of the underlying defect are not well understood.

Scientists in the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto, Team Leader), led by Mayumi Nagashimada, have now uncovered defects in a specific gene are associated with a group of neurocristopathies of the autonomic nervous system. Published in the *Journal of Clinical Investigation*, this report shows how mutations in *Phox2b* perturb the expression of another gene, *Sox10*, which plays a central role in neural crest development. Nagashimada has since taken a position at the Brain/Liver Interface Medicine Research Center in Kanazawa University.

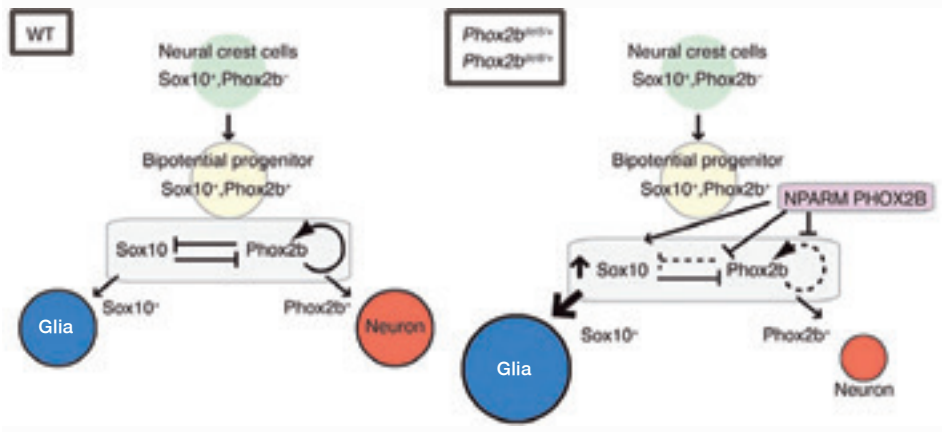
Interestingly, despite the fact that neuroblastoma is a cancer of the sympathetic nervous system while Hirschsprung's is a defect in gut innervation, these diseases sometime occur in the same patient. This is particularly the case in patients with a rare condition called central hypoventilation syndrome (CCHS), a respiratory disorder in which breathing often stops during sleep – CCHS patients show a 500 to 1000 times higher incidence of neuroblastoma and Hirschsprung's disease. Previous genetic studies have shown that a specific mutation in the gene *Paired-like homeobox 2b* (*PHOX2B*)

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



is associated with the CCHS-neuroblastoma-Hirschsprung's triad (CCHS-HSCR-NB). This gene encodes a homeodomain-bearing transcription factor, which itself has been implicated in neural crest development. Of the various mutations known to occur in *PHOX2B*, many CCHS-HSCR-NB patients exhibit frameshifts affecting the gene's open reading frame. To study the pathogenic mechanism of such mutations more closely, the team generated mice engineered to carry the same frameshift mutation in *Phox2b*.

The resultant mice proved to be viable for much of development, but showed 100% lethality in the perinatal period. Analyses of heterozygous *Phox2b* mutant offspring showed they suffered from hypoxia due to failure of spontaneous respiration, and defects in colon innervation and sympathetic ganglion formation – an extraordinarily close match to the symptoms of the CCHS-HSCR-NB association in humans.

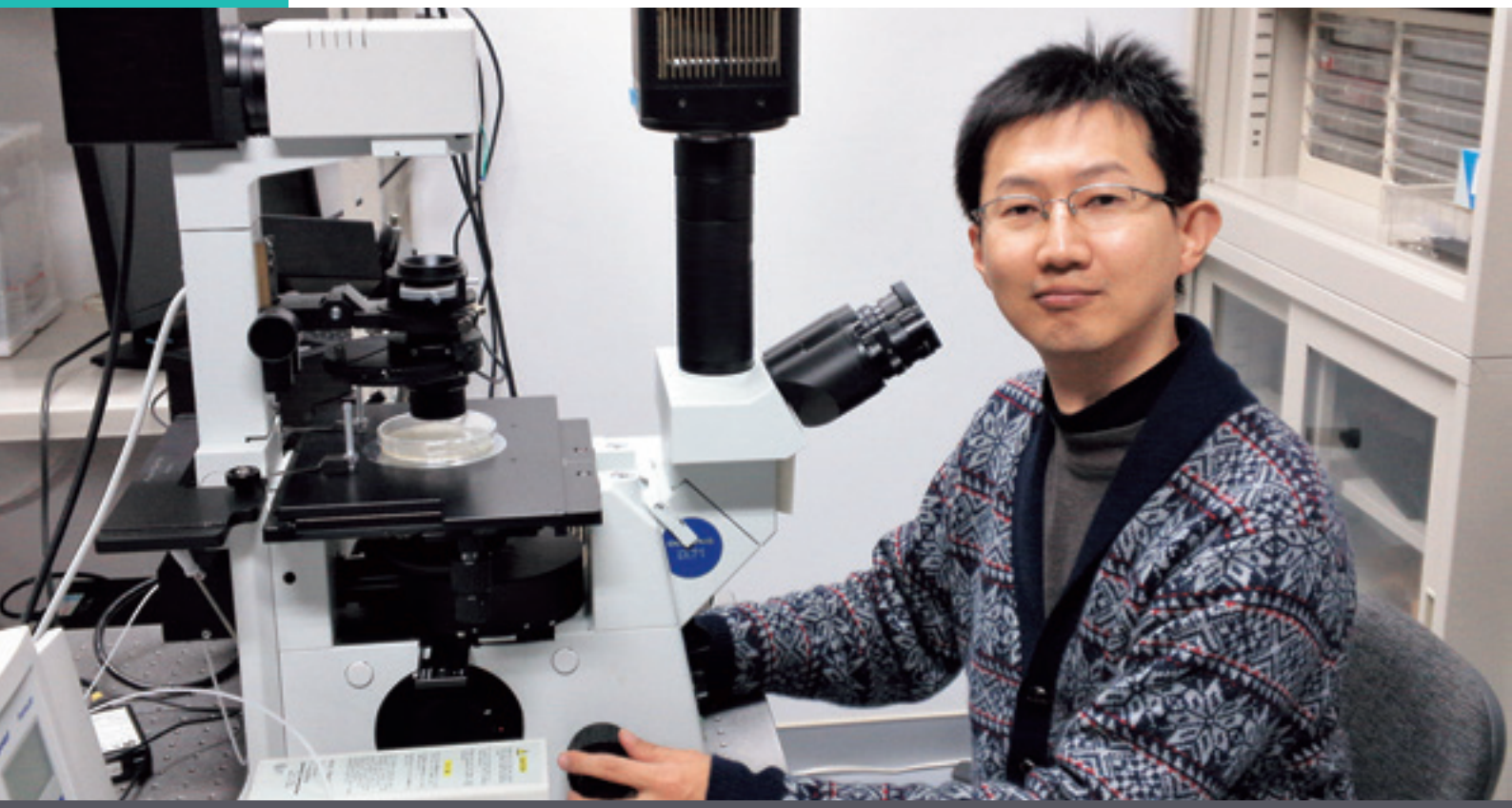


Model of how defect in Phox2b leads to Sox10 dysregulation and perturbed neuron-glia balance in neural crest-derived nervous system (right; wildtype shown on left)

In mouse, the cells of the autonomic nervous system characteristically express a pair of genes, *Phox2b* and *Sox10*; the former in differentiated neurons and the latter in glia. Nagashimada looked at the expression of these genes in two autonomic populations, enteric and sympathetic ganglia, in the mutant animals, and found that *Phox2b*-expressing cells were markedly reduced in both, while the ratio of *Sox10*$^{+}$ cells within the population was dramatically increased. Evidently, the frameshift resulted in a dysregulation of *Sox10*, freeing it from transcriptional control by *Phox2b* and resulting in disturbed self-renewal and proliferation of neural progenitors and a bias in differentiation toward glial fate. These imbalances in gene regulation subsequently manifest as the diverse and widespread symptoms observed in the mouse model, and presumably in human CCHS-HSCR-NB patients as well.

"This is a very important finding, as we have been able to identify the molecular basis for a developmental pathology of the neural crest," says Enomoto. "But this is only one of many mutations associated with neurocristopathies such as Hirschprung's and neuroblastoma, so we hope to continue developing additional mouse models in order to develop a better understanding of and, one day, treatments for these diseases."

Talin links cytoskeleton and cell membrane in migrating and dividing cells



Masatsune TSUJIOKA

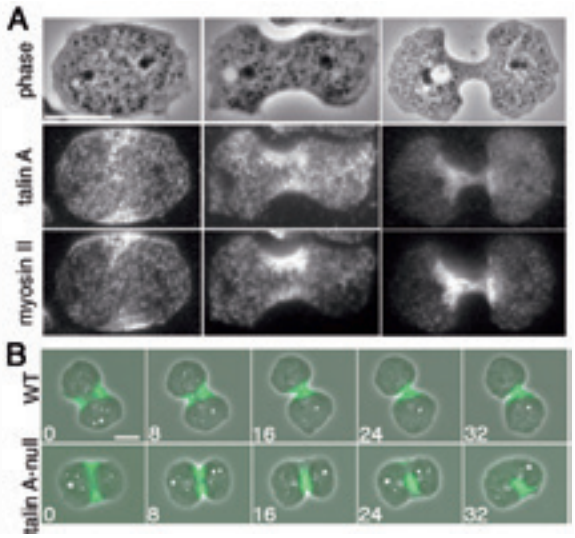
Cells can show a remarkable range of motility, creeping over substrates using a variety of pushes, pulls, stretches, and drags to get from A to B. This range of motion is achieved through the concerted efforts of motor proteins and structural complexes collectively known as the cytoskeleton. In addition to propulsion, however, cells also need to find footholds on surfaces in order to get the traction needed to advance or withdraw. Mobile single-celled organisms, such as the amoeba *Dictyostelium*, provide excellent living models for studying the molecular basis of such mechanisms, as they spend much of their lives solitary and on the crawl.

New work by Masatsune Tsujioka and colleagues in the Electron Microscope Laboratory (Shigenobu Yonemura, Laboratory Head) reveals how a protein called talin A links the cytoskeletal complex actomyosin to the plasma membrane of *Dictyostelium* cells as they move and divide. Published in the *Proceedings of the National Academy of Sciences*, this work was conducted in collaboration with colleagues at Yamaguchi and Kyoto Universities, the University of Edinburgh, Osaka University, Core Research for Evolutional Science and Technology Agency (CREST), and the RIKEN Quantitative Biology Center (QBiC).

Cortical actomyosin is a contractile fiber-like complex of filamentous actin and the motor protein myosin II, and is the driving force behind many forms of changes in cell morphology and movement. This activity requires connections between this cytoskeletal molecular complex and the membrane of the cell, but how this is accomplished has remained something of a mystery. Talin, a protein known to function in integrin-mediated cell-substrate adhesions, has been implicated as a candidate for this role, as loss of talin function has been shown in previous studies to affect bond between cells and their substrate. Knowing this, Tsujioka opted to study the question more closely in *Dictyostelium* as it possesses only a single myosin II gene, simplifying the task of

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

studying it in isolation, and it shows a remarkable range of motile behaviors.



(A) Co-localization of talin A and myosin II during cell division. (B) Defects in cell division in cells lacking talin A (bottom) compared to wildtype (top); the mutant cells show separation of contractile ring and plasma membrane (from 16 min), leading to mitotic failure.

He began by examining the subcellular localization of talin A (the *Dictyostelium* homolog of mammalian talins). Using a GFP-tagged construct and immunostaining techniques, Tsujioka found that this protein co-localized with myosin II at the back end of migrating amoebae. He next used a sophisticated piece of kit called a total internal reflection fluorescence microscope, which enables very thin sections of a sample to be visualized, to check for direct binding between the two, but was intrigued to find that while talin A and myosin II remained close to one another, they were not directly connected. Both did co-precipitate with actin, raising the possibility that this fibrous protein served as the link.

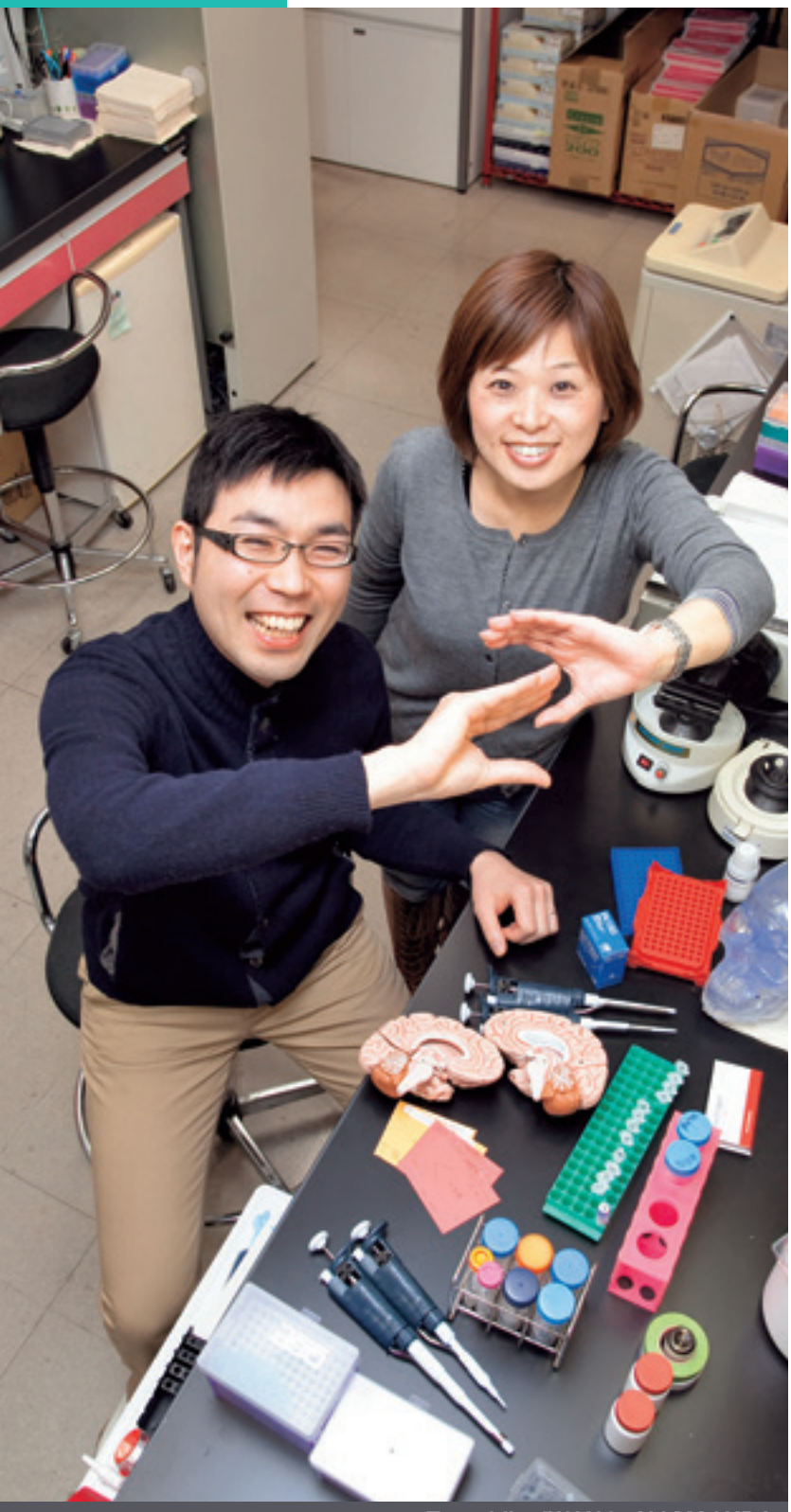
When Tsujioka expressed talin A in *Dictyostelium* cells lacking both myosin II and endogenous talin, he found that it spread throughout the entirety of polarized migrating cells, rather than just in the rear, indicating that its distribution relies on myosin. To test whether the talin A protein plays a functional role in cell motility, the team set up a system for observing *Dictyostelium* cells as they moved, taking advantage of this amoeba's well-known taste for cAMP. When talin A-null cells were presented with a chemical gradient generated by a micro-capillary, they would set off to follow it, but their posterior ends often remained stuck behind them, resembling a long tail that failed to detach from the substrate. Suspecting that this sticky predicament might be mediated by a cell-substrate adhesion molecule called SibA, they next generated double knockouts for both proteins and found that the cellular tails vanished.

Actomyosin also plays a role in mitotic cell division, so Tsujioka next asked whether talin might be at work in this process as well. As in migrating cells, talin co-localized with myosin II, in this case mid-cell near the cleavage furrow, and again talin's accumulation appeared to rely on myosin II. In dividing wildtype cells, this furrow gradually folds inward, a form of movement called ingression, eventually splitting the cell into a pair of daughter cells. In cells in which talin A was deleted, however, the furrow was wider and longer than in wildtype, and responded differently to mechanical stresses (in this case, being sandwiched tightly under a sheet of agarose), which appeared to be due to a disconnect between the actomyosin contractile ring and the plasma membrane.

As a last step, the lab conducted a structural analysis of the talin A protein to look for possible binding sites that might account for its apparent coupling role. They found that while the talin C-terminal contains an actin binding site, explaining its bond with actomyosin, a locus on its N-terminal domain bound with a phospholipid called PtdIns(4,5)P2. PtdIns(4,5)P2 is a known talin activator in mammals and is also enriched in the tail ends of Dictyostelium migrating cells, suggesting that this might be the molecule that activates talin A, which conveys contractile force in migrating and dividing cells.

"The fact that a similar separation between the plasma membrane and the underlying actin has been seen to occur in talin mutant cells in mammals as well suggests that that this discovery of a novel function for the molecular in *Dictyostelium* may be evolutionarily conserved," says Tsujioka. "We hope to dig deeper in future analyses of this new function and to identify other molecules involved in constructing and regulating these links."

Prox1 controls differentiation of hippocampal granule cells



Tomohiko IWANO, Aki MASUDA

As is true of many brain structures, the hippocampus, which plays a central role in memory and learning, is made up of multiple cell types organized into domains controlling different functions. Distinct from many other brain regions, however, the hippocampus is a site of active neuronal generation in the adult. Its distinctive pyramidal neurons are contained mainly in the cornu ammonis (CA), while another hippocampal region called the dentate gyrus (DG) is characterized by granule cells. While it is known that the survival of these granule cells relies on the activity of specific transcription factors, how they arise during the differentiation of the hippocampus has remained unknown.

In a recent study, Tomohiko Iwano and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) have now shown that the transcription factor Prox1 specifies the identity of postmitotic granule cells in mouse, while steering them away from a pyramidal fate. Published in *Development*, this work reveals how this single factor contributes to generating the cellular diversity of the hippocampus.

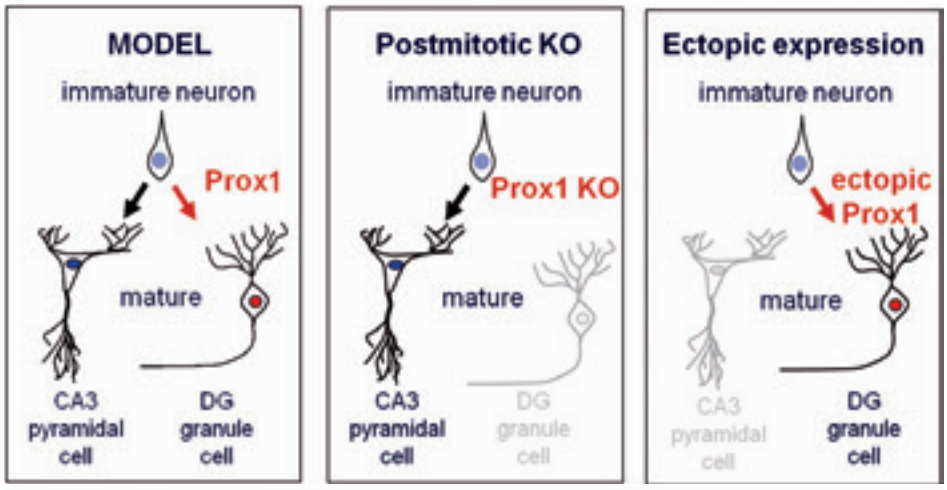
For neurons, granule cells are tiny, but they play a major role in diverse brain regions, including memory formation in the dentate gyrus. Taking a lead from previous studies that showed Prox1 to be involved in maintaining granule cell survival, Iwano sought to gain a better understanding of whether this transcription factor might play a role in the differentiation of these neurons as well. As the Prox1 gene is constantly switched on in granule cells, the group first generated conditional knockouts allowing them to selectively switch it off at any stage in development. When they looked at DG neurons in which Prox1 was homozygously deleted in the postmitotic stage, the prospective granule cells lost the expression of all markers of both mature and immature neurons of that type.

Even more interestingly, however, these conditional Prox1 mutant cells in the DG began to exhibit gene expression patterns and morphology reminiscent of pyramidal cells, the typical neuronal subtype found in a specific CA subregion of the hippocampus – a remarkable transformation for cells which might otherwise be thought of as terminally differentiated. Iwano found that granule cells exhibited similar expression levels of at least 18 marker genes characteristic of CA3 pyramidal neurons after postmitotic knockout of Prox1, and began to show typical pyramidal dendrite morphology as well.

Iwano T, et al. Prox1 postmitotically defines dentate gyrus cells by specifying granule cell identity over CA3 pyramidal cell fate in the hippocampus. *Development* 139.3051-62 (2012)

By looking at the effects of loss of Prox1 function at various stages in hippocampal development (which occurs mainly from late embryonic to neonatal stages of the animal, although granule cells continue to be generated throughout life in the DG), the group was able to show that at an advanced stage of hippocampal development and even in adulthood, Prox1 knockout was able to convert incipient granule cells to pyramidal-like neurons.

A key aspect of a neuron's function is its connectivity with other parts of the brain, both by accepting inputs and by projecting its axon to a specific site. Granule cells and pyramidal neurons ordinarily project to different destinations, so Iwano next checked whether Prox1-deficient DG cells would mimic not only pyramidal cells' gene expression and morphology, but their circuit-forming behavior as well. He found that while some of the Prox1-homozygous granule cells retained their characteristic axonal destinations, the range of projection was greatly expanded to include sites typical of those of CA3 pyramidal neurons.



Prox1 steers mature neural precursor cells toward a granule cell fate.

Having determined the effects of Prox1 depletion, the group next tried the converse experiment to observe how its overexpression would affect hippocampal cell fate. Iwano watched primary cultures of cells electroporated with a *Prox1* plasmid, they exhibited a much stronger tendency to gene expression and morphology typical of granule cells, as opposed to control cultures in which the pyramidal fate predominated, suggesting that Prox1 overexpression promotes granule cell fate at the expense of pyramidal. Electroporation of the same plasmid in vivo had equally pronounced effects in inducing the granule cell phenotype. Thus, the group indicated that both depletion and acquisition of Prox1 expression at postmitotic hippocampal cells could induce a conversion of these cells' neuronal fate (from granule cell to pyramidal, and from pyramidal to granule, respectively), demonstrating that Prox1 is a kind of on-off switch for pyramidal vs. granule neurons, even in at advanced stages of neuronal maturation.

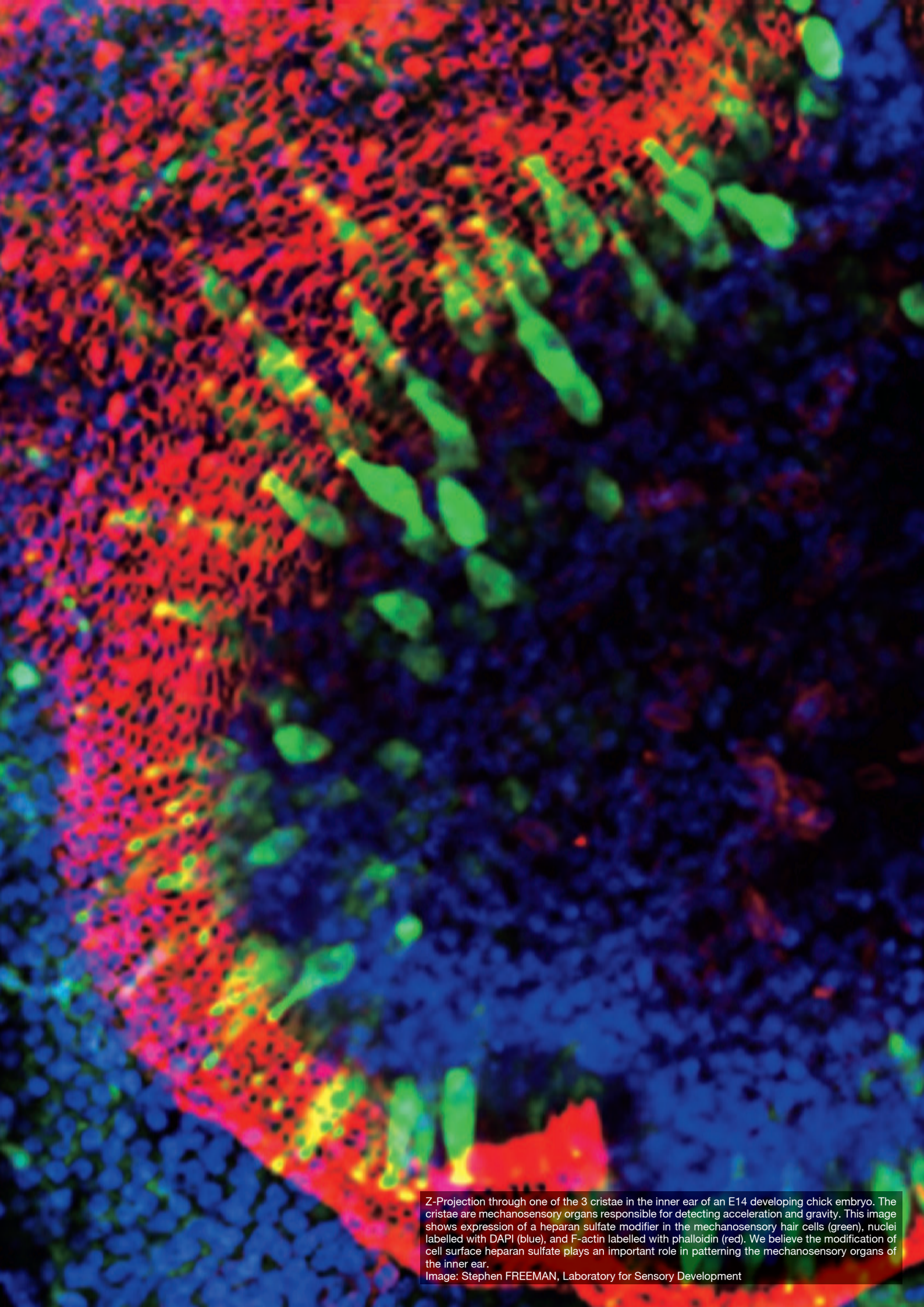
"It has been thought that pyramidal neurons and granule cells in the hippocampus are of totally different origin, but our finding reveals that their destinies are not so securely fixed, at least not until right before their maturation by the integration into the circuit, which takes about three weeks after their birth," says Matsuzaki. "This may be a property common to other neurons as well, which ensures the flexibility and tunability of neural circuit formation during both brain development and adult neurogenesis."

2012 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
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Inoue Prize for Science	Inoue Foundation for Science
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Tsukahara Nakaakira Memorial Award	Brain Science Foundation
Hiroki R. Ueda	Project Leader	Systems Biology	Tsukahara Nakaakira Memorial Award	Brain Science Foundation
Mototsugu Eiraku	Deputy Unit Leader	Four-dimensional Tissue Analysis	MEXT Prize	Ministry for Education, Culture, Sports, Science and Technology
Erina Kuranaga	Team Leader	Histogenetic Dynamics	Development, Growth & Differentiation Wiley-Blackwell Prize 2012	Wiley-Blackwell
Masatoshi Takeichi	Director of the RIKEN Center for Developmental Biology		2012 Citation Laureate	Thomson ISI
Teruhiko Wakayama	Team Leader	Genomic Reprogramming	Award for Excellent Poster Presentation	Asian Reproductive Biotechnology Society
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Yamazaki-Teiichi Prize	Foundation for Promotion of Material Science and Technology of Japan
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Takeda Medical Prize	Takeda Science Foundation



Z-Projection through one of the 3 cristae in the inner ear of an E14 developing chick embryo. The cristae are mechanosensory organs responsible for detecting acceleration and gravity. This image shows expression of a heparan sulfate modifier in the mechanosensory hair cells (green), nuclei labelled with DAPI (blue), and F-actin labelled with phalloidin (red). We believe the modification of cell surface heparan sulfate plays an important role in patterning the mechanosensory organs of the inner ear.
Image: Stephen FREEMAN, Laboratory for Sensory Development

Mammalian Epigenetic Studies

Masaki OKANO Ph.D.

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

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Recent Publications

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

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

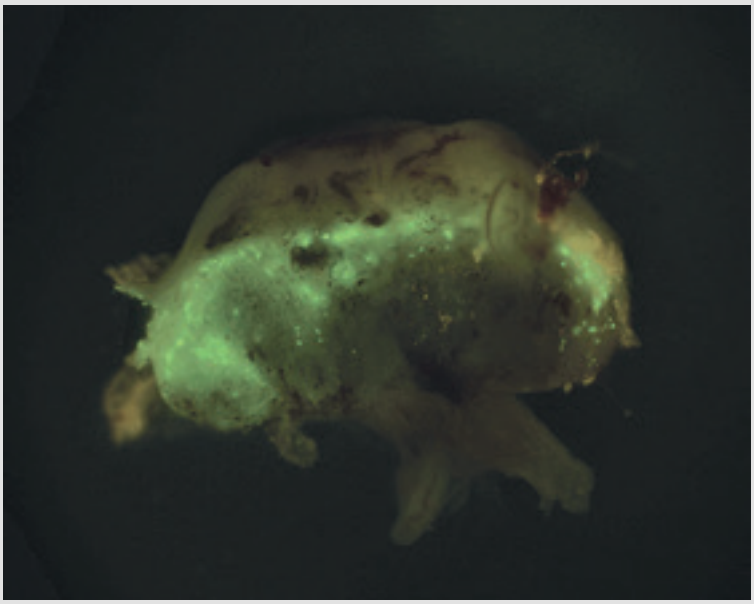
Kumaki Y, et al. QUMA: quantification tool for methylation analysis. *Nucleic Acids Res* 36. W170-5 (2008)

Takebayashi S, et al. Major and essential role for the DNA methylation mark in mouse embryogenesis and stable association of DNMT1 with newly replicated regions. *Mol Cell Biol* 27. 8243-58 (2007)

Sharif J, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450. 908-12 (2007)

The development and physiological function of multicellular organisms relies on the ability of their component individual cells, which as a rule contain identical genetic information, to express subsets of genes in a selective manner. In order to maintain lineages of cells of different types, the gene expression programs established in ancestral precursor cells must be transmitted stably to many generations of progeny. The fundamental mechanisms by which patterns of gene expression are preserved across cycles of cell division without a change to the underlying genome sequence are commonly referred to as “epigenetic” processes. These processes produce chemical modifications to and structural remodeling of chromatin, nuclear structures that store the cell’s DNA, thereby allowing individual cells to regulate the switching on and off of the expression of specific genes with great precision and flexibility.

Our laboratory studies epigenetics by focusing on a particular set of molecules that act in the methylation of DNA, with the goal of identifying their roles in the regulation of chromatin function in embryonic development and the processes of lineage commitment and plasticity in cell differentiation. We conduct phenotype analyses of mice in which various genes involved in DNA methylation have been knocked out, as well as biochemical and cell biological studies of embryonic stem (ES) cells to explore how dynamic changes in DNA methylation function as regulatory factors in mammalian embryogenesis and cell differentiation, and the means by which DNA methylation works to maintain cell proliferation and chromosome function. We anticipate that a deeper understanding of these questions will lead to new insights in the context-specificity of the DNA methylation process, as well as the roles of methylation in development, health, and regeneration.



Placenta tissue in a chimera between a nuclear transfer embryo using a Dnmt1$^{-/-}$Dnmt3a$^{-/-}$Dnmt3b$^{-/-}$ ES cell nucleus (green) and a wildtype embryo. Cells without DNA methylation contribute to extraembryonic lineages.

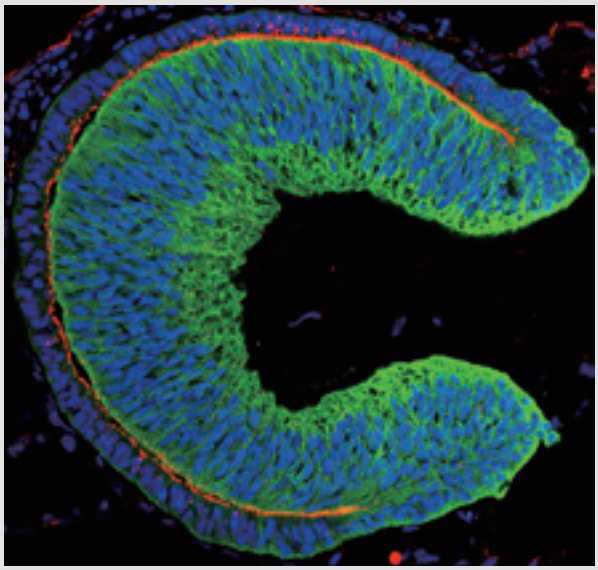
Organogenesis and Neurogenesis

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 axis is significant in neural development in that the central nervous system forms on the dorsal side of the body in all vertebrate orders. This process is dictated by the effects of a number of signaling factors that diffuse from organizing centers and direct dorsal ectoderm to maintain a neural fate. These molecules, which include Noggin, Chordin, Follistatin and their homologs, participate in elaborate signaling networks in which factors collaborate and compete to initiate the embryonic nervous system.

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

Our group is now also actively developing effective methods of inducing neuralization in mammals, work which it is hoped will contribute to basic research by providing in vitro experimental systems for use in the analysis of mammalian neurogenesis. In addition, this approach has potential for application in the treatment of neurodegenerative disorders, such as Parkinson's disease. Using a system developed in our lab, we have succeeded in inducing mouse and human embryonic stem (ES) cells to differentiate into a range of specialized neuronal types, including those of the cerebral cortex, cerebellar cortex and retina.

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



Self-organized formation of optic cup from human ES cells

Yoshiki SASAI M.D., Ph.D.

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

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

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Recent Publications

Nakano T, et al. Self-Formation of Optic Cups and Storable Stratified Neural Retina from Human ESCs. *Cell Stem Cell* 10.771-85 (2012)

Suga H, et al. Self-formation of functional adeno-hypophysis in three-dimensional culture *Nature* 480. 57-62 (2011)

Eiraku M, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 472. 51-56 (2011)

Kamiya D, et al. Intrinsic transition of ES cell differentiation into neural progenitors. *Nature* 470. 503-509 (2011)

Muguruma K, et al. Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. *Nat Neurosci* 13. 1171-80 (2010)

Early Embryogenesis

Guojun SHENG Ph.D.

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

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

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Part-Time Staff
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Recent Publications

Shin M, et al. Activin/TGF-beta signaling regulates Nanog expression in the epiblast during gastrulation. *Mech Dev* 128.268-78 (2011)

Nagai H, et al. Embryonic development of the emu, Dromaius novaehollandiae. *Dev Dyn* 240. 162-175 (2011)

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Shin M, et al. Notch mediates Wnt and BMP signals in the early separation of smooth muscle progenitors and blood/endothelial common progenitors. *Development* 136. 595-603 (2009)

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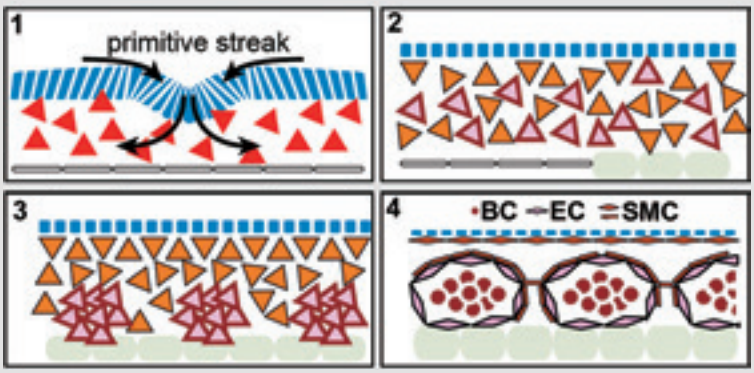
Nakazawa F., et al. Negative regulation of primitive hematopoiesis by the FGF signaling pathway. *Blood* 108.3335-43 (2006)

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 ectoderm cells during gastrulation through a process called epithelial to mesenchymal transition (EMT). During EMT, mesoderm cells leave epithelial-shaped ectoderm 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 ectoderm 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.



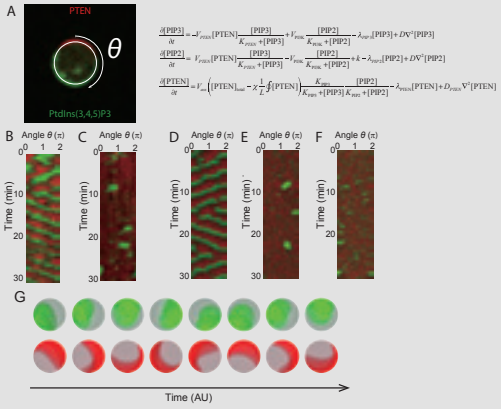
The circulatory system of the early chicken embryo (E5 shown here) is complex and contains embryonic, allantoic, and yolk sac sub-systems. The diversity of blood cells during this period is revealed by the transcriptomic analysis of non-red blood cells in circulation.

Physical Biology

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

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

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



(A) Spontaneous asymmetry formation of phosphatidylinositol 3,4,5-trisphosphate (PtdIns (3,4,5) P3, green) and PTEN (red), and an explanatory theoretical model.(B, C) Spatiotemporal diagrams of the membrane distributions.(D-F) Stochastic numerical simulations of the theoretical model. (G) Temporal evolution of the distributions on the cell surface calculated from the theoretical model. (Shibata, et al. 2012).

Tatsuo SHIBATA Ph.D.

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

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Recent Publications

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

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Nishikawa M. and Shibata T. Nonadaptive fluctuation in an adaptive sensory system: bacterial chemoreceptor. *PLoS One* 5.e11224 (2010)

Retinal Regeneration

Masayo TAKAHASHI M.D., Ph.D.

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Masayo Takahashi received her M.D. from Kyoto University in 1986, and her Ph.D. from the same institution in 1992. After serving as assistant professor in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1996, where she discovered the potential of stem cells as a tool for retinal therapy. She returned to Kyoto University Hospital in 1998, and since 2001 served as an associate professor at the Translational Research Center in the Kyoto University Hospital. She joined the CDB as a team leader of the retinal regeneration research team in 2006. Her clinical specialty is retinal disease - macular diseases and retinal hereditary diseases in particular. Her aim is to understand these diseases at a fundamental level and develop retinal regeneration therapies.

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Recent Publications

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

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

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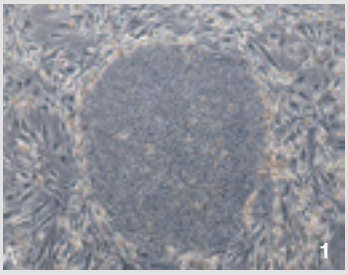
Haruta M, et al. Induction of photoreceptor-specific phenotypes in adult mammalian iris tissue. *Nat Neurosci* 4.1163-4 (2001)

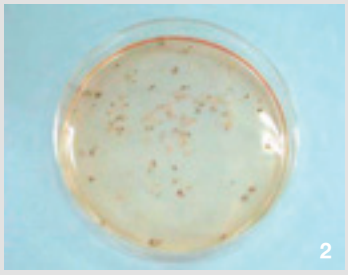
The retina has been called the “approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that in adult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

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

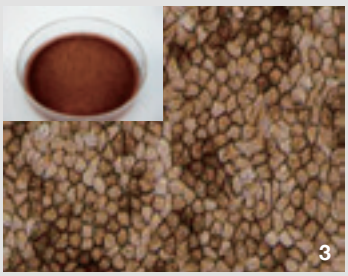
Therapeutic applications cannot be developed from basic research alone; the clinical approach – a thorough understanding of the medical condition to be treated is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily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



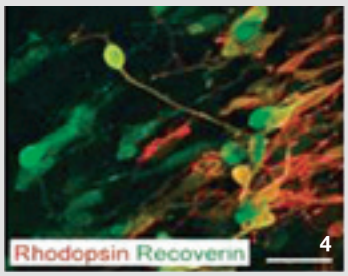
Human iPS cells



Retinal pigment epithelial cells differentiated from iPS cells



Purified retinal pigment epithelial cells



Photoreceptor cells differentiated from iPS cells

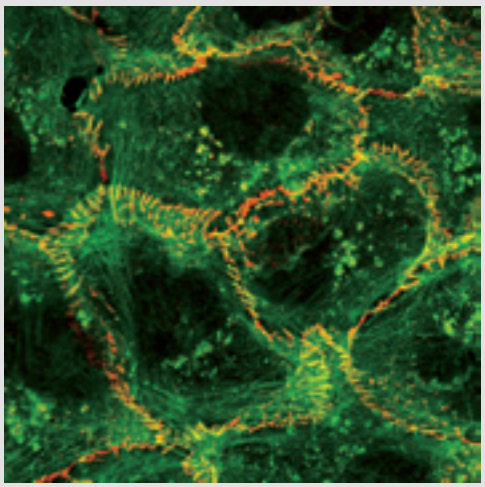
Cell Adhesion and Tissue Patterning

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

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

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

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



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

Masatoshi TAKEICHI Ph.D.

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

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Recent Publications

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

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Systems Biology

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

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

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Recent Publications

Jolley C. C, et al. A design principle for a posttranslational biochemical oscillator. *Cell Rep* 2.938-50 (2012)

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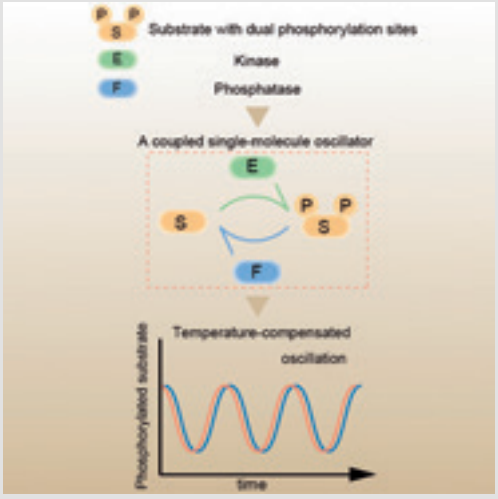
Recent large-scale efforts in genome-sequencing, expression profiling and functional screening have produced an embarrassment of riches for life science researchers and biological data can now be accessed in quantities that are orders of magnitude greater than were available even a few years ago. The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

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

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



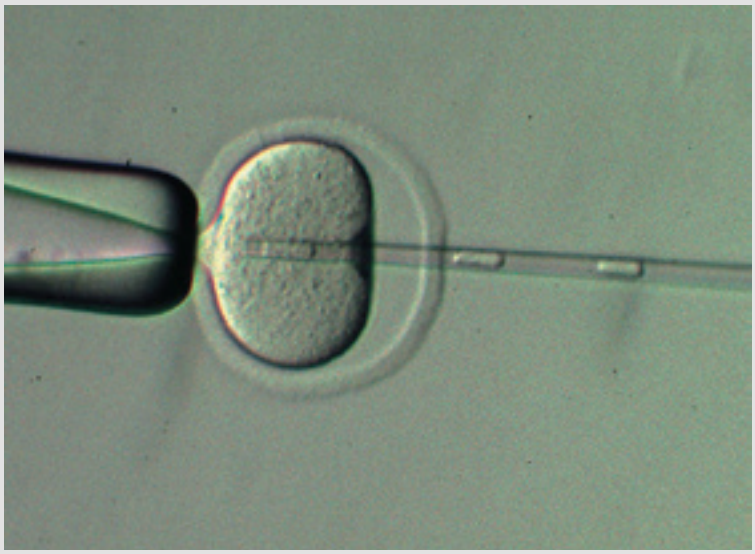
Post-translational processes such as protein phosphorylation are vital for circadian rhythms in many organisms. In cyanobacteria, circadian proteins can be incubated with ATP to form an in vitro post-translational oscillator (PTO) that operates in the absence of transcription and translation. It is still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

Genomic Reprogramming

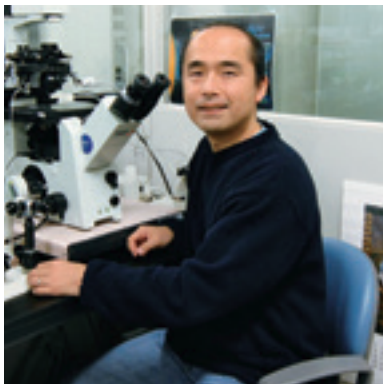
A theoretically limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual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

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

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



Transfer of a somatic nucleus into an enucleated egg



Teruhiko WAKAYAMA Ph.D.

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

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Recent Publications

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N-cadherin keeps cornea pumping

Vassil S. VASSILEV

The cornea of the eye serves a dual role, protecting the sensitive interior tissue from the environment and supporting the lens by focusing incoming light. With a thickness of only 0.5 mm in humans, the cornea nonetheless has a complex stratified structure, including epithelial, stromal, and endothelial layers. The innermost of these, the endothelium, separates the corneal stroma from the aqueous humor of the eye's interior, acts as a point of entry for nutrients into the non-vascular overlying tissue, and as a pump that siphons away excess fluids from the corneal interior. Defects in endothelial function can thus lead to accumulations of fluid that cloud the otherwise transparent cornea, leading to blurring of vision. Although it is known that this selectively permeable barrier is created by junctions between endothelial cells, the molecular mechanisms behind it have remained unclear.

A new study by Vassil S. Vassilev in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) and others in CDB now show that N-cadherin plays an important role in maintaining corneal integrity. In an article published in *Investigative Ophthalmology and Visual Science*, the group shows that loss of N-cadherin in mouse leads to defects in endothelial cell junctions, leading to stromal edema and a range of other defects in the cornea.

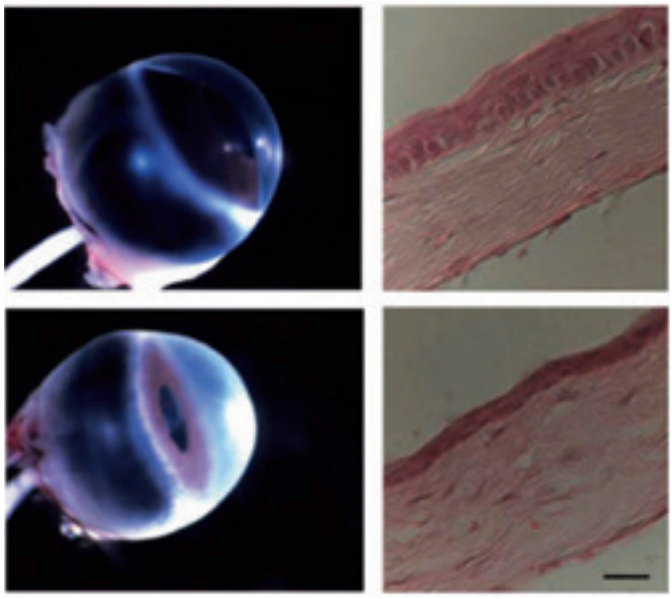
Corneal endothelial cells are bound to their neighbors in monolayers by two forms of cell-cell adhesion: tight junctions and adherens junctions, which together are known as the apical junction complex. Tight junctions are established by multiple types of adhesion molecule and form a physical barrier between cells, while adherens junctions are formed by classic cadherin adhesion molecules, which work to stabilize the links between adjacent cells and to regulate cell morphology through interactions with the cytoskeleton. In the cornea, the N-cadherin gene is highly expressed in the fetal through adult stages, which led the group to investigate the exact role of this molecule in corneal function.

Vassilev began by generating a conditional knockout mouse in which the N-cadherin gene was deleted specifically in neural crest cells, the main embryonic source of the corneal endothelium. After confirming that N-cadherin expression was lost in mutant corneal endothelial junctions, the group next examined the tissue structure and found it to be much more opaque than wildtype cornea. Interestingly, although N-cadherin was selectively ablated from the endothelium, the effects of its loss were seen in the other corneal layers. In the stromal layer, col-

Vassilev V. S, et al. Loss of N-cadherin from the endothelium causes stromal edema and epithelial dysgenesis in the mouse cornea. *Invest Ophthalmol Vis Sci* 53.7183-93 (2012)

lagen fibrils became unevenly spaced and fluid accumulated, resulting in edema, while the epithelium grew thinner and exhibited delamination and abnormal apoptosis.

But how does loss of an endothelial cadherin perturb the tissue architecture of other corneal layers? Suspecting the answer might lie in (fine) structural changes of the endothelial layer, Vassilev et al. compared the cytoskeleton of mutant and unmodified cells by staining for F-actin (which connects with cadherins via catenin linker molecules). In wildtype endothelium, actin molecules ring the cells' circumference, maintaining the structure of cell-cell junctions. In the conditional N-cadherin knockouts, however, the actin cytoskeleton was malformed, leading to defects in nuclear morphology and cellular adhesion. Additionally, the group discovered defects in endothelial tight junctions, finding that expression of the tight junction marker ZO-1 was significantly downregulated and the junctions disorganized. Tests of the ion pumping function of mutant endothelial cells further revealed that their ability to transport sodium and water across endothelium layer was disturbed. This suggested loss of the endothelial barrier function leading to abnormal distribution of electrolytes and the excessive accumulation of fluids in the corneal tissue.



Loss of N-cadherin leads to turbidity of corneal tissue (bottom left; wildtype shown top left). Stromal layer is edematous and epithelium is thinner in mutant cornea (bottom right, compared to wildtype (top right)

"In vitro studies had suggested that cadherin-based adherens junctions can be important for the maintenance of tight junctions, but here we were able to show that this is the case in vivo as well," says Takeichi. "In this study, we showed how the adherens junction plays a critical role in the exquisite architecture that gives the cornea its transparency. We next hope to use this conditional knockout system to investigate the function of N-cadherin in other neural crest-derived tissues."

New role for Pax2 in placode maintenance

Sabine FRETER

Thickenings in ectodermal tissue in the early embryo, known as placodes, serve as the developmental starting points for a great many neural and sensory tissues. The region that gives rise to the inner ear, known as the otic placode, and the epibranchial placode, which generates craniofacial neurons, are induced by the FGF signaling pathway acting on a shared domain called the posterior placodal area (PPA). Studies in a variety of model organisms have suggested that the Pax family of genes may also function in the formation of these placodes, or the PPA as a whole, but the evidence has remained equivocal.

Now, Sabine Freter and colleagues in the Laboratory for Sensory Development (Raj Ladher, Team Leader) in collaboration with the Genome Resource and Analysis Unit have shown that *Pax2* functions not as an inducer, but as a regulator of cell proliferation, in the otic and epibranchial placodes. Published in *Developmental Dynamics*, the report adds a new twist to our understanding of *Pax* family genes in tissue maintenance.

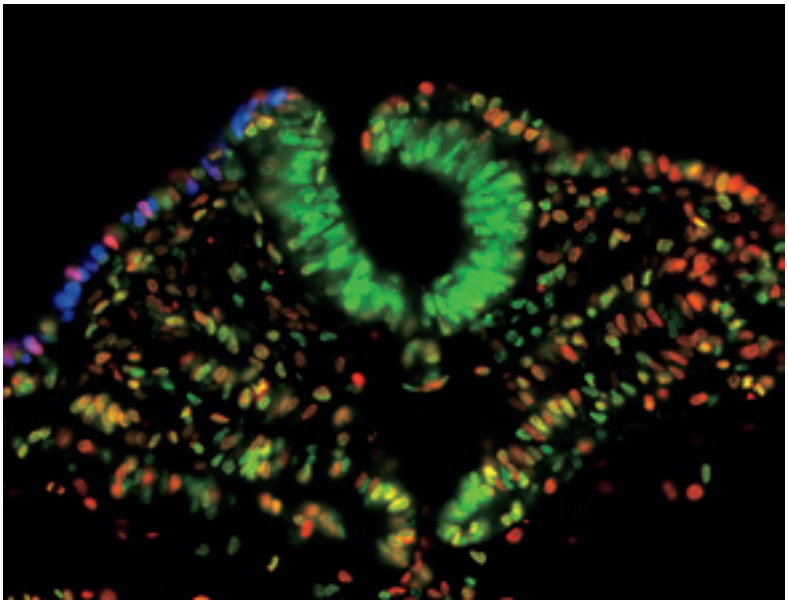
Early work in mouse, zebrafish, and frog indicated that the gene *Pax8* is expressed earlier than Pax2 in the nascent inner ear, and it was thought that either or both might play an inductive role in otic placode or PPA formation. The understanding of the function of these *Pax* genes was muddled somewhat when subsequent studies revealed that *Pax8* mutants showed no inner ear phenotype, and to add to the confusion, analyses of chick development revealed that loss of *Pax2* function had no effect on induction of the PPA. Seeking to get to the bottom of the question, Freter sought to study the role of *Pax2/8* in chicken otic development. Interestingly, after attempts to clone the *Pax8* gene and to identify it through searches of the chick genome, the team was unable to identify the orthologous sequence in chick, or in the genomes of other related species, suggesting it had been evolutionarily lost. *Pax2*, however, is highly conserved, indicating that it is likely the sole *Pax* gene functioning in PPA development in birds.

Freter next used RNAi to interfere with *Pax2* function, but found that this had no effect on PPA formation. Similarly, overexpression of the gene also left the PPA apparently unaffected. Suspecting that the true role of *Pax2* might be in inner ear differentiation rather than induction, the team looked at a later stage in development, when inner ear-specific genes normally begin to be expressed. *Pax2* knockdown resulted in the complete loss of expression of *Soho1*, an early marker of inner ear, but did not appre-

Freter S, et al. Pax2 modulates proliferation during specification of the otic and epibranchial placodes. *Dev Dyn* 241.1716-28 (2012)

ciably affect the patterning of the otocyst, although it did cause a reduction in size. Its overexpression, in contrast, inhibited inner ear differentiation.

The epibranchial placode, which contributes to various cranial ganglia, also derives from the PPA, so Freter investigated whether *Pax2* inhibition would affect its formation or differentiation as well. She found that, as in the otic placode, repression of *Pax2* function had no effect on the expression of genes associated with the induction of this placode, but dramatically reduced expression of genetic markers of committed neuronal progenitors. And, as in the inner ear, overexpression of *Pax2* interfered with epibranchial differentiation as well.



Pax2 electroporation (blue cells) reduces uptake of the thymidine homologs, BrdU (green) and EdU (red) in the inner ear precursor.

Seeking to better understand the mechanism by which Pax2 affects the otocyst and epibranchial tissue, the team next investigated the possibilities that its effects could be due to altered cell death or differentiation. The absence of caspase upregulation following Pax2 knockdown suggested that an effect on programmed cell death could be ruled out. The cell cycle, however, was slowed significantly by Pax2 inhibition, causing an overall reduction of mitotic activity in affected cells. Interestingly, overexpression of Pax2 had no appreciable effect on cell cycle.

“Given that Pax is known to work in the maintenance of precursor cells in other tissues, such as skeletal muscle, these latest results point to the possibility that Pax plays a general role in cell division and the maintenance of an undifferentiated state,” says Ladher.

CAMSAPs regulate microtubule dynamics in epithelial cells

Nobutoshi TANAKA, Wenxiang MENG

Microtubules organize themselves differently in different types of dividing cells. While they grow radially out from the centrosome during interphase in many cell types, this is not the case in epithelial cells, in which their minus-ends are mainly anchored in the cytoplasm instead. Despite its importance to mitotic dynamics, however, the means by which the distribution of centrosomal and non-centrosomal microtubules is established and maintained in different cells has a remained a mystery.

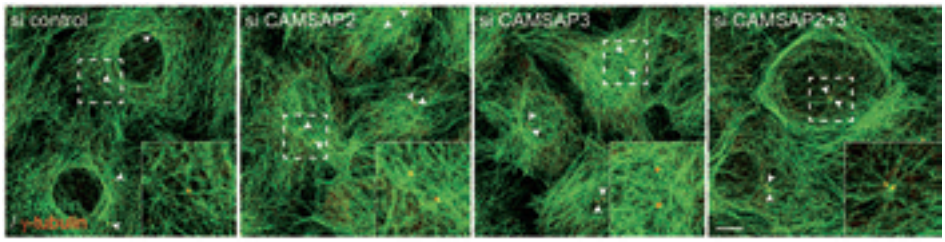
A study by Nobutoshi Tanaka and others in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) has now shown that a pair of molecules, CAMSAP3 (also known as Nezha) and CAMSAP2, work together to regulate and maintain noncentrosomal microtubule organization in cultured human cells. Published in the *Proceedings of the National Academy of Sciences*, this work reveals the mechanism by which interphase epithelial cells establish this distinct form of cytoskeletal organization.

Microtubules are polarized molecular chains, in which the terminal site of growth by polymerization is called the plus end; the minus end is found at the other pole. Minus ends have been shown to anchor in the centrosome, from which microtubules extend radially, a process mediated by numerous molecular factors. But some microtubules stabilize without tethering to the centrosome, by mechanisms that remain poorly understood. Seeking to gain more insight into this these noncentrosomal microtubules, Tanaka focused on CAMSAP3, which the Takeichi group had previously shown to bind to microtubule minus ends, and a related molecule CAMSAP2.

The group first looked at the localization of the two molecules and found that both tended to clump together in small clusters throughout the intracellular space. When they simultaneously visualized the plus ends of microtubules by immunostaining, they noted that the CAMSAP clusters tended to be located at the opposite poles, suggesting an affinity for minus-ends. Having established a physical connection, Tanaka et al. next sought after the functional role. The plus-end protein, EB-1, which they used in their immunostaining experiments, is known to bind to microtubule plus ends and contribute to their polymerization. Using specific siRNAs to knock down the function of each of the CAMSAPs, the group found that loss of the function of either molecule resulted in a reduction of EB1 radiating growth. This effect was amplified when both CAMSAPs were knocked down simultaneously, suggesting that these proteins promote stable extension of microtubules.

Tanaka N, et al. Nezha/CAMSAP3 and CAMSAP2 cooperate in epithelial-specific organization of noncentrosomal microtubules. *Proc Natl Acad Sci U S A* 49.20029-34 (2012)

In wildtype epithelial cells in low-density cultures, most microtubules are arrayed in a pattern surrounding the nucleus. The centrosomes locate at random sites, and only rarely nucleate radial microtubules. But the loss of function of either CAMSAP3 or -2 triggered a rearrangement in which the microtubules densely covered the nucleus. Even more intriguingly, the double knockdown of both CAMSAPs caused some epithelial cells to develop uncharacteristic centrosomal radiation of microtubules. Cells lacking either CAMSAP also showed post-translational changes in the tubulin protein, which may account for the altered microtubule dynamics.



In control cells cultured at low density, microtubules are distributed encircling the nucleus (left), and are not seen radiating from centrosomes (stained red, arrowheads). Knockdown of either CAMSAP2 or CAMSAP3 (middle panels) triggers accumulation of microtubules at centrosome sites, while simultaneous knockdown of both induces the radial polymerization of microtubules from centrosome sites.

The arrangement of microtubules growing from CAMSAP sites appears to have functional consequences as well. In CAMSAP-depleted cells, early endosomes accumulated around the centrosome, while in control cells these organelles are dispersed throughout the cytoplasm. The Golgi apparatus, which is normally distributed in the perinuclear region, was more widely distributed in CAPSAM-knockdown cells.

“While this series of experiments points to some very interesting possibilities, we will need to study these phenomena more closely in epithelial tissue in vivo rather than low-density cell culture,” notes Takeichi. “As epithelial cells show distinct microtubule orientation toward their apical surfaces, we may be able to detect whether CAMSAPs play regulatory role in the establishment of this polarity, and how their depletion affects cell morphology and behavior in intact tissue.”

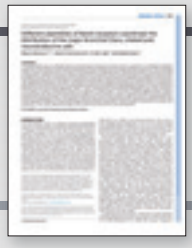
Ruling the airways: Notch controls bronchial cell fates and distributions

Mitsuru MORIMOTO, Junko SAKAI, Yuka NODA

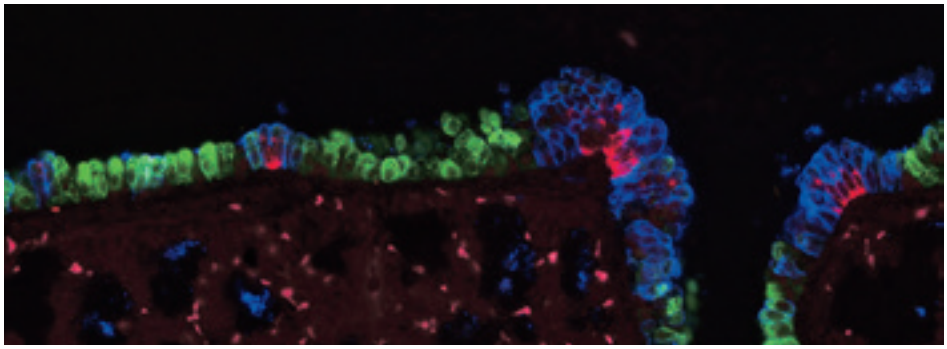
Nestled deep within the body, the epithelial lining of the respiratory system is nonetheless seriously exposed. Its direct contact with environmental air necessitates protective mechanisms that both seal off the respiratory tract from other compartments of the body and neutralize microbial invaders. This is achieved by the coordinated action of the functionally specialized various cell types that make up the lining of the airway. These respiratory cell populations include major ciliated cells, exocrine Clara cells, and neuroendocrine (NE) cells, all of which are generated by a common epithelial progenitor cell type during embryogenesis. One interesting feature of lung histology is that the relative distributions of these cell types varies along the proximal-distal axis, with ciliated cells capable of sweeping away microscopic particles and organisms more common near the mouth, and secretory Clara cells that help maintain humidity and bronchiole structure increasing in deeper regions of the lung. Neuroendocrine cells are less common than ciliated and Clara cells, and are found in clusters throughout the lung epithelium. Despite their importance, the mechanisms by which these spatial changes in lung cell distribution are regulated remain largely a mystery.

Mitsuru Morimoto, Team Leader of the Laboratory for Lung Development, working with colleagues at the National Institute of Genetics and Washington University of St. Louis, has now shown that Notch signaling controls the differentiation and distribution of these three lung epithelial cell types in mouse. Published in *Development*, this work sheds new light on how the diversity of lung cells is established and maintained.

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)



The Notch signaling pathway is triggered by interactions between ligands and receptors bound within cell membranes, making it a much more local affair than signaling involving secreted molecules that diffuse away from cells. Morimoto had previously noted that when Notch signaling is reduced during development, the number of Clara cells in the lung epithelium decreases, while ciliated cells expand in number. Other work had also shown that the Notch pathway ligand Jag1 is expressed in ciliated cells, which it was suggested might induce Clara cell differentiation in neighboring cells. To work out the details of how Notch works in lung cell fate determination, Morimoto in this most recent study tested how ablating the Notch receptors Notch1-3 affected the relative distributions of epithelial cell types in the airway. His first finding came as something of a surprise – Clara cell differentiation relies on Notch2, not Notch1 as previously believed.



Clara cells (green), neuroepithelial cells (red) and SPNC cells (blue) in fetal mouse airway epithelium. Clara cells are distributed throughout the airway, while NE cells are observed in clusters. SPNC cells are induced in a Notch-dependent manner, and appear to regulate NE cell cluster size.

Morimoto further observed that simultaneous deletion of all three receptors caused the size of neuroendocrine cell clusters to increase, an effect not seen when these factors were knocked out individually or in other combinations, suggesting that Notch1-3 have additive functions in the regulation of NE cell differentiation. Even more intriguingly, Notch activity was not observed in the neuroendocrine cells themselves, but rather in a distinct population of their neighbors, apparently representing a hitherto unidentified cell type, which the team dubbed SPNC cells, after a characteristic gene expression profile (SSEA-1^{+} peri-pNEB N1ICD^{+} CC10^{-}). Morimoto found that these SPNC cells are maintained by juxtacrine Notch signaling from adjacent NE cells, suggesting that once neuroendocrine cells have been induced, they function to maintain local SPNC cells, which in turn provide signals that regulate the size of NE cell clusters.

“The combinatorial effects of Notch factors in the airway epithelium may be the mechanism underlying the distribution of ciliated, Clara and neuroepithelial cells. While the ciliated/Clara selection is sensitive to dosage of Notch2, NE cells are regulated synergistically by the combination of Notch1-3, which may account for various pattern of ciliated/Clara cells and the uniform distribution of NE cell cluster throughout the epithlieum,” says Morimoto. “In future studies, we will be interested in looking more deeply into how the Notch signaling works in Clara cell differentiation, and inducers of NE cells as well.”

Craniofacial development holds key to hagfish phylogeny

Yasuhiro OISHI, Shigeru KURATANI

The position occupied by cyclostomes, such as lampreys and hagfish, within the phylogenetic tree has been a controversial question for decades. Distinct from gnathostomes (vertebrates possessing a jaw), the cyclostomes are jawless, and have only a single nostril-like facial structure, as opposed to the gnathostome's two. This solitary nostril (an olfactory organ known as the nasohypophyseal duct) is itself the focus of a debate over the evolutionary relationship between hagfish and lampreys; while the duct dead-ends in the lamprey, it opens into the pharynx in hagfish. There are also disagreements within the research community as to the developmental source of the adenophophysis, which opens into the nasohypophyseal duct in hagfish - specifically whether it is endodermal in origin, as suggested by some previous studies, or ectodermal, as is the case for both lampreys and the gnathostomes. Finding answers to these questions has long been frustrated by the difficulty in obtaining hagfish embryos for detailed examination.

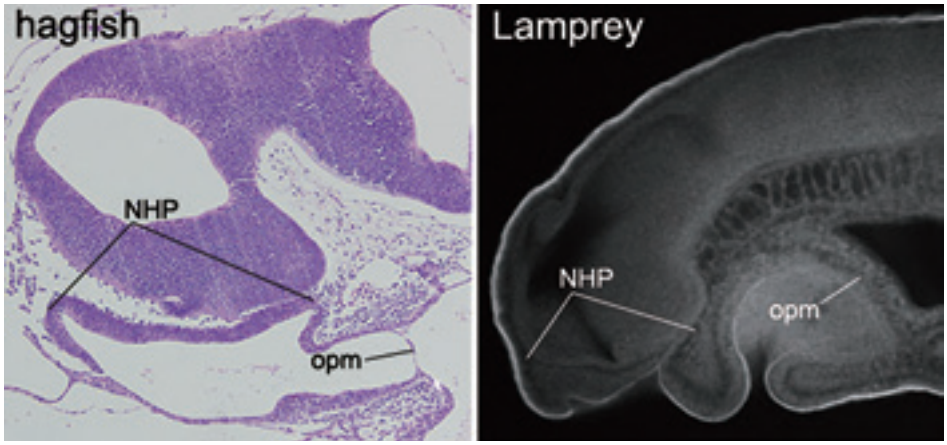
New work by Yasuhiro Oisi and others in the Laboratory for Evolutionary Morphology (Shigeru Kuratani, Group Director) in collaboration with colleagues in CDB and Academia Sinica (Taiwan), provides morphological evidence that reveals that development of the olfactory organs and adenohypophysis is roughly homologous in hagfish and lampreys. Published in *Nature*, this study solidifies our understanding of hagfish as close relatives of their cyclostome kin, and sheds new light on the evolution of vertebrate craniofacial structure.

Several years ago, the Kuratani lab develop a system for breeding the hagfish *Eptatretus burgeri* in captivity, a breakthrough that provided the lab with a much-needed supply of embryos after a nearly century-long drought that forced hagfish biologists to rely on studies published in the 19th century. Oisi used this resource to conduct a staged series examination of embryos to examine the development of the NHP and adenohypophysis, and to resolve their germ layer origin.

Observation of embryos at the mid-neurula stage suggested that the adenohypophysis arise from a transient ectodermal tissue, similar to other vertebrates including lamprey, and not from the endoderm as had previously been reported. The ectodermal origin of the adenohypophysis was further supported by gene expression profiles consistent with an ectodermal source, and by following changes in gene expression and morphology across the relevant development stages, the group was further able to exclude the possibility that the hagfish ad-

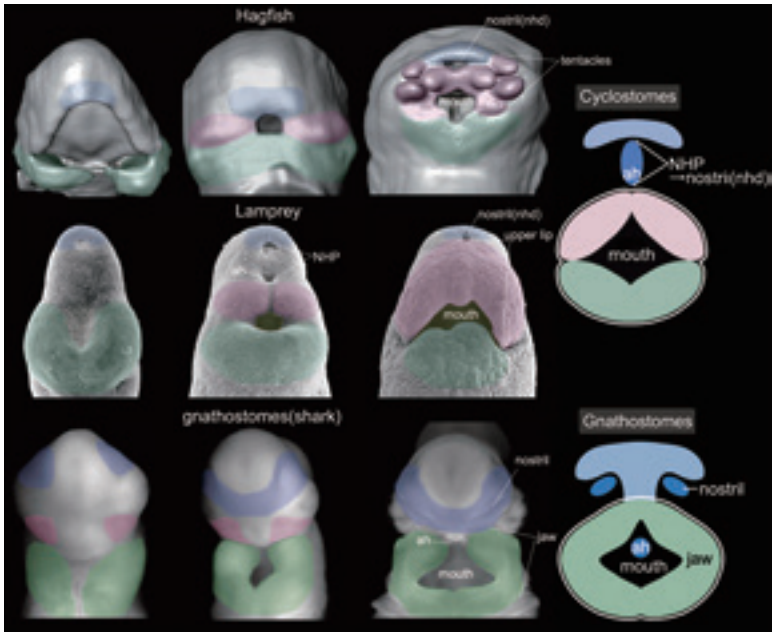
Oisi Y, et al. Craniofacial development of hagfishes and the evolution of vertebrates.
Nature 493.175-80 (2013)

enohypophysis differentiates from the prechordal plate (endodermal thickening). This suggests that the developmental differences between hagfish and lampreys are smaller than had been suspected, as well as a commonality in important aspects of craniofacial development between both extant vertebrate groups: cyclostomes and gnathostomes.



Sagittal sections of hagfish and lamprey heads. NHP is of ectodermal origin in both animals, and located anterior to the oropharyngeal membrane (opm), which defines ecto/endodermal boundary.

In lampreys, the nasohypophyseal duct forms from a median complex of placodes known as the nasohypophyseal plate (NHP), which corresponds to a pair of discrete placodes - hypophyseal and nasal – found in gnathostomes. The differences in the gross morphology of the adult hagfish had led some to suspect that its development might differ significantly from that in lamprey. Oisi set out to test this hypothesis by studying changes in genetic markers of NHP and the gnathostome placodes. He found that, as in lamprey, the hagfish NHP takes the form of a single median placode, suggesting the structures are conserved across the cyclostomes. The distinct morphological features observed in the craniofacial structures in adults of the two taxa thus appear to be attributable not to differences in their developmental origins, but to hagfish-specific modifications at subsequent embryonic stages.



Comparison of vertebrate heads. Hagfish and lamprey share embryonic craniofacial features. This pattern does not appear during any developmental stage in other living gnathostomes. ah, adenohypophysis; nhd, nasohypophyseal duct.

Animal Resources and Genetic Engineering

Genetically engineered mice are an important resource used in biomedical research. The progress and quality of research is greatly dependent on how efficiently mutant mice can be generated, propagated, and housed. However, researchers today tend not to engage in routine generation of mutant mice. The major function of our laboratory is to develop mutant mice for research in the fields of developmental biology and regenerative science, and to maintain and enhance the experimental rodent resources in the CDB.

Yasuhide FURUTA Ph.D.

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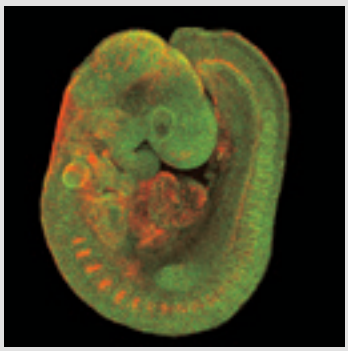
Behnoush KHALEDIAN
Prem Swaroop YADAV

Genetic Engineering Unit

Shinichi AIZAWA Ph.D.

The Genetic Engineering Unit works with research labs within the CDB, as well as other labs in Japan and throughout the Asia-Pacific region to develop mutant mice useful for the study of development and regeneration. In these joint development projects, we receive sequence information for genes of interest from our collaborators, and perform all subsequent stages of the development from construction of the targeting vector to generation of chimeras, making about 100 new knockout mutants every year. In addition, we develop new bioimaging technologies to aid in the visualization of mutant mouse embryos at the tissue, cell and organelle levels.



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

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Mayo SHIGETA
Aki SHIRAISHI
Norie TANAKA
Tomoko TOKUNAGA
Sachi YAKAWA

Assistant
Yuki TSUJI

Animal Resource Unit

Kazuki NAKAO Ph.D.

The Animal Resource Unit maintains and cares for CDB's laboratory mouse and rat resources in a Specific Pathogen Free (SPF) environment. We also handle shipping and receiving of mutant mice both within the CDB and with other domestic and overseas institutions. In addition, we provide pregnant females, fertilized mouse eggs, and services for colony expansion and strain cryopreservation. We also develop technologies for the study of reproductive biology.

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

Recent Publications

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

Shinohara R, et al. A role for mDia, a Rho-regulated actin nucleator, in tangential migration of interneuron precursors. *Nat Neurosci* 15.373-80, S1-2 (2012)

Niisato E, et al. CRMP4 suppresses apical dendrite bifurcation of CA1 pyramidal neurons in the mouse hippocampus. *Dev Neurobiol* 72.1447-57 (2012)

Tao H, et al. Nuclear localization of Prickle2 is required to establish cell polarity during early mouse embryogenesis. *Dev Biol* 364.138-48 (2012)

Nakae J, et al. Novel repressor regulates insulin sensitivity through interaction with Foxo1. *EMBO J* 31.2275-95 (2012)

Kanemaru K, et al. Epidermal phospholipase Cdelta1 regulates granulocyte counts and systemic interleukin-17 levels in mice. *Nat Commun* 3.963 (2012)

Nishimura R, et al. Osterix regulates calcification and degradation of chondrogenic matrices through matrix metalloproteinase 13 (MMP13) expression in association with transcription factor Runx2 during endochondral ossification. *J Biol Chem* 287.33179-90 (2012)

Iwano T, et al. Prox1 postmitotically defines dentate gyrus cells by specifying granule cell identity over CA3 pyramidal cell fate in the hippocampus. *Development* 139.3051-62 (2012)

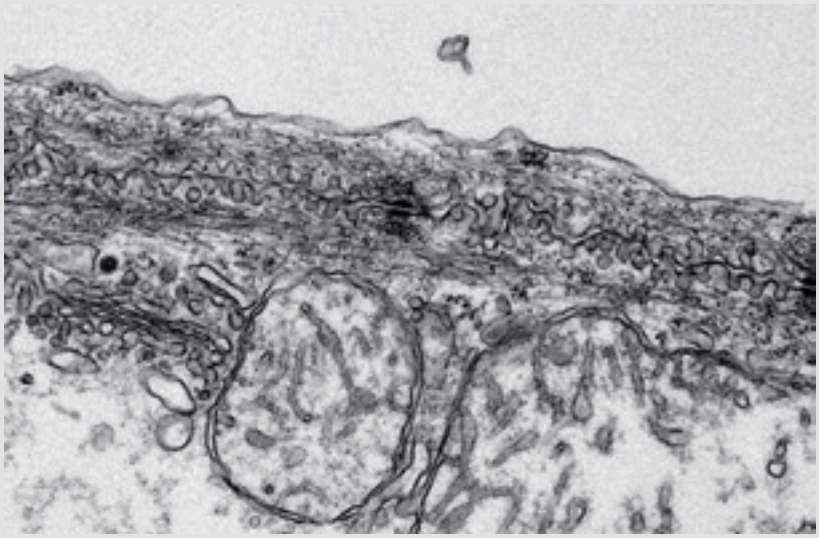
Electron Microscope

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

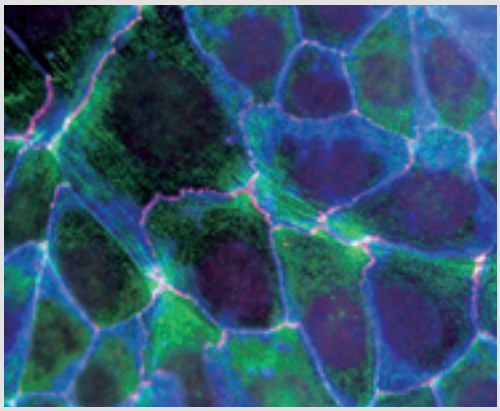
Shigenobu YONEMURA Ph.D.

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

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



Cells organizing the notochord of zebrafish embryo



α-catenin molecules stretched by forces produced by Myosin Ⅱ (green) are selectively labeled with α18 antibody(red).

Staff

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Research Fellow
Akira ONODERA
Ayuko SAKANE

Student Trainee
Yu AMANO

Assistant
Mai SHIBATA

Recent Publications

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

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

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

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

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

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

Bio-imaging

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

Shigeo HAYASHI Ph.D.

Staff

Unit Leader
Yuko KIYOSUE

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Togo SHIMOZAWA

Technical Staff
Tomoko HAMAJI

Part-Time Staff
Emiko MAEKAWA

Agency Staff
Taisaku NOGI

Recent Publications

Shimozawa T, et al. Improving spinning disc confocal microscopy by preventing pinhole crosstalk for intravital imaging. *Proc Natl Acad Sci U S A* in press (2013)

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

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

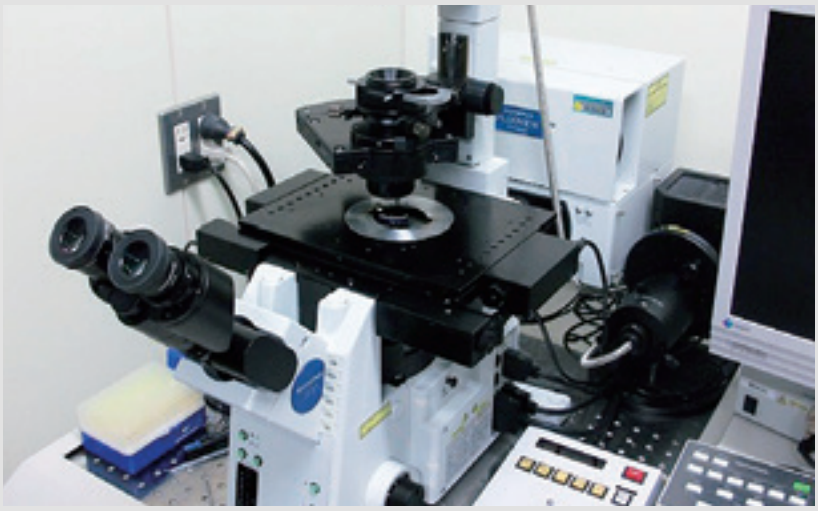
Optical Image Analysis Unit

Yuko KIYOSUE Ph.D.

This unit runs the CDB's common-use Imaging facility.

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

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



Inverted microscope maintained by the Optical Image Analysis Unit

Genomics

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

Fumio MATSUZAKI Ph.D.

Genome Resource and Analysis Unit

Shigehiro KURAKU Ph.D.

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

Our main sequencing platform, illumina HiSeq 1000, in operation since spring 2012

Our long-standing solution, Roche 454 FLX+, in operation since 2007

Staff

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Kaori TATSUMI

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Nathalie FEINER

Part-Time Staff
Naomi KAWASHIMA
Ayako TANIGUCHI

Recent Publications

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

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

Matsubara K, et al. Intra-genomic GC heterogeneity in sauropsids: evolutionary insights from cDNA mapping and GC3 profiling in snake. *BMC Genomics* 13.604 (2012)

Freter S, et al. Pax2 modulates proliferation during specification of the otic and epibranchial placodes. *Dev Dyn* 241.1716-28 (2012)

Functional Genomics Unit

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

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

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Takeya KASUKAWA
Itoshi NIKAIDO

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Kenichiro UNO

Assistant
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Recent Publications

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

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

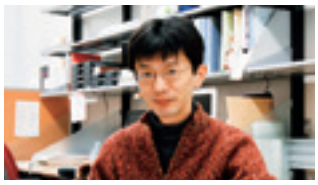
Masumoto K.H, et al. Acute induction of Eya3 by late-night light stimulation triggers TSHßexpression in photoperiodism. *Curr Biol* 20. 2199-206 (2010)

Isojima Y, et al. CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc Natl Acad Sci U S A* 106. 15744-9 (2009)

Proteomics

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

Shigeo HAYASHI Ph.D.



Staff

Lab Head
Shigeo HAYASHI

Unit Leader
Akira NAKAMURA

Research Specialist
Reiko NAKAGAWA

Recent Publications

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

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

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

Satoh R, et al. Role of the RNA-binding protein Nrd1 in stress granule formation and its implication in the stress response in fission yeast. *PLoS One* 7.e29683 (2012)

Hatanaka A, et al. Fub1p, a novel protein isolated by boundary screening, binds the proteasome complex. *Genes Genet Syst*. 86.305-14 (2011)

Zou P, et al. p57(Kip2) and p27(Kip1) cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70. *Cell Stem Cell* 9.247-61 (2011)

Mass Spectrometry Analysis Unit

Akira NAKAMURA Ph.D.

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

In the most recent fiscal year, the unit received more than 30 spectrometry requests and nearly 900 samples for use in identification.

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

Human Stem Cell Technology

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

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

Human Stem Cell Technology Unit

Yoshiki SASAI M.D., Ph.D.

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

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Part-Time Staff
Yoshinori NAKAI

Recent Publications

Ohgushi M. and Sasai Y. Lonely death dance of human pluripotent stem cells: ROCKing between metastable cell states. *Trends Cell Biol* 21.274-82 (2011)

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

Four-dimensional Tissue Analysis Unit

Yoshiki SASAI M.D., Ph.D.

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

Staff

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Junior Research Associate
Yuiko HASEGAWA

Recent Publications

Eiraku M, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 472. 51-56 (2011)

Eiraku M. and Sasai Y. Mouse embryonic stem cell culture for generation of three-dimensional retinal and cortical tissues. *Nature Protocols* 7. 69-79 (2011)

Science Policy and Ethics Studies Unit

Douglas SIPP

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

Staff

Unit Leader
Douglas SIPP

Recent Publications

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

Sipp D. Pay-to-participate funding schemes in human cell and tissue clinical studies. *Regenerative Medicine* 7.105-111 (2012)

Yuan W, et al. Stem cell science on the rise in china. *Cell Stem Cell* 10.12-15 (2012)

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

The bustling heart of Kobe includes a variety of distinct neighborhoods. The downtown area of Sannomiya sits square in the city's center, and its range of international restaurants and 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 stand in contrast to the colorful Chinatown that's right next door. A short walk north from city center lies the old foreign settlement of Kitano, whose clapboard houses and well-kept parks are a perfect retreat from the dynamism downtown.

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

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

RIKEN Kobe Institute

The RIKEN Institute was established in April 2000 as an organizational framework for the newly launched Center for Developmental Biology (CDB), which conducts a wide range of research, from fundamental studies of development and stem cells, to cutting-edge work with the potential to make a contribution to regenerative medicine. In April 2007, the Kobe Institute welcomed a new institution, the Molecular Imaging Research Program, which carries out research into bioimaging technologies such as positron emission tomography. In autumn 2008, this program was redesignated as the Center for Molecular Imaging Science (CMIS). In April 2011, the Kobe Institute established its newest research center, the Quantitative Biology Center, which will focus on systems, simulation, and computational approaches to life sciences phenomena.

The RIKEN Kobe Institute seeks both to help develop a foundation of knowledge into biological phenomena and, through translational research efforts conducted with the Institute for Biomedical Research and Innovation and other nearby institutions, to help bridge basic science to novel applications in medical therapy and drug discovery, thereby contributing to the health and welfare of the people of Japan.

The Kobe Institute administrative structure comprises the Research Promotion Division and the Safety Center.

Kobe Institute Administrative Structure

- **Kobe Research Promotion Division**
  - Planning
  - Finance
  - General Affairs
    - Human Resources
    - Facilities
  - Computer Networks
  - Office for Research Communications
  - Library
- **Kobe Safety Center**
  - Institutional Review Board

Center for Molecular Imaging Science

The Center for Molecular Imaging Science (CMIS) was established in October 2008 as an expansion of the Molecular Imaging Research Program, which was launched by RIKEN in July 2005. Molecular imaging is the only non-invasive technique for quantitative monitoring of changes in concentration or distribution of target molecules in living organisms. This is made possible through the integration of multiple fields, including chemistry, physics, molecular biology, pharmaceutical science, medical science, engineering, and computer science. Our Center brings together researchers from these diverse areas to work on translational projects that span basic research, such as compound design, and the development of instruments, animal research, and clinical research. This collaborative strategy makes our institution very unique within the Japanese research system.

Quantitative Biology Center

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

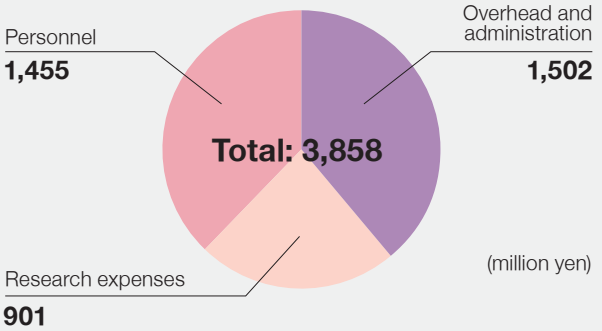
Research Promotion Division

The Kobe Institute Research Promotion Division (KRPD) provides a full range of administrative services required by CDB labs, with sections responsible for planning, finance, general affairs, human resources and facilities, as well as support for scientific meeting logistics, computer and information networks, research communications, and the CDB library.

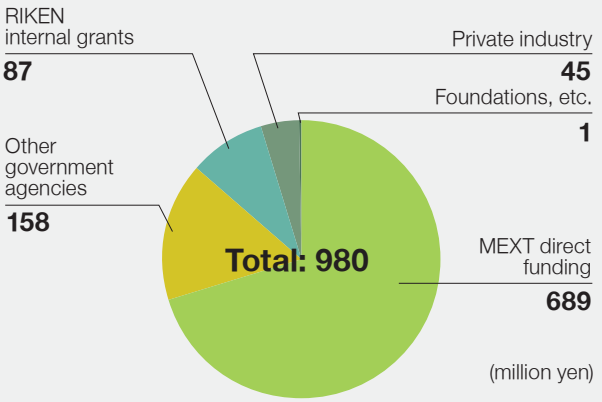
Safety Center

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

2012 CDB Budget



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



2012 CDB Staff

Laboratory heads	31
Deputy leaders	4
Research scientists	94
Research associates	8
Technical staff	91
Assistants	20
Visiting scientists	112
Student trainees	32
Part-time staff	46
Other	30
Total	468
Research Promotion Division*	78

*Administrative staff for Kobe Institute

2012 CDB Symposium

Quantitative Developmental Biology

March 26-28, 2012

The RIKEN CDB held its tenth annual symposium, “Quantitative Developmental Biology” on March 26–28. Around 160 participants from 14 countries gathered to interact and review experimental, computational, and theoretical approaches to the study of principles of development. In addition to the 30 talks over the course of the three-day program, days 1 and 2 included poster sessions in which over 70 poster presenters discussed their work.

This year’s event was co-organized by the CDB’s Shigeo Hayashi and Tatsuo Shibata , as well as Suzanne Eaton of the Max Planck Institute of Molecular Cell Biology and Genetics (Germany), Shigeru Kondo of Osaka University, and Shuichi Onami of the RIKEN Quantitative Biology Center. Additional support for the meeting was provided by 11 organizations.

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

Session 1

Suzanne Eaton (Max Planck Institute of Molecular Cell Biology and Genetics, Germany)
Edwin W. Munro (The Univeristy of Chicago, USA)

Session 2

Shigeo Hayashi (RIKEN CDB, Japan)
Kaoru Sugimura (Kyoto University, Japan)

Session 3

Cornelis J. Weijer (University of Dundee, UK)
Darren Gilmour
(European Molecular Biology Laboratory, Germany)
Martin Behrndt
(Institute of Science and Technology Austria, Austria)

Session 4

Tadashi Uemura (Kyoto University, Japan)
Philipp Khuc Trong (University of Cambridge, UK)

Session 5

Shigeru Kondo (Osaka University, Japan)
Cheng Ming Chuong (University of Southern California, USA)
Philip J. Murray (University of Oxford, UK)

Session 6

Stuart A. Newman (New York Medical College, USA)
Przemyslaw Prusinkiewicz (The University of Calgary, Canada)
Dina A. Faddah (MIT/Whitehead Institute for Biomedical Research, USA)

Session 7

Frank Jülicher (Max Planck Institute for Physics of Complex Systems, Germany)
Hidehiko Inomata (RIKEN QBiC, Japan)
Eugene Myers (HHMI, Janelia Farm Research Campus, USA)

Session 8

Shuichi Onami (RIKEN QBiC, Japan)
Ralf Schnabel (TU Braunschweig, Germany)
James Sharpe (Center for Genomic Regulation, Spain)

Session 9

Tatsuo Shibata (RIKEN CDB, Japan)
Orion D. Weiner (University of California, San Francisco, USA)
Tetsuya Nakamura (Osaka University, Japan)

Session 10

Jérôme Solon (Center for Genomic Regulation, Spain)
Christopher S. Chen (University of Pennsylvania, USA)
Satoshi Sawai (The University of Tokyo, Japan)

Session 11

Masako Tamada (Sloan-Kettering Institute, USA)
Buzz Baum (MRC Laboratory for Molecular Cell Biology, UK)
Lance Davidson (University of Pittsburgh, USA)

2013 CDB Symposium

The Making of a Vertebrate

March 4–6, 2013

The eleventh annual symposium “The Making of a Vertebrate” will be held on March 4–6, 2013 in the CDB Auditorium. This symposium will focus on the current understanding of developmental mechanisms in vertebrates at the cell and molecular levels, focusing not only on what we have learned, but what remains unknown and avenues to future discovery. Discussion will center on the latest developments in such topics as the fundamental structures of the vertebrate body and evolutionary mechanisms leading to the emergence of diversity.

Invited Speakers

Shinichi Aizawa
(RIKEN CDB, Japan)
Detlev Arendt
(EMBL, Germany)
Edward M. De Robertis
(HHMI/UCLA, USA)
Denis Duboule
(Univ. of Geneva & EPFL, Switzerland)
Hiroshi Hamada
(Osaka Univ., Japan)
Edith Heard
(CNRS, France)
Andreas Hejnol
(Sars Intl Centre for Marine Molecular Biol, Norway)
Masahiko Hibi
(Nagoya Univ., Japan)
Nicholas D. Holland
(UC San Diego, USA)
Hidehiko Inomata
(RIKEN CDB, Japan)
Naoki Irie
(RIKEN CDB, Japan)
Fumitoshi Ishino
(Tokyo Medical and Dental Univ., Japan)
David Kingsley
(Stanford Univ., USA)
Shigeru Kuratani
(RIKEN CDB, Japan)
Chris Lowe
(Stanford Univ., USA)
Miguel Manzanares
(CNIC, Spain)
Hitoshi Niwa
(RIKEN CDB, Japan)
Luis Puelles
(Univ. of Murcia, Spain)
Toshihiko Shiroishi
(NIG, Japan)
Billie J. Swalla
(Univ. of Washington, USA)
Hiroyuki Takeda
(The Univ. of Tokyo, Japan)

CDB Seminars

The RIKEN CDB makes special efforts to provide a full and diverse series of invited seminars by scientists from around the world. To date, the Center has hosted more than 650 such talks, in addition to numerous meetings, distinguished lectures, internal forums, and colloquia. The following speakers presented CDB Seminars in the period from January to December 2012.

Date	Title	Speaker
01-23	Two types of Notch signaling cooperate in epithelial patterning during lung organogenesis	Mitsuru MORIMOTO
02-06	A gene network regulating temporal changes in neural stem cell function	Jinsuke NISHINO
02-10	The stem cell niche is giving me goosebumps, and more	Hironobu FUJIWARA
02-23	The role of genetic programs and activity in the development of cortical interneuron diversity	Gordon FISHELL
03-02	Mitotic microtubules in mammalian neurogenesis	Felipe MORA-BERMÚDEZ
03-07	Dissecting the role of morphogenesis in the origins of the first two cell lineages in the mouse embryo	Robert Odell STEPHENSON
03-08	Secreted semaphorins from degenerating larval ORN axons direct adult projection neuron dendrite targeting	Lora B. SWEENEY
04-05	Spatial regulation of VEGF receptor endocytosis in angiogenesis	Masanori NAKAYAMA
04-10	What songbirds can tell us	Kentaro ABE
05-11	PDGF signaling pathways in development and homeostasis	Phil SORIANO
06-01	Microfabricated substrates as a tool to study cell mechanotransduction & collective cell migration	Benoît LADOUX
06-18	Cell culture engineering toward large scale chondroprogenitor production from human pluripotent stem cells	Naoki NAKAYAMA
06-18	Non-canonical Wnt signaling maintains hematopoietic stem cell through flamingo and Frizzled8 in the niche	Ryohichi SUGIMURA
06-18	The power of cell autonomous growth in the absence of ERK signalling is gained just before implantation	Thorsten BOROVIAK
06-19	BMP signaling and spinal cord development	Naihe JING
06-29	Conserved and diversified roles of eIF5-mimic proteins in eukaryotes	Katsura ASANO
07-03	Symmetrical divisions dedicated to generating distinct cone photoreceptor types	Sachihiro SUZUKI
07-05	Investigation of the physical and functional links between the APC/C and the centrosome in *Drosophila*	Yuu KIMATA
07-13	Dissecting the route of human cellular reprogramming	Kazutoshi TAKAHASHI
07-13	Chromosome and spindle pole-derived signals generate an intrinsic code for spindle position and orientation	Tomomi KIYOMITSU
07-19	Systematic single-cell analysis of development: worm and beyond	Zhirong BAO
07-23	Molecular mechanisms of the initial kinetochore-microtubule interaction in early mitosis	Shinya KOMOTO

Date	Title	Speaker
08-27	Divergent tissue dynamics of neural and non-neural ectoderm in the zebrafish	Stephen YOUNG
09-03	Intersection of transcriptional and signalling activity for head morphogenesis in mouse embryos	Patrick TAM
09-18	How do misfolded proteins and altered RNA metabolism cause ALS?	Robert BALOH
10-03	The exit from ground state pluripotency	Austin SMITH
10-31	Identifying a novel small-molecule inhibitor of the giant AAA+ ATPase using fission yeast chemical genetics	Shigehiro A. KAWASHIMA
10-31	How to publish in *Science*	L. Bryan RAY
11-12	Annotation of the chicken W sex chromosome using RNA-seq reveals candidate female-determining genes	Craig SMITH
11-12	Angiogenesis in the zebrafish embryo: From single cell imaging to single cell functional analysis; a new approach	Markus AFFOLTER
11-13	Specification and patterning of the neural plate border - special focus on lens and olfactory placodes	Lena GUNHAGA
11-19	Making fate decisions at the exit of pluripotency: what ES cells tell us about the embryo	Alfonso MARTINEZ-ARIAS
11-22	Cellular and molecular mechanisms of lineage segregation during mesoderm development	Chaya KALCHEIM
11-26	Phylogenomics in the light of ever-growing sequencing data	Toni GABALDON
11-27	Transposon technologies towards iPS cell gene therapy	Akitsu HOTTA
11-30	Migrating transient neurons: organizing activity in patterning of the cerebral cortex	Alessandra PIERANI
12-04	From mechanical instabilities of epithelial tissues to morphogenesis, stem cell dynamics and cancerogenesis	Edouard HANNEZO
12-06	Systemic control of hematopoietic progenitors in *Drosophila*	Utpal BANERJEE
12-07	How can microtubules establish protein patterns at the cell wall?	Núria Taberner CARRETERO
12-10	Haploid ES cells as a tool for forward genetic approaches in mammals	Martin LEEB
12-10	Actin-driven chromosome transport in starfish oocytes	Masashi MORI
12-17	Genetic screens in haploid mouse ES cells to determine mechanisms of drug toxicity and resistance	Stephen PETTITT
12-25	*Cis*-element evolution of the *Dlx* genes as an underlying mechanism in toolkit gene co-option in vertebrate appendages	Kenta SUMIYAMA
12-28	Wnt/Dkk negative feedback loop regulates sensory organ size in the lateral line system of fish	Hironori WADA
12-28	Mechanisms of nuclear reprogramming by eggs and oocytes	Kei MIYAMOTO

About RIKEN

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

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

RIKEN Website

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

RIKEN Research

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

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

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On The Cover

Caudal view of *Drosophila* male terminalia at 24 hr after puparium formation. All nuclei (magenta) and cells in posterior compartment of each segment (green) are shown.
Image: Ayako ISOMURA, Laboratory for Histogenetic Dynamics

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