RIKEN Center for Developmental Biology

2010 Annual Report

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

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

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

Message from the Director

The end of 2010 brings us to the end of our first decade of research at the RIKEN Center for Developmental Biology, a decade that has seen well over 1,000 publications from nearly 50 laboratories and over a thousand research staff from dozens of countries that have made the CDB their home. Our work has spanned an exciting and broad range of fields, from fundamental aspects of developmental cell and molecular biology, to research into stem cell maintenance and differentiation, to systematic approaches to genetic regulatory networks, to fresh new insights into evolutionary phenomena. It has been an exciting time for developmental biology and associated fields, and we have been fortunate to play a part on the global scientific stage.

This home is not, however, intended to be a permanent one for the majority of our research programs, but rather an incubator and accelerator for talented young scientists to work in a collegial environment with a strong concentration of peers in related disciplines. Our policy has always been one of encouraging positive turnover, in which older labs continually transition outward to make way for the fresh ideas and approaches that new generations bring. In real terms, this means that the CDB is now in the process of rebirth and renewal, mirroring in our own structure one of the central themes of our research, as new labs focusing on mechanisms of growth control, sensory circuitry, and physical biology joined the institute in the past year.

Despite the transitional phase we have entered, research at the CDB remains highly productive, with numerous high profile scientific articles and awards attained by our staff in 2010. Highlights include new findings in the molecular machinery that harnesses forces at the cell-cell junction, techniques for steering pluripotent stem cells toward specific neuronal fates, studies of asymmetric cell division, and insights into the evolution of the insect wing. Our record for conducting leading research has won recognition for many of our investigators from organizations devoted to the advancement of the sciences in Japan and abroad.

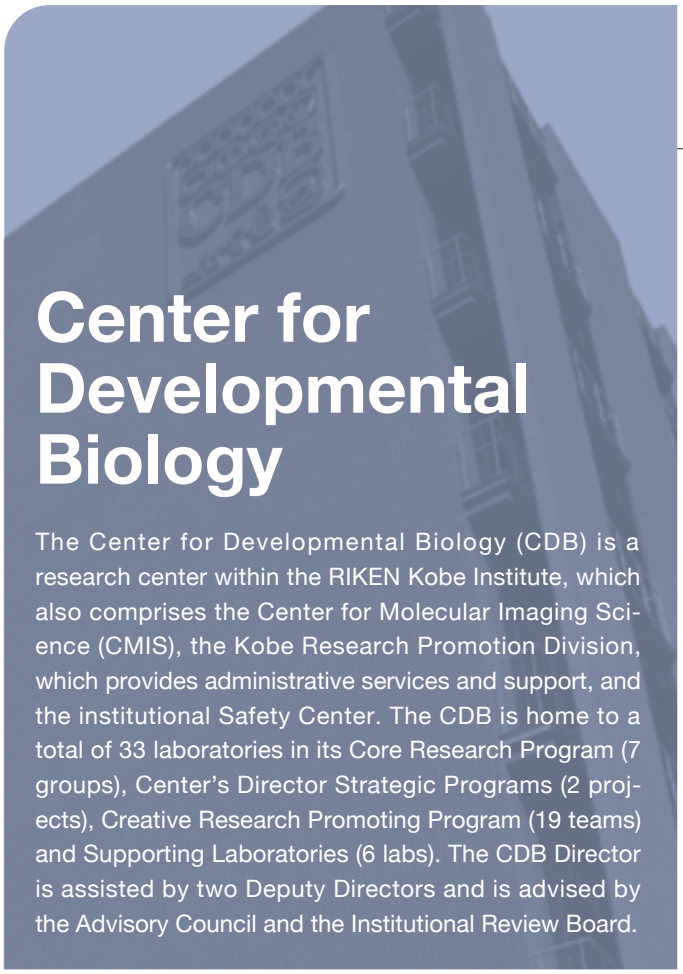
The CDB was also honored to receive visits by then Japanese Prime Minister Yukio Hatoyama and Her Royal Highness, Princess Srindhorn of Thailand in 2010, highlighting the high level of recognition our institute has begun to attract both in Japan and overseas. We strive to remain in the public eye at ground level as well, hosting numerous programs and events intended to promote a better understanding of science and more opportunities for communication between our research staff and the general public. Our annual Open House event received more visitors in 2010 than in any previous year, and our educational programs for students and high school teachers remain popular science learning events in the Kansai region of Japan.

Indeed, our location in the city of Kobe, in the heart of western Japan continue to provide opportunities for interaction and collaboration at the laboratory and institutional level. The RIKEN Kobe Institute, of which the CDB is the founding Center, is now home to the Center for Molecular Imaging Science (CMIS), which focuses on positron emissions tomography technology and applications, and the newly established Quantitative Biology Center (QBiC), which will work on computational and systematic approaches to fundamental biological questions of complexity and dynamics. In addition, RIKEN is readying the launch of its Next-Generation Supercomputer facility only a few minutes from the CDB, bringing a new dimension of computational power and expertise to the area. Kobe also boasts numerous biomedical and biotechnology companies, research institutes, and foundations, and the new Kobe City Medical Center is set to open in spring of next year, which will enhance the possibilities for translational research, very much in line with our original mission of helping to build a foundation in science for the field of regenerative medicine.

We can look back proudly on 2010 as another successful year for the institute, which could only be achieved through the dedicated and collaborative efforts of our research and administrative staff, and the support and cooperation of our colleagues around the world. As we enter our second decade, I would like to thank all those who have helped make the RIKEN CDB what it is today, and ask for your continuing support as we work to open up new frontiers in development.

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 Kobe Research Promotion Division, which provides administrative services and support, and the institutional Safety Center. The CDB is home to a total of 33 laboratories in its Core Research Program (7 groups), Center's Director Strategic Programs (2 projects), Creative Research Promoting Program (19 teams) 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

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 allows 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.

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

- Margaret Buckingham
  Institut Pasteur, France
- Steve Cohen
  EMBL, Germany
- Hiroshi Hamada
  Osaka University, Japan
- Haifan Lin
  Yale University, USA
- Yo-ichi Nabeshima
  Kyoto University, Japan
- Austin Smith
  University of Cambridge, UK
- Toshio Suda
  Keio University, Japan
- Yoshimi Takai
  Osaka University, Japan
- Patrick Tam
  Children's Medical Research Institute, Australia
- Christopher Wylie
  Cincinnati Children's Hospital Research Foundation, USA

Director

Deputy Directors

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 places great emphasis on cooperation and international collaboration.

- Neuronal Differentiation and Regeneration
  Hideki ENOMOTO M.D., Ph.D.
- Neocortical Development
  Carina HANASHIMA Ph.D.
- Vertebrate Axis Formation
  Masahiko HIBI M.D., Ph.D.
- Cell Lineage Modulation
  Toru KONDO Ph.D.
- Sensory Development
  Raj LADHER 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.
- Mammalian Molecular Embryology
  Tony PERRY Ph.D.
- Mammalian Germ Cell Biology
  Mitinori SAITOU M.D., Ph.D.
- Embryonic Induction
  Hiroshi SASAKI Ph.D.
- Cell Fate Decision
  Hitoshi SAWA Ph.D.
- Early Embryogenesis
  Guojun SHENG Ph.D.
- Developmental Genomics
  Asako SUGIMOTO Ph.D.
- Retinal Regeneration
  Masayo TAKAHASHI M.D., Ph.D.
- Genomic Reprogramming
  Teruhiko WAKAYAMA 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
  Shinichi AIZAWA Ph.D.
  - Genetic Engineering Unit
    Shinichi AIZAWA Ph.D.
  - Animal Resource Unit
    Kazuki NAKAO Ph.D.
- Electron Microscope
  Shigenobu YONEMURA Ph.D.
- Bio-imaging Laboratory
  Shigeo HAYASHI Ph.D.
  - Optical Image Analysis Unit
    Yuko MIMORI-KIYOSUE Ph.D.
- Genomics
  Fumio MATSUZAKI Ph.D.
  - Genome Resource and Analysis Unit
    Hiroshi TARUI 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

Visit by Prime Minister Hatoyama

January 17

Japan's then Prime Minister, Yukio Hatoyama, visited the RIKEN CDB as part of a visit to Kobe's biomedical research park. He was greeted by CDB Director Masatoshi Takeichi, Group Director Yoshiki Sasai, and Team Leader Masayo Takahashi, and introduced to the Center's work using embryonic and induced pluripotent stem cells, as well as fundamental developmental and regenerative phenomena.

RIKEN Kids' Lab

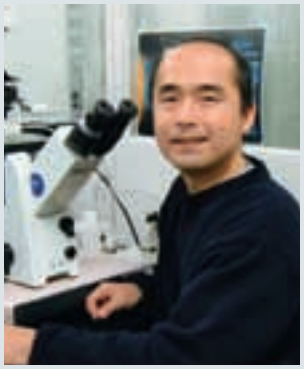
March 23

The CDB announced the opening of its "RIKEN Kids Lab: The wonders of development and regeneration" in the Kobe Science Museum. This child-friendly space creates an environment for discovery and fun, with graphic and hands-on activities relating to topics such as the development of the body from a single cell, and the exciting future promise of stem cells in medicine.

Frontiers in Organogenesis

March 23 – 25

The RIKEN CDB hosted its eighth annual symposium, "Frontiers in Organogenesis," bringing together nearly 200 participants from 16 countries to discuss recent advances in the study of the formation of functional, complex biological structures. The meeting featured a wide range of themed sessions, including patterning, imaging, evolution, and the dynamics of organogenic phenomena.



Team Leaders win Ministry Prizes

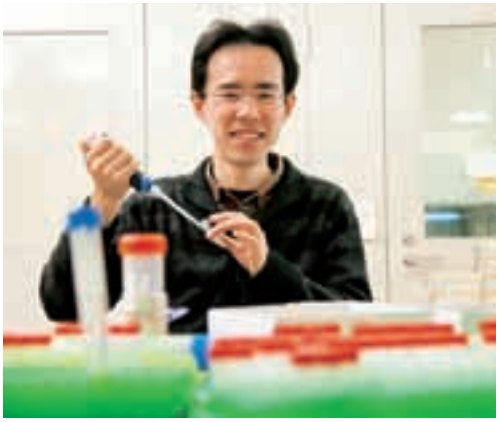
April 5

The Ministry of Education, Culture, Sports, Science and Technology (MEXT) announced that CDB Team Leaders Teruhiko Wakayama and Jun-ichi Nakayama has been awarded Commendations for Science and Technology for their contributions to the advancement of their fields. Wakayama received the Prize for Science and Technology for Research for his work in somatic cell nuclear transfer, and Nakayama received the Young Scientist's Prize for his research on how dynamic structural changes to chromatin regulate gene expression.

Takeshi Imai joins as Team Leader

July 1

The Center welcomed its newest Team Leader, Takeshi Imai, who heads the Laboratory for Sensory Circuit Formation. His research will focus on the organization of the olfactory system in mouse.



CDB postcards a hit with developmental biologists

July 2

The RIKEN CDB debuted a set of decorative postcards incorporating motifs from Japanese art and fashion into depictions of 16 organisms used in developmental biology. The printed cards were distributed at scientific meetings and are no longer available, but the data can be downloaded from the CDB website: www.cdb.riken.jp.

2010 Highlights

Intensive Lecture Program for Grad School Affiliates

August 19 – 20

Every summer, the Center holds a two-day program for interested students enrolled in affiliated graduate and medical schools in the Kansai region. Nearly 150 students gathered for talks and demonstrations of some of the advanced methods and technologies used by CDB labs.

Summer school for HS students

August 24, 26

The CDB held its annual summer school program for area high school students. The pair of one-day programs, consisting of lectures and demonstration experiments, focused on *C. elegans* research. More than 20 young learners joined in practicing techniques for observing, collecting and working with these tiny organisms.

New Research Unit Leader, Tatsuo Shibata

October 1

The Laboratory for Physical Biology was launched under the leadership of Tatsuo Shibata, and as part of the Center Director's Project on Systems Biology. The lab will use concepts and methodology from mathematical in the study and elucidation of emerging questions in biology.

Lessons in development for high school teachers

October 2 – 3

The CDB held its third recurrent course in developmental biology for teachers of high school biology in conjunction with the Japanese Society of Developmental Biologists. This program is intended to give teachers an opportunity to learn hands-on techniques in embryology that can be shared with their classes.

Visit by HRH Princess Sirindhorn of Thailand

October 11

Princess Maha Chakri Sirindhorn of Thailand paid a visit to the CDB during a tour of the city of Kobe. After a brief introduction to the Center's history, research mission, and organization, Her Royal Highness heard presentations on the work being done in the Laboratory for Retinal Regeneration.

RIKEN Kobe Open House

November 20

This year saw more visitors to the Center's annual Open House than ever before, with more than 1,700 people turning out to enjoy talks, demonstrations and exhibits on development, regeneration, evolution and stem cells, as well as tours of the nearby Center for Molecular Imaging Science and Next Generation Supercomputer.

Group Director Shigeru Kuratani wins science prizes

November 10

Shigeru Kuratani was awarded the Hyogo Prefectural Science Prize in recognition of his work on the evolutionary mechanisms that led to the innovation of the turtle carapace, and the Alexander Kowalevsky Medal, granted annually by the St. Petersburg Society of Naturalists (Russia) for contributions in the fields of evolutionary developmental biology and comparative zoology.

Annual Retreat in Sasayama

November 29 – 30

Scientific and administrative staff gathered for an overnight stay in the mountains of Sasayama for active discussion of the latest developments in CDB research. This once-a-year event serves as a forum for unfettered exchange among the Center's many labs.

A pair of paths to the insect wing

Ai AKIMOTO-KATO, Nao NIWA

The emergence of the wing is a critical innovation that underlies the evolutionary success and diverse speciation of insects. Two rival hypotheses have been proposed to explain the emergence of the first insect wings, one suggesting that it arose *de novo*, the other that it represents a modification of a pre-existing structure. Given the scarcity of early and intermediate examples in the fossil record, the resolution of this question will require a forensic examination of wing-related genes in basal insects and their relatives.

Work led by Nao Niwa and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) now suggests a possible solution based on genetic evidence that collates the two competing propositions into a single theory of insect wing origins. Working with scientists from Nagoya and Shinshu Universities and the University of Tsukuba, Niwa proposes that the expression of wing-related genes in limb branch primordial regions drifted over time in such a way as to integrate into the dorsal body wall, which already showed potential for flat sheet-like outgrowth.

Niwa's study was born from a comparison of the advantages and limits of two previous models of wing evolution. The first, referred to as the paranotal hypothesis, posits that the wing emerged as a novel extension of the thoracic tergum (the dorsal body wall), while its alternate, the limb branch hypothesis, suggests that wings are actually modifications of dorsal limb branches that were already in place. However, neither of these models can fully explain the morphological features of the insect wing on its own.

The Hayashi group used knowledge of the anatomy and developmental genetics of a winged insect to draw comparisons with two key insect species: a wingless basal insect (the bristletail *Pedetontus unimaculatus*) and

Niwa N, et al. Evolutionary origin of the insect wing via integration of two developmental modules.
Evolution & Development 12.168-76 (2010)

a primitive winged insect (the mayfly *Ephoron eophilum*). These models for comparison have specific morphological traits that have previously been suggested to represent forerunners to the early wing via adaptations to dorsal limb branches. The bristletail develops a rod-like outgrowth known as a stylus from thoracic and abdominal appendages, while the mayfly nymph shows articulated tracheal gill structures associated with abdomen. However, little is known about the genetic aspects of their specification and growth.

The fruit fly *Drosophila melanogaster*, in contrast, is one of the most widely studied organisms in biology, and we have a detailed understanding of its wing growth at the genetic level. The gene *wingless (wg)* plays key roles inducing the wing disc primordium in the embryo and later promoting growth of the wing pouch. The wing does not form in the absence of *wg* activity, as indicated by the gene's name. *vestigial (vg)* also plays a role in growth control and specification of wing tissue. *apterous (ap)* specifies the flat shape of the wing by controlling subdivision of the wing into dorsal and ventral compartments.

Scanning electron micrograph of the bristletail, *Pedetontus unimaculatus*, showing stick-like stylus attached to the base of limbs, and tergal plates covering the lateral side of the body. Expression analysis of wing-related genes revealed the two structures share key properties of wing development, suggesting a dual origin of the insect wing.

Niwa et al. analyzed gene expression patterns in the primordia of bristletail stylus and mayfly tracheal gill, and found that *wg* and *vg* are expressed, results that are consistent with the limb branch hypothesis. However the expression of *wg* and *vg* is less regionalized than it is in the *Drosophila* wing disc, suggesting that positional information required for dorsal-ventral subdivision is missing. Interestingly, *wg* and *vg* expression was also detected in the tergum of mayfly and bristletail, accompanied by *ap* expression. In bristletail, the *ap* expression domain formed a sharp border, marking the margin of the tergal extension that forms the prominent flat lateral extensions. The expression of *wg*, *vg*, and *ap* in the tergum supports the paranotal hypothesis. Given these new insights into the paranotal and limb branch hypotheses, the authors propose a dual role for *wg-vg* signaling; an inductive role in the dorsal limb branch and growth-promoting role in the tergal extension, where *ap* specifies the tergal margin in the dorsal body wall. They speculate that the inductive signal may have drifted dorsally over evolutionary time toward the outgrowth of tergal extension, and that a synergistic effect may account for the rapid advent of the wing once the two intersected. If this is correct, it would indicate that the two current theories of wing origins are both partially correct, but incomplete, and that the early wing may in fact have arisen from a combination of paranotal and limb branch components.

"The evolutionary origin of the insect wing has fascinated many biologists," says Hayashi. "I am very pleased that we can now propose a model that provides a plausible explanation for previously unanswered questions and serves as a basis for future molecular studies."

Epigenetic factors hold cell fate to the script

Yukimasa SHIBATA

DNA famously plays a central role in determining the body's form and function, typically through the transcription of genes and the translation of these transcripts to proteins. Controlling this expression of genes such that they are only "switched on" at the right times and in the right places requires reliable and precise regulatory mechanisms. The results of such regulatory activities result in the establishment and maintenance of cells with specific functional identities, or fates, but how is this spatiotemporal transcriptional control achieved?

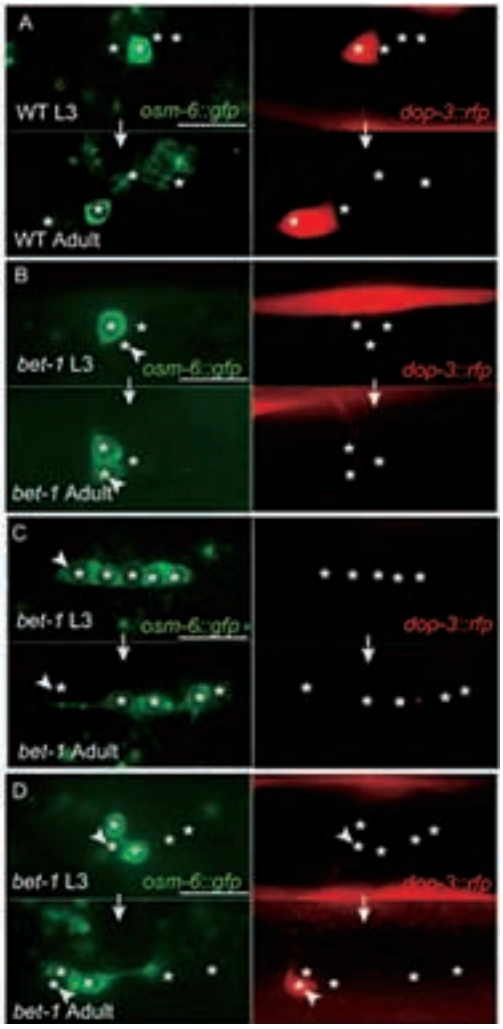
Yukimasa Shibata and others in the Laboratory for Cell Fate Decision (Hitoshi Sawa, Team Leader) were interested in possible roles for epigenetic mechanisms in the upkeep of cell fate in *C. elegans*, as a body of previous work had pointed to functions for such phenomena as DNA methylation and histone modifications in cell type-specific transcriptional regulation in various model systems. In a study published in the journal *Development*, they showed that a pair of protein factors implicated in histone acetylation plays crucial roles in setting up and stably maintaining cell fates. Acetylation is one of a number of modifications known to modify histone protein activity and configuration, and to affect the expression of genes in associated regions of DNA within chromatin complexes.

The team identified the first of their molecules of interest in a mutation screen, which gave the first hint that the histone-associated bromodomain factor BET-1 might play a role in cell fate stability. A mutant allele yielded phenotypes in which cell-fate maintenance is disrupted. The result of these aberrations was seen, for example, in the mistaken adoption of inappropriate cell fates by progeny of tail neural precursor cells lacking *bet-1* function, with the result that neuroblasts gave rise to hypodermal cells (in wildtype animals, these neuroblasts produce neural cells).

Shibata Y, et al. Double bromodomain protein BET-1 and MYST HATs establish and maintain stable cell fates in C. elegans. *Development* 137.1045-53 (2010)

In an extremely wide range of cell lineages, loss of BET-1 resulted in the ectopic expression of cell type-specific markers and more than usual numbers of specific type of cells. In at least one case, they traced this effect to the regulation of the expression of a cell-fate determinant by BET-1, a general mechanism that they suspect may be common to other lineages as well.

Armed with evidence of the ability of *bet-1* mutation to cause cell fate transformations in the somatic, germ, and neural lineages, the Sawa team suspected that this might also be true in other cell populations. To test this possibility, they observed the expression of marker genes over time in the hopes of gleaning insights into the effects of loss of *bet-1* function on cell fate stability. Again, they found that differentiated cells switch between fates in mutants, but not in wildtype. Further tests using heat-shock treatment to conditionally rescue the phenotype suggested that BET-1 establishes and maintains stable cell fates.



Comparison of wildtype (A) and *bet-1* mutants (B–D) reveals altered numbers of cells expressing cell type-specific markers genes.

Pursuing the role of histone acetylation in cell fate establishment and maintenance, Shibata next looked at a family of acetyltransferases known as MYST HATs, and found that blocking their function produced phenotypes closely resembling those of the *bet-1* mutants. Distribution analyses showed that the localization of *bet-1* within the nucleus depends on MYST factors, highlighting a possible functional relationship between the two.

These findings shed new light into roles for epigenetic processes in establishing and maintaining cellular identity, possibly through the regulation of the expression of cell fate determinant genes. As analogs of BET-1 and MYST proteins have been implicated in expression of developmental genes in other organisms, such as the fruit fly *Drosophila melanogaster*, these factors may have a more generally conserved function in cell fate maintenance and the prevention of wayward differentiation.

Anchors down: New roles for Wnt and Src in nuclear localization

Kenji SUGIOKA

When cells divide, they often produce daughters with different identities, or “fates.” This asymmetric division is an intricate process involving the sorting of intracellular components to different sides of the mitotic cell so as to ensure that they are allocated differentially to its progeny. A growing body of work has revealed that such polarization relies on signals both from within and outside of the cell, and typically involves mechanical action by elements of the mitotic machinery, such as centrosomes. The roundworm *C. elegans* has served as one of the most powerful systems for studying these phenomena, shedding light on the mechanisms that underlie asymmetric cell division.

Research by Kenji Sugioka in the Laboratory for Cell Fate Decision (Hitoshi Sawa, Team Leader) examined how the positioning of daughter cell nuclei, a critical event in asymmetric division, is regulated by signaling proteins. Published in the journal *Genes to Cells*, this work reveals roles for the Wnt and Src signaling pathways in ensuring that cells in the early embryo split up their bequests appropriately.

Very early in development, when the roundworm embryo is made up of only four cells, many fundamentally important decisions are being made that will determine whether the embryo develops in the normal fashion. One of these four, known as the EMS cell, is the source of the lineages that give rise to tissues such as muscle, gut, and the nervous system. Given its importance, the asymmetric division of EMS has been intensively studied at the structural and molecular levels, and it is known that this activity is directed by cues from its neighboring P2 cell in the form of MOM-2 (Wnt) and other signals.

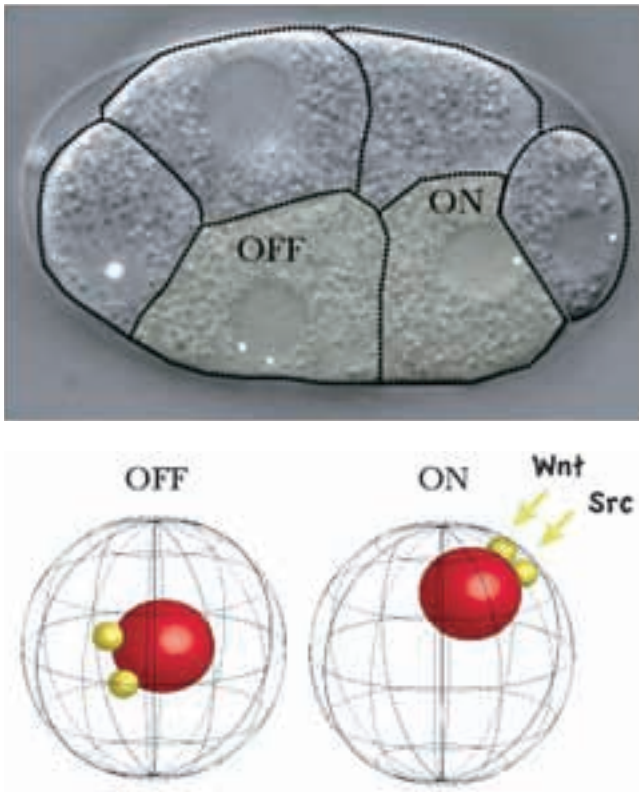
Sugioka used 4D live imaging to watch as embryonic cells divided, and made an intriguing observation. First, the centrosomes of the anterior and posterior daughters of the EMS cell behaved differently, with the posterior centrosomes generally moving, along with the nucleus, to anchor in the posterior cortex, while their anterior counterparts rarely did so. Looking at other asymmetrically dividing cells, he found that this posterior nuclear anchoring was widely conserved.

Moving back to the EMS cell, Sugioka began to search for a mechanism. Previous work had shown that the EMS cell receives signals from its posterior neighbor, the P2 cell. These signals are mediated by at least two distinct pathways: MOM-2/Wnt through the Wnt/ß-catenin pathway, and MES-1 through the tyrosine kinase SRC-1.

Sugioka K and Sawa H. Regulation of asymmetric positioning of nuclei by Wnt and Src signaling and its roles in POP-1/TCF nuclear asymmetry in Caenorhabditis elegans. *Genes Cells* 15.397-407 (2010)

To determine whether these signals play a role in nuclear anchoring, Sugioka looked for abnormal nuclear localization in posterior daughters of mutant or RNAi-treated cells, and found that loss of Wnt or SRC function was associated with anchoring defects.

But these factors are also involved in the orientation of mitotic spindles, meaning that the effect on nuclear anchoring could be secondary. To rule this out, he quantitatively monitored spindle orientation in three dimensions, and found that even when these structures were oriented within the normal range in MOM-2 or SRC-1 RNAi embryos, nuclear cortical anchoring remained abnormal.



Nuclear localization in asymmetrically dividing cells of the early *C. elegans* embryos (yellow). The cortical anchoring of the nucleus is dependent on the activity of centrosomes (white) regulated by Wnt and Src signaling.

What then is the function of anchoring the nucleus to the posterior cortex? The team speculated it might have a relationship with asymmetric localization of another factor, POP-1, which is linked with distinct transcriptional activity and cell fate determination. By measuring the proximity of the nucleus to the cell cortex in mutants lacking a protein required for centrosomes attachment to the cortex, they found that POP-1 asymmetry could be maintained even in the absence of nuclear anchoring. On looking more closely, using mutants in which asymmetric cell fate determination and POP-1 localization were only partially defective, he was able to show a strong correlation between the proximity of the nucleus to the cortex with POP-1 asymmetry, suggesting that while nuclear cortical attachment itself is not essential, it may support normal asymmetry by holding the nucleus close to the cell cortex.

“We know that, in many organisms, the nucleus is often found off-center, but the underlying mechanisms and significance are for the most part still unknown,” says Sugioka. “By showing how an extracellular signal can drive nuclear localization, and how the positioning of the nucleus near the cortex makes it more accessible to signals, we have added what I think will be an important piece to this puzzle.”

New chromatin factor in DNA repair pathway

Noriyo HAYAKAWA, Tomohiro HAYAKAWA, Yasuko OHTANI

Maintaining the integrity of the genome is a mission-critical role for the cell's many DNA repair mechanisms. Errors of varying severity can creep into the code in the form of substitutions, deletions, reversals and extra copies, but ruptures of the double helix itself can be even more catastrophic. Such double-stand breaks set off alarms, as they can lead to massive rearrangements of long stretches of the genome, and eukaryotes have evolved an arsenal of detection, repair, and abort mechanisms to deal with this contingency. Homologous recombination is one tactic used to salvage broken DNA, by making a template for the damaged sequence from a sister chromatid; a very similar process takes place during meiosis.

Studies of homologous recombination have revealed a number of the molecular factors that underlie the process, with the genes *BRCA1* and *BRCA2* (whose loss of function is a well-known predictor of breast cancer) playing central roles. The proteins encoded by these genes are bound together in a complex with other factors known as RAD51 and PALB2, which respectively form single-strand filaments of DNA and protein that are used in initiating the recombination event, and help recruit BRCA2 to chromatin containing damaged DNA. The chromatin-side target of the BRCA complex, however, remains unclear.

A study by Tomohiro Hayakawa of the Laboratory for Chromatin Dynamics (Jun-ichi Nakayama, Team Leader), Fun Zhang in Paul Andreassen's lab at Cincinnati Children's Research Foundation, and others added a new link to that chain, showing that the chromodomain protein MRG15 binds directly to PALB2, and is required for homologous recombination. Published in *The Journal of Cell Science*, this work provides new insight into how the DNA damage response network interacts with the genome through its nucleoprotein packaging.

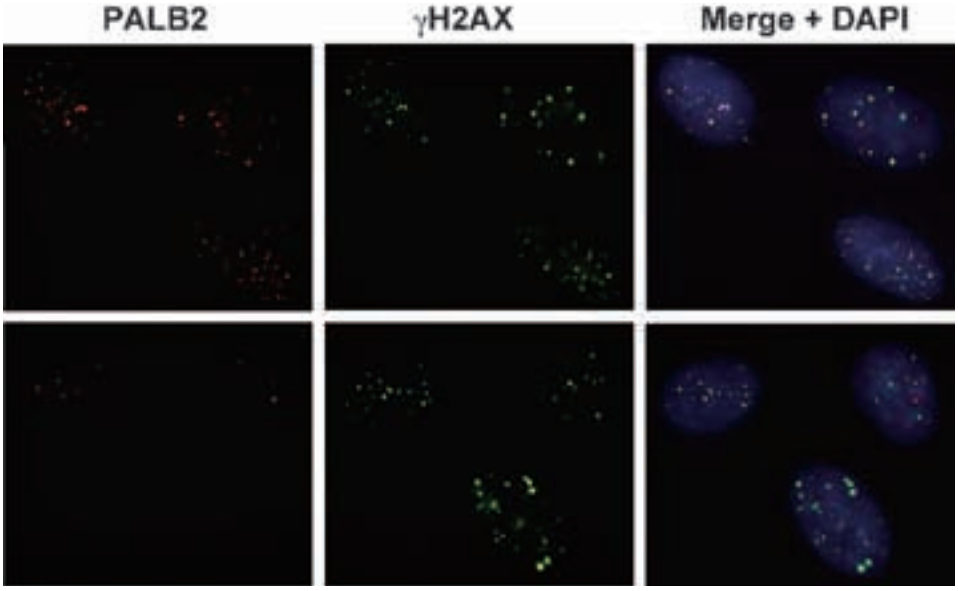
Hayakawa T, et al.MRG15 binds directly to PALB2 and
stimulates homology-directed repair of chromosomal breaks.
J Cell Sci 123.1124-30 (2010)

Knowing that a molecular relative of MRG15 complexes with PALB2, Hayakawa and Zhang started by testing for a possible functional link using immunoprecipitation, and found that the two proteins show affinity for each other. Further experiments using Western blotting revealed that MRG15 indeed interacts with the entire BRCA complex.

Testing next for function, the team found that MRG15 plays a vital role in homologous recombination; depletion of the protein caused a significant drop in the efficiency of this DNA repair mechanism. Interestingly, MRG15 also appeared to be tied to cellular resistance to mitomycin C (MMC), a potent DNA crosslinker that activates not only homologous recombination, but multiple DNA repair pathways. MMC resistance is mediated by the BRCA complex, and Hayakawa and Zhang found that loss of MRG15 function caused hypersensitivity to the crosslinking agent, similar to the phenotype when either BRCA2 or PALB2 are knocked down.

The recruitment of the BRCA complex to the site of DNA damage has been shown to rely on PALB2, so the researchers looked for a possible role for MRG15 in this targeting. Using immunoblotting to look at BRCA complex binding to chromatin in wild type and irradiated cells in which MRG15 was expressed normally or silenced by siRNA, they found that the DNA repair complex was associated with chromatin at much higher frequencies in untreated cells (see Fig.). They speculate that once PALB2 has attached to MRG15 in the chromatin, it then recruits the other elements of the BRCA complex, enabling homologous recombination and possibly, other DNA repair mechanisms to proceed.

“Several lines of study have shown how dramatically chromatin structure can change over the course of development,” says Hayakawa, “but we still know very little about what roles MRG15, PALB2, and the BRCA complex might play in such rearrangements. We’re hopeful that by studying these interactions we will gain a better understanding of developmental processes.”



PALB2 (red) localizes with damaged DNA (green) in cells expressing MRG15 (top), but not when its function is blocked (bottom).

Genetic controller of cell senescence found

Yuki KUJURO

One of the main functions of somatic (or adult) stem cells is to keep the body's systems in working order by generating replacements for cells that have been damaged or aged. As the body grows older, however, its stem cells gradually lose their ability to self-renew through cell division, a phenomenon known as cell senescence. A number of cell cycle mechanisms have been linked to this form of cellular aging, but its molecular basis remains poorly understood.

A recent report by Yuki Kujuro and others in the Laboratory for Cell Lineage Modulation (Toru Kondo, Team Leader) identifies a new genetic inducer of cell senescence in the nervous system. The gene, *Ecrg4*, encodes a secreted protein that triggers the degradation of cell cycle factors in oligodendrocyte progenitor and neural precursor cells, marking it as an important link in the aging of the brain.

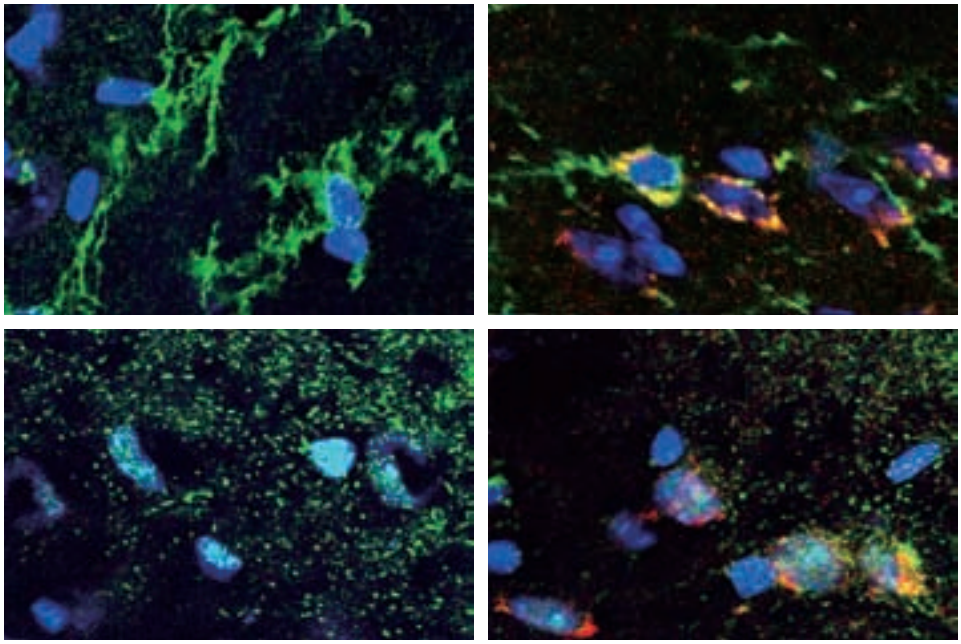
Kujuro began by observing the aging process in oligodendrocyte progenitor cells (OPCs), and found a number of irreversible changes in their morphology and internal regulation that take place when they are cultured in a high concentration of serum, which serves as an experimental surrogate for in vivo aging. Looking next at changes in gene expression, she found more than a thousand were up- or down-regulated on high serum culture, more than three hundred of which remained in that state even after the culture medium was switched back to a normal serum level.

Among the genes caught in this screen, *Ecrg4*, caught the team's interest for several reasons. The difference between its expression levels in senescent and non-senescent cells was greater than for any other gene, and its expression was also activated in senescing mouse embryonic fibroblasts, shown for the first time in this report. In addition, its expression is down-regulated in tumors, which are notorious for their age-defying ability to self-renew indefinitely.

To test for function, the team transfected rat OPCs with *Ecrg4* and watched for signs of senescence. Compared to control populations, the transfected cells showed significantly higher activation of senescence markers. Subsequent experiments showed that the gene's effect is likely achieved through a combination of the degradation of cell cycle factors, cyclin D1 and D3, and dephosphorylation of Rb.

Kujuro Y, et al. Esophageal cancer-related gene 4 is a secreted inducer of cell senescence expressed by aged CNS precursor cells. *Proceedings of the National Academy of Sciences.* 107.8259-64 (2010)

Having established a solid case for the involvement of *Ecrg4* in OPC senescence in vitro, Kujuro next looked at the mouse brain to see if it had similar effects in vivo. She found that while the gene showed only weak expression in the brains of young mice, it was much higher in aged brain, specifically in the regions where OPCs and neural precursor cells reside. Interestingly, *Ecrg4* was upregulated even in terminally differentiated neurons, which have ceased dividing and should therefore be immune to senescence.



Expression of Ecrg4 (red) is induced in oligodendrocytes (green, top panels) and neural stem cells (green, bottom panels) as aging progresses from 2 months (left) to 20 months (right). Nuclei are stained blue.

"The question of 'Why do we age?' is a longstanding mystery, and since the discovery of stem cells, people have speculated that it may be due in part to natural wear and tear affecting their ability to function," says Kondo. "In this study, we identified one mechanism relating to the aging of neural stem and progenitor cells, and we're hopeful that this will lead us to a better understanding to diseases of the nervous system."

2010 Events

The RIKEN CDB strives to engage with the public through a variety of media, including its website, media coverage and direct interactions. This final form of outreach takes the form of frequent site visits by groups and delegations from around the Japan, and in our annual Open House.

In addition to these public engagement activities, the Center also organizes events for bringing scientists together outside of the laboratory environment, such as its annual Retreat.

Kagaku Ichiba and RIKEN Kids' Lab

In February, the RIKEN CDB jointly organized a science exchange event, "Kagaku Ichiba," with the Kobe Science Museum, where it was held. This event, supported by a grant from the Japan Science and Technology Agency (JST), was held in an effort to promote science to the general public and attended by people of all ages, and featured lectures and hands on exhibits for kids of all ages.

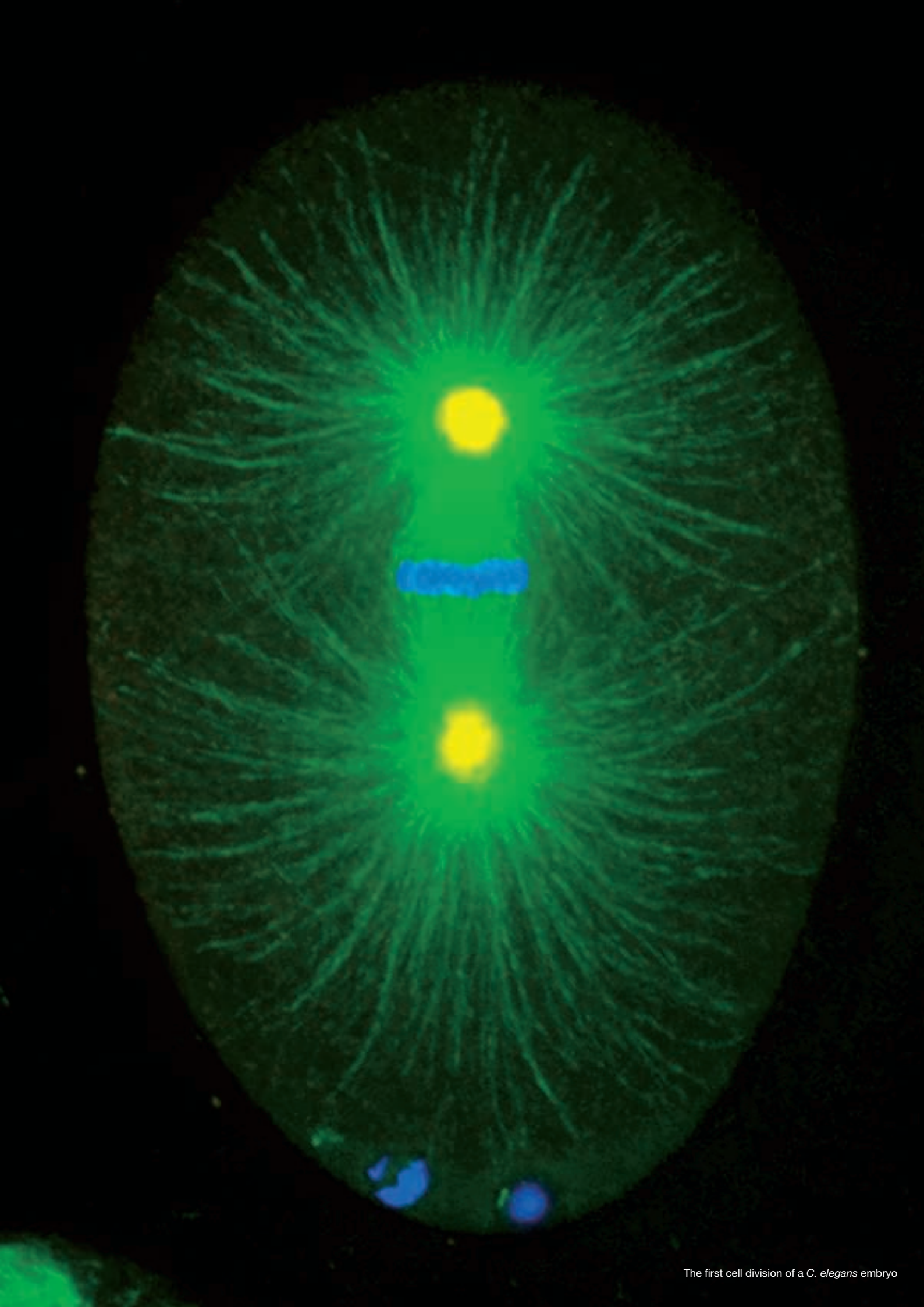
In March, the CDB opened the RIKEN Kids Lab in the Kobe Science Museum. This space provides an environment for discovery and fun, with graphic and hands-on activities relating to topics such as the development of the body from a single cell, and the exciting future promise of stem cells in medicine. Additional exhibits include an introduction to green fluorescent protein (GFP) and the fantastic regenerative ability of flatworms known as planarians.

2010 Open House

The 2010 CDB Open House, held in conjunction with the RIKEN Kobe Institute's Center for Molecular Imaging Science and the RIKEN Next Generation Supercomputer R&D Center, was the most well-attended event in the CDB's history, with over 1700 visitors on a single day in November. The event featured a commemorative talk by Kobe Institute and CDB Director, Masatoshi Takeichi, marking ten full years of the Center's history, and numerous open laboratories, where PIs, postdocs, technicians and students welcomed the curious and explained their work. Themes for this year's special exhibits included stem cells and regenerative medicine, and cell and body morphology. For younger visitors, a number of games and arts and crafts activities were available.

2010 Retreat

The CDB held its annual retreat for its research staff in the mountain setting of Sasayama. CDB lab leaders, research scientists and student trainees gathered in a closed meeting to share and discuss the latest developments from their labs, and to get better acquainted through social activities and casual conversation away from the bench. This year, CDB Advisory Council member Stephen Cohen of the Institute of Molecular and Cell Biology in Singapore joined the Retreat as well, adding a voice of broad international scientific experience.



The first cell division of a *C. elegans* embryo

Vertebrate Body Plan

Shinichi AIZAWA Ph.D.

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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. In January 2007, he was appointed President of the Japanese Society of Developmental Biologists. He also serves as editor for the journal, *Mechanisms of Development.*

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Publications

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

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

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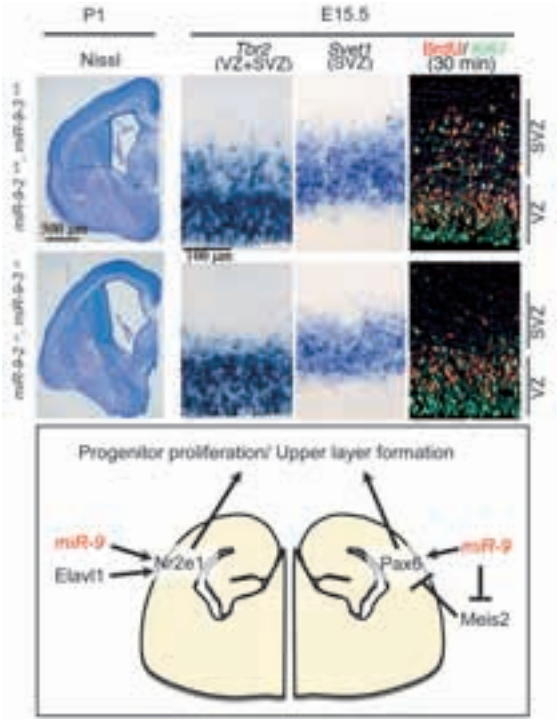
All vertebrate species share certain features in the development of the brain, in that all vertebrate brains comprise four regions—telencephalon, diencephalon, mesencephalon and metencephalon (or cerebellum)—an organizational trait that is thought to have appeared with the advent of the vertebrate lineage. 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.



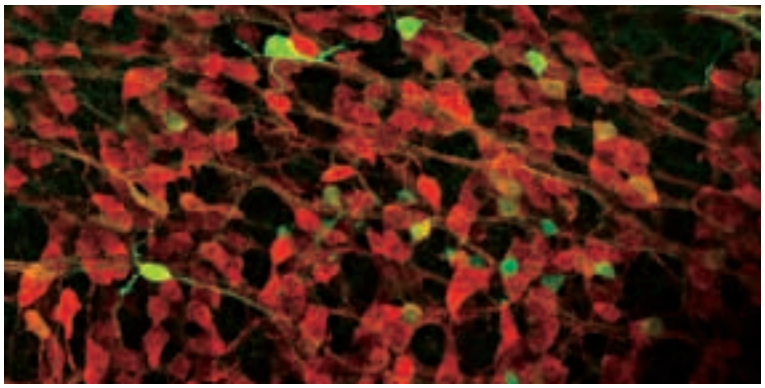
microRNA-9 is required for neural progenitor proliferation and upper cortical plate formation: Together with Elavl1, microRNA-9 targets the Nr2e1 mRNA 3'UTR to enhance its expression. microRNA-9 also indirectly inhibits Pax6 expression by suppressing Meis2 expression. Both Nr2e1 and Pax6 are essential for upper cortical plate formation.

Neuronal Differentiation and Regeneration

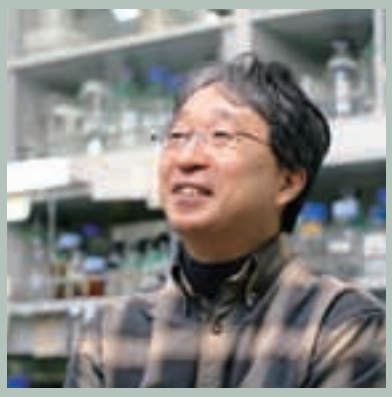
The architecture of the nervous system, which underlies much of the dynamism of animal life, is frequently described as complex, but closer examination reveals great beauty as well. The construction of this exquisite and elaborate structure involves the birth, growth, and death of countless individual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

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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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Publications

Uesaka T, et al. Neural precursor death is central to the pathogenesis of intestinal aganglionosis in Ret hypomorphic mice. *J Neurosci* 30. 5211-8 (2010)

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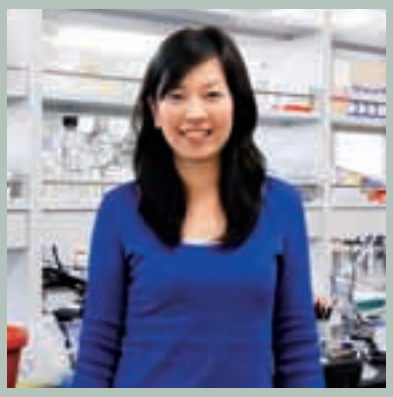
Neocortical Development



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 post-doctoral fellow in the laboratory of Eseng Lai at Memorial Sloan-Kettering Cancer Center, Cell Biology Program from 1999 to 2002, where she was engaged in developmental neuroscience. She moved to Skirball Institute Developmental Genetics Program in 2002 in Gord Fishell's laboratory where she extended her research to cell specification in the neocortex. She was appointed team leader at the CDB in September 2007.

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Publications

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

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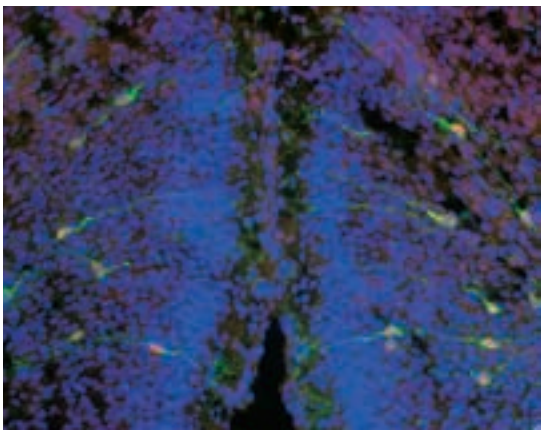
The neocortex, by far the most complex structure of all vertebrate brain systems, is organized into cytoarchitectonic groups of neurons that process unique sensory inputs such as pressure, light, and sound. Nonetheless this highly ordered structure is generated from a relatively simple sheet of neuroepithelium 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 establish cytoarchitectonic boundaries? 3) To what extent does the refinement of functional areas rely on environmental inputs? To investigate these questions we utilize the 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 repressors that function cell-autonomously to prevent neurons from acquiring an earlier neuronal phenotype. These results suggest that cortical intrinsic programs in which neuron fate is established by temporal changes in gene expression may have been 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.



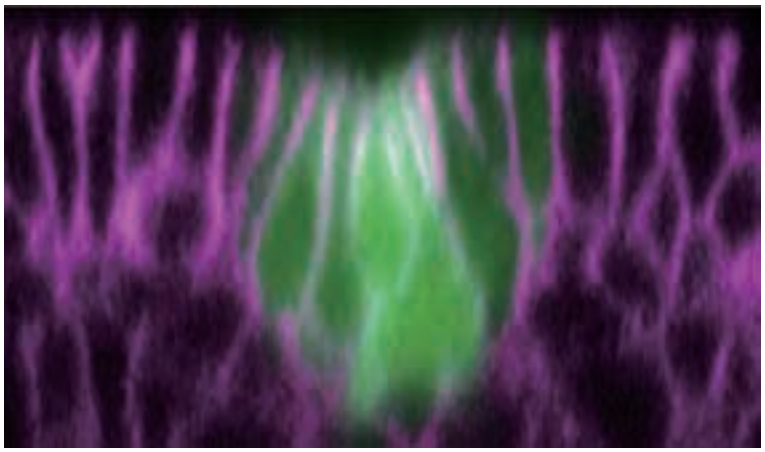
Recruitment of late-born neocortical neurons expressing membrane-targeted GFP (green) and nuclear lacZ (red) to the motor cortex.

Morphogenetic Signaling

The main research interest in my lab is focused 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 and spermatids, and higher level organization in insect limbs.



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 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.

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 postdoctoral research associate in Matthew Scott's lab at the University of Colorado before returning to Japan to work in the National Institute of Genetics. He became an associate professor at the same Institute in 1994, and professor in 1999. Also in 1999, he received the incentive prize of the Genetics Society of Japan for Genetic Studies on *Drosophila* Development. He was named group director of the Morphogenetic Signaling research group at the RIKEN CDB in May 2000. His current research interests are dynamic aspects of cell adhesion, cell migration and cell morphogenesis in *Drosophila*.

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Publications

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

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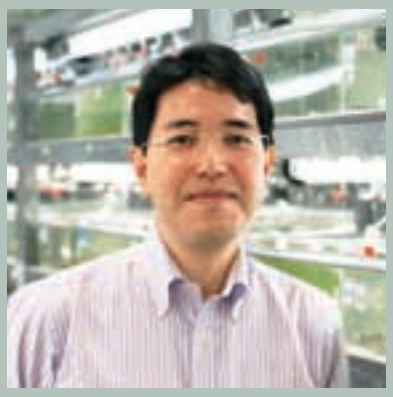
Vertebrate Axis Formation



Masahiko HIBI M.D., Ph.D.

Masahiko Hibi received his M.D. from Hiroshima University School of Medicine in 1988, and his Ph.D. from Osaka University in 1992. From 1992 to 1995, he worked as a postdoctoral fellow in Michael Karin's lab in the University of California, San Diego Department of Pharmacology, and then returned to Japan as a research associate in the Division of Molecular Oncology at Osaka University Medical School. He was appointed associate professor in the same division in 1999, where he remained until he assumed his position as team leader at the RIKEN CDB. He took a professorship at Nagoya University in 2009.

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Publications

Tanabe K, et al. Atypical PKC regulates primary dendrite specification of cerebellar Purkinje cells by localizing Golgi apparatus. *J Neurosc*.30. 16983-92 (2010)

Shimizu T, et al. Zinc finger genes *Fezf1* and *Fezf2* control neuronal differentiation by repressing *Hes5* expression in forebrain. *Development* 137. 1875-85 (2010)

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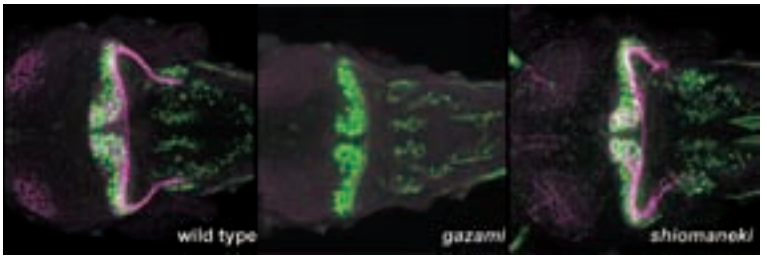
During early vertebrate development, the embryonic dorso-ventral (DV) and anterior-posterior (AP) axes are established through a series of inductive signals that prefigure and determine the concerted movements and differentiation of a group of cells. The dorsal organizer plays a major role in the process of axis formation. In amphibian and teleost fish embryogenesis, the program for dorsal organizer formation begins soon after fertilization. In these species, dorsal determinants are believed to be initially localized to the vegetal pole and later transported to the dorsal side of the embryo.

Although the molecular nature of the dorsal determinants has not been elucidated, they are known to activate the canonical Wnt pathway and thereby lead to the expression of genes involved in the induction of the dorsal organizer. The dorsal organizer generates secreted signaling molecules that participate in the generation of the DV axis in the mesoderm and endoderm, and the formation of the neuroectoderm. We are working to identify the molecules that are involved in the formation and function of the dorsal organizer. This search led us to identify the protein Sizzled as a negative feedback regulator of BMP signaling that cooperates with the dorsal organizer protein Chordin to regulate DV axis formation. We also found that Syntabulin, a linker of the kinesin I motor protein, is involved in microtubule-dependent transport of dorsal determinants.

Neural patterning and neurogenesis as a model of cell fate determination, a process that is linked to axis formation, is also a question of interest to our team. Neuronal tissues are generated in a stepwise manner in vertebrates; these steps include neural induction, AP patterning and neurogenesis. In amphibian and teleost embryos, neuroectoderm is initially induced by the dorsal organizer proteins. The induced neuroectoderm is anterior in character and is subsequently subjected to posteriorization and regionalization. Accordingly, the central nervous system becomes highly ordered along the AP axis, which is regionalized to forebrain, midbrain, hindbrain and spinal cord compartments in a head-to-tail direction. Our studies have revealed that two groups of genes play important roles in the AP patterning of neural tissue. The zinc-finger genes *Fezf1* and *Fezf2* are expressed in the anterior forebrain and control the AP patterning of forebrain by repressing the caudal forebrain fate, while the *caudal*-related genes *cdx1a* and *cdx4* are expressed in the posterior neural tissue and control the formation of posterior spinal cord by repressing anterior fate. We are also extensively studying the mechanisms that establish the complex structure of the cerebellum.



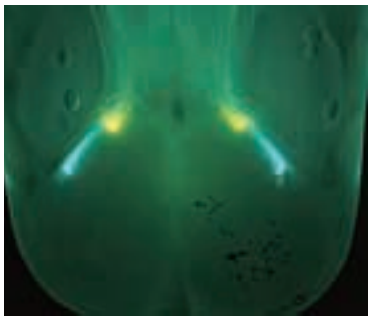
Development of cerebellar neurons in zebrafish larvae at 5 days post fertilization. Purkinje neurons (green) receive axons of granule cells (magenta) in wild type. Loss and abnormal extension of granule cell axons are observed in *gazami* and *shiomaneki* mutants, respectively.

Sensory Circuit Formation

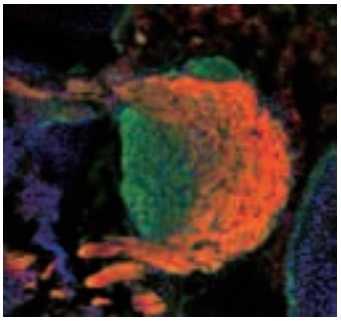
The mammalian nervous system is composed of enormous numbers of neurons, but how do these cells take on diverse fates and organize and array themselves during development? In recent years, it has become clear that the mouse olfactory system provides an excellent platform for addressing these questions experimentally. In this system, there are around 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 neuron.

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.

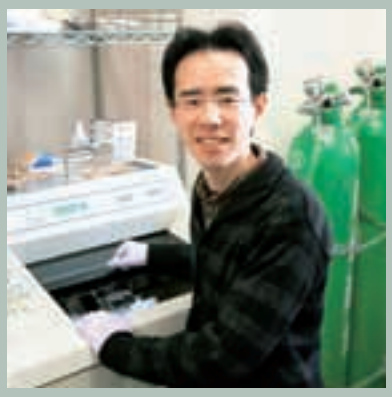
Our lab will seek to develop a better understanding of odorant receptor-dependent axon projection of olfactory sensory neurons, and the formation of neuronal circuitry in the olfactory bulb dependent on inputs from these neurons. We will also seek to develop next-generation genetic tools to aid in our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



Axonal projection of olfactory sensory neurons to the olfactory bulb. Cyan, olfactory sensory neurons expressing I7-CFP; Yellow, olfactory sensory neurons expressing I7-dnPKA-YFP.



A graded olfactory map forms in the absence of the olfactory bulb. Fibrocellular mass of a bulb-less mutant mouse, stained with anti-gap43 (green) and anti-Neuropilin-1 (red).



Takeshi IMAI Ph.D.

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

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

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Cell Lineage Modulation

Toru KONDO Ph.D.

Toru Kondo received his B.Sc. from Waseda University and his M.S. and Ph.D. from the Osaka University Institute for Molecular and Cellular Biology. He worked as a postdoctoral fellow in Shigekazu Nagata's lab at Osaka Bioscience Institute from 1994 to 1998 and in Martin Raff's lab at University College London MRC Laboratory for Molecular Cell Biology from 1998 to 2001. He returned to Japan to take a position as an associate professor at Kumamoto University in 2001 and moved to Cambridge University Centre for Brain Repair in 2002 to take a group leader position. He was appointed team leader in 2005. He was appointed professor at Ehime University in 2009.

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Hide T, et al. Combination of a Ptgs2 inhibitor and an EGFR signaling inhibitor prevents tumorigenesis of oligodendrocyte-lineage derived glioma-initiating cells. *Stem Cells (2011) In press*

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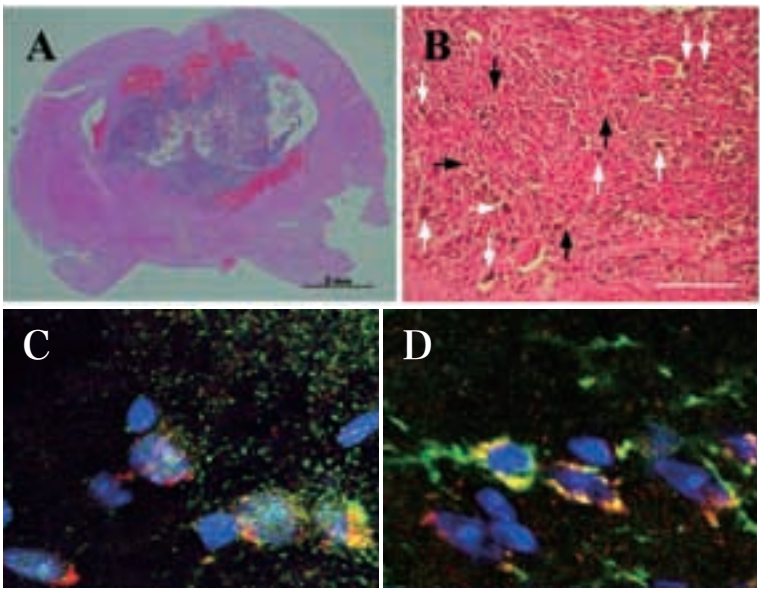
Mankind has long wondered whether it might be possible to regenerate body tissues and structures that have been lost. Recent research has shown that even the adult body contains a diverse range of tissue-specific somatic stem cells that serve to maintain the function and integrity of tissues, opening a promising avenue toward possible applications in regenerative medicine. But the limits on the number of somatic stem cells present in any individual, coupled with the limited availability of donors, have heightened interest in the development of new, alternative means of generating stem cells.

Research in our lab seeks to develop a better understanding of the molecular mechanisms involved in the reacquisition of “stemness” (stem cell characteristics) using the dedifferentiation of oligodendrocyte progenitor cells (OPCs), which are abundantly present in the central nervous system, into neural stem-like cells, knowledge which it is hoped may lead to new techniques for the generation of neural stem cells. We are also interested in two other related subjects: one is the characterization of stem cell-like cancer cells (sometimes called “cancer stem cells” or “cancer initiating cells”), found in malignant glioma, and to find markers and targets for them, potentially leading to the development of novel anti-cancer therapies. The other is to develop a better understanding of the molecular mechanisms of cellular senescence, which are involved in both the defects of tissue-specific stem cells and the suppression of tumorigenesis.



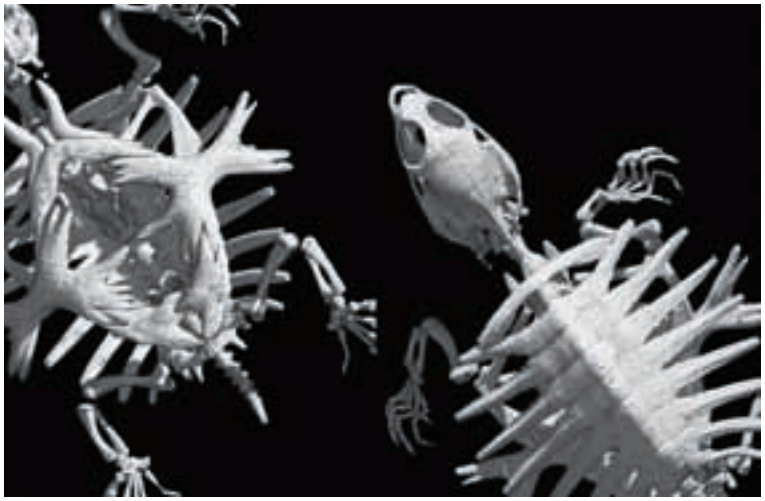
Neural stem/precursor cells may be cells-of-origin for brain disorders. Brain tumor formed by induced mouse glioma-initiating cell, NSCL61, shows a phenocopy of human GBM (A and B). Neural stem cells (C, green: Musashi1) and oligodendrocyte precursor cells (D, green: NG2) in the aged mouse brain are positive for a new senescence marker, Ecrg4 (red).

Evolutionary Morphology

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 hope 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

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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Nagashima H, et al. Evolution of the turtle body plan by the folding and creation of new muscle connections. *Science* 325. 193-6 (2009)

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Sensory Development

Raj LADHER Ph.D.

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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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Ladher R K, et al. From shared lineage to distinct functions: the development of the inner ear and epibranchial placodes. *Development* 137, 1777-1785 (2010)

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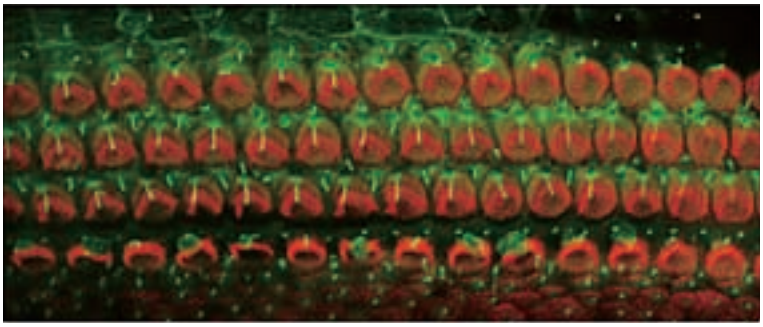
Organogenesis is a monumental undertaking. From siting a given organ in its correct location, to confirming that it is correctly engineered and constructed, to ensuring the necessary functional connections with other organs, the embryo has a daunting task before it; indeed, perhaps we should be surprised 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 as well, 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.



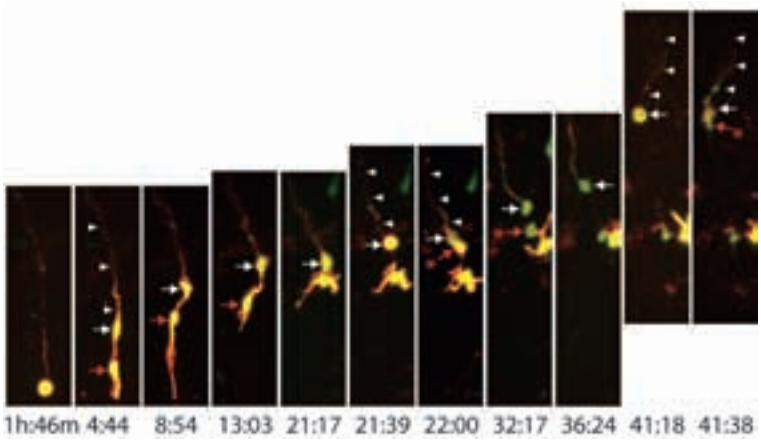
Ciliated mechanoreceptors, or inner ear hair cells, transduce sound information in the organ of corti, part of the mammalian cochlea. Shown are the 4 rows of inner ear hair cells that make up the organ of corti, stained with alpha-tubulin (to show kinocilia in green) and phalloidin to show the actin based stereocilia (in red).

Cell Asymmetry

Our group seeks to explore the molecular mechanisms underlying the organization of cells into highly ordered structures in the developing brain, where neural stem cells generate a large number of neurons of different fates at appropriate points in time. Asymmetric cell division plays an essential role in this process. We have focused our study on the control of asymmetric division and cell polarity in neural precursor cells, and their roles in brain development in invertebrate (*Drosophila*) and vertebrate (mouse) systems.

Asymmetric cell division, in which daughter cells of different types are produced, is a process fundamental to the generation of cells of divergent types during proliferation. This type of division requires the polarized organization of mitotic cells when it occurs cell-autonomously, and depends on asymmetric microenvironments when the process is non-cell autonomous. *Drosophila* neural stem cells, called neuroblasts, provide an excellent model system for the investigation of fundamental aspects of asymmetric division, including the cell polarity that promotes it. Neuroblasts typically undergo asymmetric divisions to produce two daughter cells: another neuroblast, and a smaller ganglion mother cell (GMC) to which neural fate determinants such as Numb and the Prospero transcription factor are asymmetrically partitioned. However, we do not yet understand a number of fundamental aspects of the asymmetric division of neuroblasts. We are addressing these issues using genetic screens for mutations affecting the asymmetric division of neuroblasts.

The vertebrate brain comprises a considerably larger number of neurons arranged in a vastly more complex functional network than that in *Drosophila*. This complex organ develops from the two dimensional epithelial sheet that forms the neural tube, in which neuroepithelial cells initially proliferate and subsequently yield neurons from neural stem cells. Hence the transition from the proliferative to neurogenic phase is thought to be critical for determining brain size. Although previous studies have proposed that neural stem cells divide symmetrically to proliferate and asymmetrically to produce neurons, the mechanism by which neural stem cells switch their mode of division from symmetric to asymmetric and the manner in which they simultaneously generate self-renewable and differentiating daughter cells are not well understood. We are investigating these problems to understand the principles that organize the cellular architecture of the vertebrate brain.



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.

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Fumio Matsuzaki received his B. Sc. from the Department of Biochemistry and Biophysics at the University of Tokyo in 1979, and his doctorate from the same institution in 1984, for his work on the characterization of the erythrocyte cytoskeletal structure. He spent the period from 1984 to 1988 as a postdoctoral fellow, first in the Department of Cell Biology at the Tokyo Metropolitan Institute of Medical Science, then in the laboratory of Gerald Edelman at the Rockefeller University. He returned to Japan in 1988 to take a position as a section chief in the Department of Molecular Genetics in the National Institute of Neuroscience. In 1998, he was appointed professor in the Department of Developmental Neurobiology at Tohoku University and remained there until taking his current position as group director at the RIKEN CDB.

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Shitamukai 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 in press*

Kitajima A, et al. Progenitor properties of symmetrically dividing Drosophila neuroblasts during embryonic and larval development. *Dev Biol* 347. 9-23 (2010)

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α-catenin relies on force

Shigenobu YONEMURA

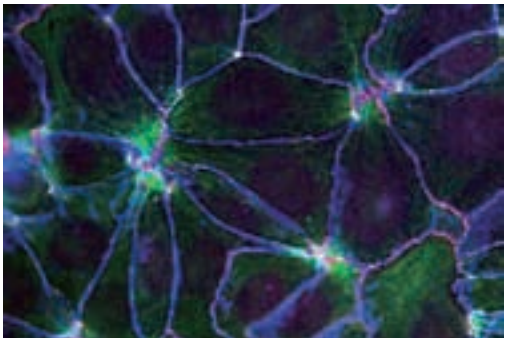
Near their apical surfaces, epithelial cells are bound to their neighbors through molecular interactions that collectively give rise to an active space known as the adherens junction. In addition to holding cells of the same type in close proximity, these junctions are believed to act as sensors for mechanical forces pushing and tugging on the cellular membrane, allowing it to respond and maintain its shape and integrity through rearrangements of the cytoskeleton. It is suspected that cytoplasmic elements that attach to cadherins, the primary cell-cell adhesion molecules, are responsible for this regulatory activity, but recent research has cast doubt on the most popular contender, a protein called α-catenin, as the elusive molecule behind this mechanism.

Now, however, a study by Shigenobu Yonemura, head of the Electron Microscope Laboratory, and colleagues has reinstated α-catenin as the leading candidate for the link between the adhesion machinery and the cytoskeleton. This latest work, published in *Nature Cell Biology*, reveals that the protein recruits a second factor, vinculin, known to interact with the cytoskeletal molecule actin in a manner determined by mechanical forces.

The key molecular complex in the adherens junction is known as the cadherin-catenin complex, which forms when a group of catenin molecules (ß, α, and p120) attaches to the cytoplasmic tail of any one of the many classic cadherin adhesion molecules. α-catenin is also known to bind with actin filaments and multiple actin-binding elements (including vinculin), which seemingly made it a natural candidate as the linker between cell adhesion and the cytoskeleton. But recent evidence suggesting that α-catenin does not simultaneously bind both the cadherin complex and actin filaments in vivo put its role in doubt.

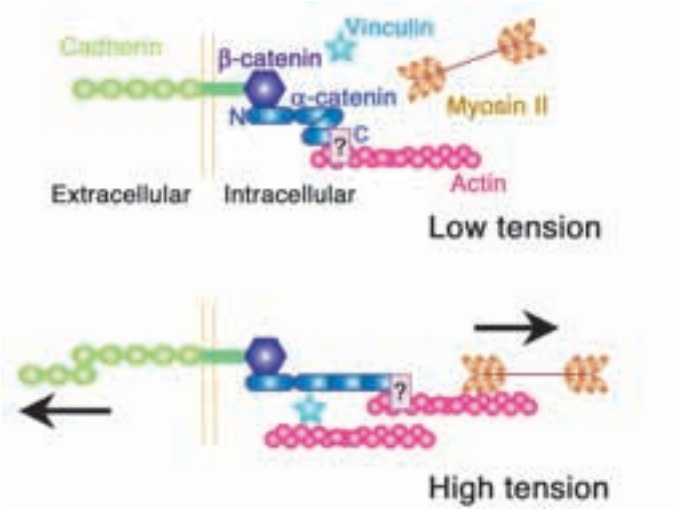
Yonemura S, et al. α-Catenin as a tension transducer that induces adherens junction development. . *Nat Cell Biol* 12, 533 - 542 (2010)

Intrigued by this problem, Yonemura and colleagues looked at a number of epithelial cell types in vitro for clues into the mechanism. Observing that vinculin (but not α-catenin) accumulates at adherens junctions only in the presence of the force-generating protein myosin II, the team conjectured that α-catenin's binding to vinculin might be dependent on the activity of myosin II. Dissection of the protein's molecular structure revealed three active regions: the amino terminus, which binds ß-catenin; the carboxy end, which binds various actin-binding factors; and a central region where vinculin-binding was thought to occur. Experimenting with various combinations of the presence and absence of α-catenin and force generation, Yonemura found that, contrary to the original speculation, vinculin molecules do not bind α-catenin in a force-dependent manner, but instead that a central region of the protein inhibits vinculin binding and that the carboxy terminus is required for the force-dependent alleviation of this inhibition.



When α-catenin molecules (blue) distributing evenly along the cell-cell interface are stretched by myosin II (green)-derived forces applied at adherens junctions, they become bound to a monoclonal antibody to α-catenin (red), suggesting force-dependent changes in α-catenin conformation.

Seeking to pinpoint its functional regions, the team generated a series of α-catenin mutants lacking various regions, and identified the vinculin-binding region (residues 325–360) and the region responsible for releasing the inhibition of vinculin binding (residues 697–906). From this structural understanding, Yonemura developed a model for force-dependent binding between α-catenin and vinculin, in which the application of mechanical force exposes the region of the α-catenin protein that releases the inhibition of vinculin, allowing its recruitment. Further examination of α-catenin dynamics in the absence and presence of cytoskeleton-generated forces revealed that its turnover was lower in presence of myosin II activity (which generates force), in line with predictions from the functional model. And, importantly, the force-dependent mechanism was shown to be in effect in membrane-bound α-catenin as well, indicating that even when it functions as part of the cadherin-catenin complex, α-catenin is still capable of responding to forces.



Model of force-dependent vinculin binding to α-catenin. N and C represent the N and C termini of α-catenin, respectively. A possible unknown linker is indicated with a question mark.

Morphogenesis of ball-and-socket joint revealed

Reiko TAJIRI

Insects wear their skeletons on the outside, but despite their external location, these chitinous cuticles must perform many of the same functions as vertebrate bones. In addition to their protective functions, exoskeletons also provide structural support and defined range of motion for limbs. This last role is the duty of the joints, which, as in vertebrate limbs, come in various configurations and play central roles in such behaviors as mobility and feeding. Indeed, an entire phylum of invertebrates, the Arthropoda, was originally named after its members' "jointed feet." But while the physiology of vertebrate joints has been extensively studied, much less is know about their development.

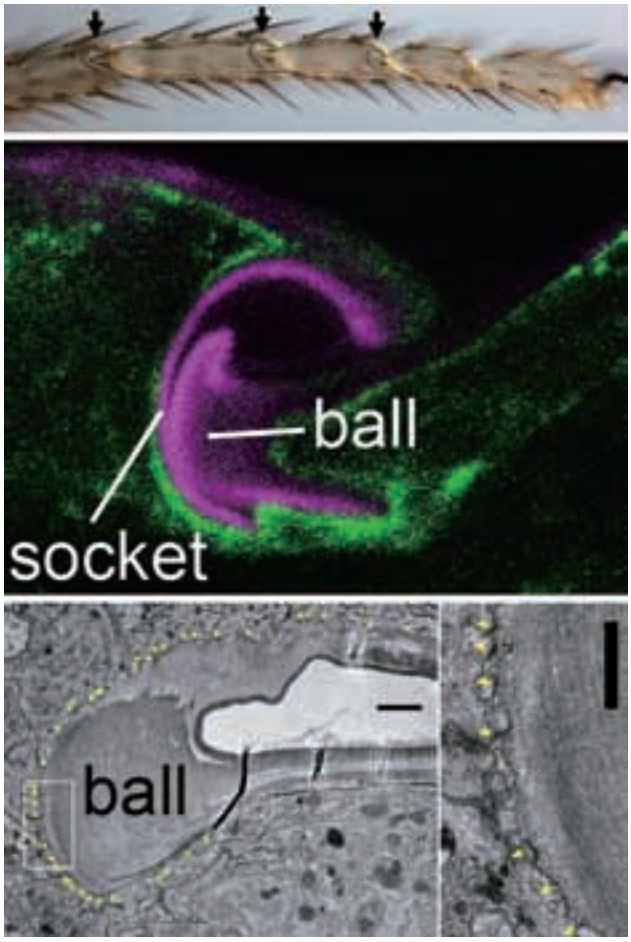
Reiko Tajiri in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) in collaboration with the Electron Microscopy Laboratory (Shigenobu Yonemura, Laboratory Head) chose the leg of the fruit fly *Drosophila melanogaster* as a model to study the formation of ball-and-socket joint skeletons. Using a combination of cell labeling and imaging technologies, the group showed that this structure forms through a sequential process involving extensive cell migration and tissue remodeling.

The study began with some basic observations, as the development of joint cuticles is largely unexplored. After characterizing the morphology and cell arrangements in the mature joint by optical and electron microscopy, Tajiri looked at the same region earlier in development to watch how these come to be. The leg primordium begins as grooved epithelium that gradually lengthens, with points of constriction at the future joints. The joints themselves form when cells on the dorsal side constrict more tightly and fold inward in a proximal direction, deepening the furrow into an empty cavity. Once this cavity has formed, the cells that line it begin to deposit cuticle, forming the ball at the deepest part of the cavity, and the socket on the dorsal side. The socket cuticle later expands ventrally toward the bottom of the cavity, and eventually covers the entire surface.

The group next turned to a structural feature known as plasma membrane plaques (PMPs), which were believed to be the sites of chitin release and of the adhesion between the cuticle and underlying cells. The question Tajiri sought to answer was, do the cuticle-forming activity move like a wave over a bed of stationary cells, or do the cells themselves move? The group used a genetic labeling method to monitor the locations of cells within the joint region at various stages, and identified three

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)

drivers of reporter gene expression in distinct, but partially overlapping, subpopulations. By tracking the behavior of the labeled cells, they determined that the apical surfaces of cells making up the ball and socket components of the joint followed the distribution of PMPs.



(Top) Distal part of the leg (tarsus) in *Drosophila melanogaster*, where segments are connected through joints (arrows). (Middle). The joint cuticle has the ball-and-socket architecture (cuticle in magenta and cell cortices in green). (Bottom) Developing ball cuticle it is surrounded by plasma membrane plaques (yellow arrows in the electron micrograph).

The next step was to see which parts of the cuticle were produced by which of the genetically marked regions. Tajiri and colleagues found that one subpopulation (expressing *fng*) was specific to the dorsal aspect of the socket, while the other two, *neur*- and *bib*-, overlapped, with *neur*-expressing cells giving rise to ventral socket while *bib*-expressing cells formed the cuticle of the ball. In contrast to the cuticle, the surfactant that lubricates the joint seemed to be manufactured more diffusely. Perhaps unsurprisingly, blockade of chitin synthesis disrupted the entire joint cuticle, but interestingly had no effect on the cuticle-secreting cells, which changed shape as usual even when the cuticle was chemically disrupted, indicating that the cuticle is unlikely to play a guidance role.

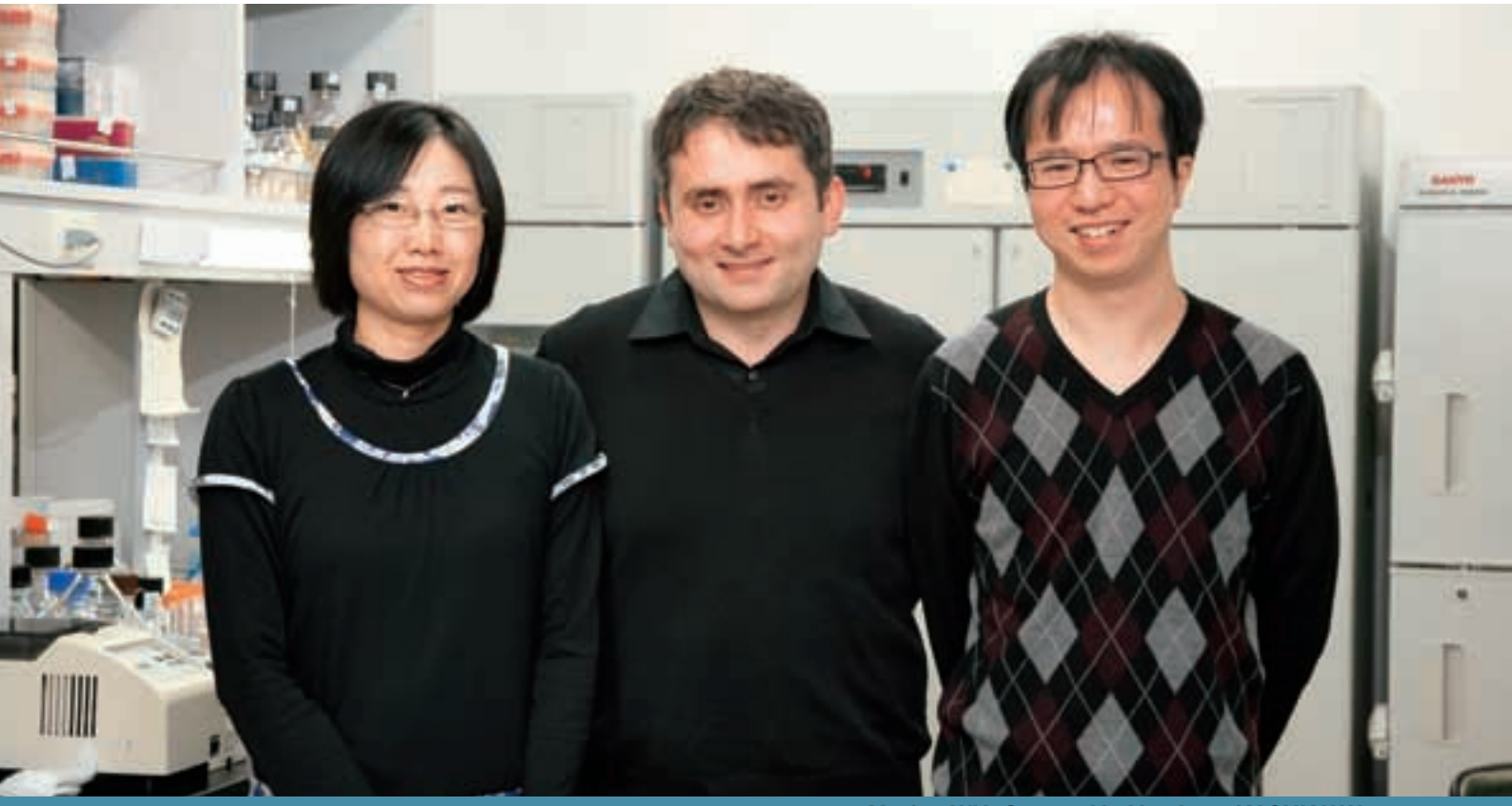
"Extracellular matrices occupy a major portion of skeletal elements of animal body, and while the roles of extracellular matrices in cell signaling have been studied extensively, much less is known about how cells instruct shaping of extracellular environment," says Hayashi. "This study opens a new direction of research into the morphogenesis of non-cellular parts of insect exoskeletons, which vary enormously in shape, color, and function."

Bird's eye view of mesodermal gene expression

The mesoderm, one of the three embryonic germ layers, is the source of a broad range of cell types, from muscle, bone and cartilage, to the reproductive system. In two major amniote groups (birds and mammals) the mesoderm arises from the ectoderm in the form of a structure known as the primitive streak. The cells in this transitory strip of mesodermal precursors are guided by positional cues that roughly translate starting points along the anterior-posterior axis of the streak to tissue destinations along the dorsal-ventral axis of the embryo. Cells from anterior sites in the primitive streak tend to give rise to dorsal structures, while more posterior streak cells contribute to ventral tissue types. The molecular underpinnings of this guidance system, however, have yet to be comprehensively studied.

Cantas Alev and colleagues in the Laboratory for Early Embryogenesis (Guojun Sheng, Team Leader), in collaboration with the members of the Laboratories for Systems Biology and Stem Cell Biology, surveyed the transcriptomic landscape of the primitive streak using genomic arrays, developing a finer understanding of the regulation of the dorsal-ventral gradient in the developing mesoderm. Published in the journal *Development*, their study of events in the early chick embryogenesis provides a valuable resource for the study of tissue patterning in amniotes.

Using more than 250 embryos collected at the full primitive streak stage (HH4), Alev et al. began by dissecting out a rectangular section containing the streak from each, and subdividing it into four equal parts along its A-P axis. Next, using a commercially available genome array of around 22,000 unique genes, they tested the segments for differences in the expression of individual genes. While they found that close to 40% of the chicken genome is expressed across the entire streak, they did identify a number of genes whose expression levels varied from seg-

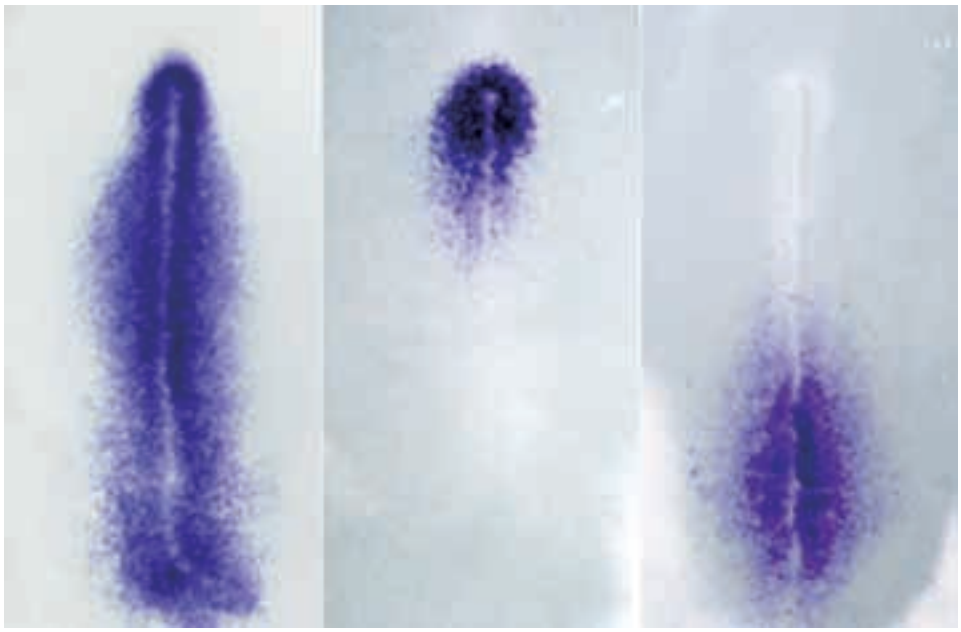


Yuping WU, Cantas ALEV, Takeya KASUKAWA

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

ment to segment. The team analyzed all genes that could be assigned a Gene Ontology classification, which is a method for categorizing genetic functional activity.

Alev found that around 14% of the expressed transcripts could be grouped into six clusters defined by the expression in various combinations of segments, such as [1,0,0,0] or [0,0,0,1], representing either dorsal-to-ventral (Group A) or ventral-to-dorsal (Group D) asymmetric expression. While many Gene Ontology categories were evenly represented across both groups, the team did find that genes involved in transcriptional regulation and receptor-mediated signaling showed biased expression, with transcriptional regulators expressed more in Group D, and signaling genes more in Group A.

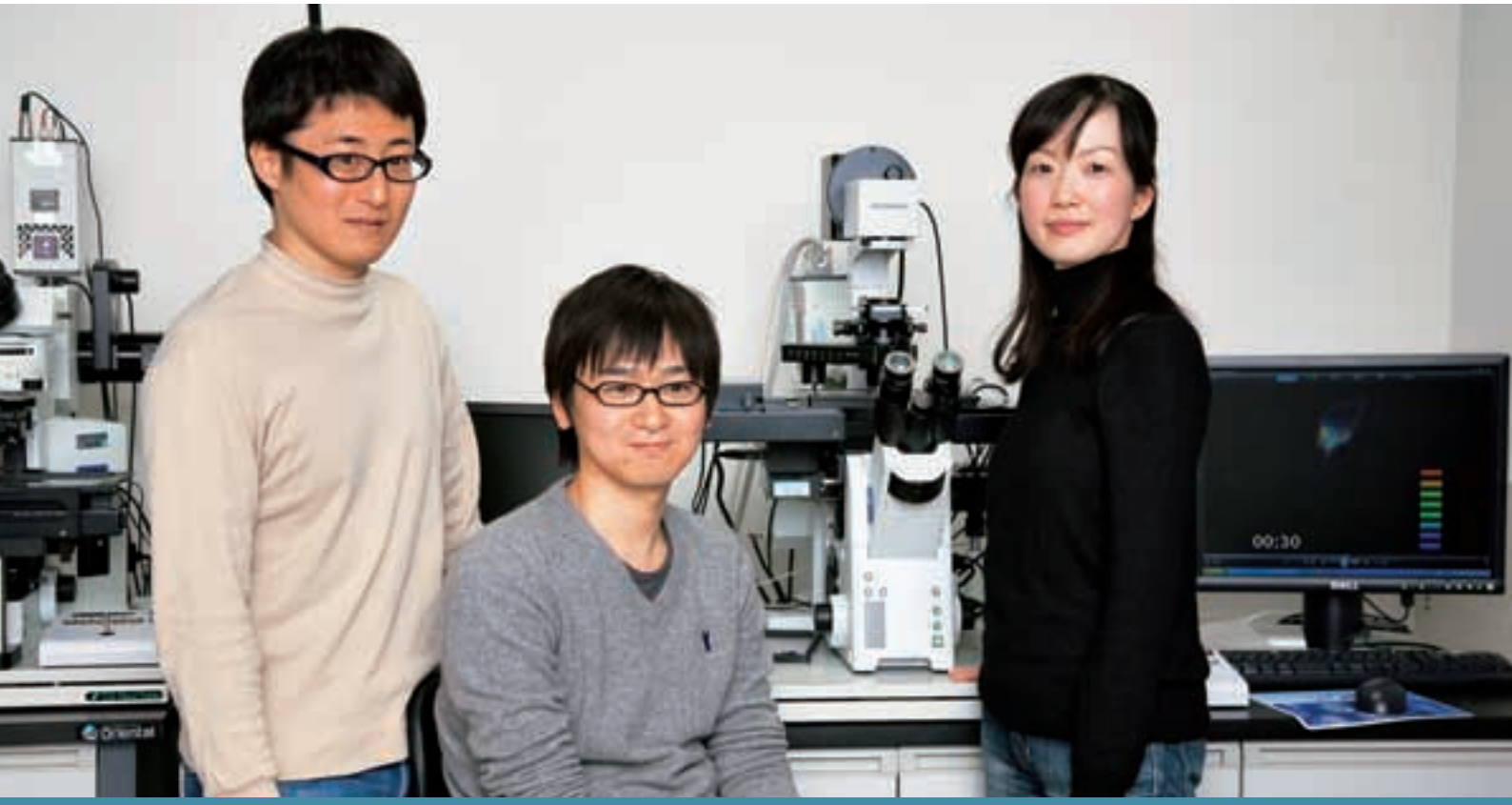


Mesoderm precursors, marked by the pan-mesodermal marker Brachyury (left panel), receive differential patterning cues along the length of the primitive streak (e.g., Chordin in the middle panel and Cdx1 in the right panel).

In their analysis, the authors focused particularly on three major developmental pathways—Wnt, TGFß/BMP, and FGF—but found that for the most part their core components were not differentially expressed. However, regulators of these pathways frequently did show either dorsal or ventral distributions. The picture that emerges from this set of findings is of a complex, multi-tiered network of genes interacting with and regulating the activity of key pathways involved in setting up the D-V axis.

"To embryologists and stem cell biologists alike, the primitive streak is a fascinating structure that defies simplification," says Sheng. "Our study offers a resource for achieving a better understanding of its molecular complexity in vivo. We're hopeful that this understanding can also help achieve efficient and targeted mesoderm lineage differentiation in translational research."

ROCK's role in hESC suicide prevention



Toshihiro ARAMAKI, Masatoshi OHGUSHI, Michiru MATSUMURA-IMOTO

Embryonic stem cells (ESCs) are known for their ability to be grown indefinitely in self-renewing culture, and for their pluripotency—the differentiative potential to give rise to all the cell types in the body. These characteristics are common to such cells in both mouse and human, but ESCs from these species also exhibit important differences, one of the most prominent of which is the greater susceptibility to cell death of human ESCs on dissociation; mouse ESCs are made of sturdier stuff, and grow happily even when isolated from their cell-mates. Although methods have been developed to prevent hESCs from self-destructing, including a breakthrough report from the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director) in 2007, the mechanisms behind its occurrence have never been explained.

A new study by led by Masatoshi Ohgushi in the same group, in collaboration with the CDB Laboratory for Pluripotent Stem Cell Studies and scientists at Kyoto University, now provides an answer to that puzzle. Published in *Cell Stem Cell*, the authors report that hESC cells die due to a hyperactivation of myosin that is triggered by loss of cell-cell adhesion and mediated by the Rho signaling pathway. These changes in molecular activity are accompanied, but not dependent on, changes in cells morphology known as "blebbing," in which the cells perform a "dance of death" as an early signal of their demise.

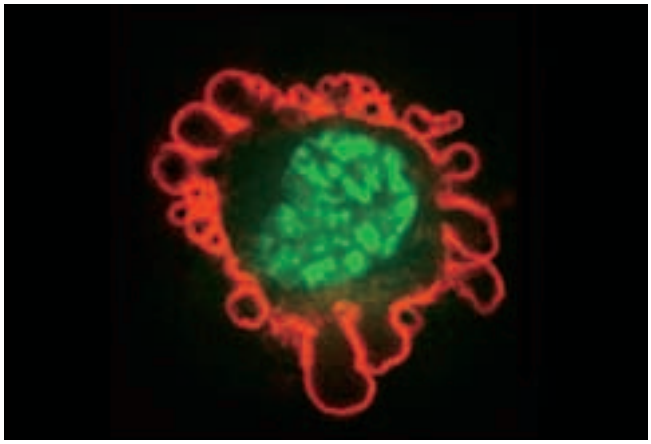
Ohgushi began by building on previous work from the same lab, which had shown that inhibition of a component of the Rho signaling pathway known as ROCK can prevent human ESCs from dying on separation from their colony. He used a combination of cell sorting and live imaging to identify features of dying hESCs, and found that quickly after dissociation the cells became motile and began to form distinct outpockets in their surface membranes, called blebs. This symptom is known to be the result of the actomyosin system, which regulates cytoskel-

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)

etal behavior, but it is ordinarily a short-lived phenomenon, a kind of convulsive last gasp in a dying cell's final minutes; in hESCs, however, it lasts for as long as a day. And interestingly, while blebbing is usually under the control of the caspase pathway that regulates canonical forms of programmed cell death, its onset in hESCs is caspase-independent, but can be prevented by ROCK inhibition.

It turns out that the reason for ROCK's role is its effect on non-muscle myosin light chain 2 (MLC2). ROCK is upregulated following dissociation of hESCs, which triggers an elevation of MLC2, hyperactivating myosin and generating the intracellular contractive forces that underlie blebbing. Interestingly, however, Ohgushi found that inhibition of blebbing did not prevent apoptosis.

The group's first supposed the true cause of this programmed cell death in hESCs on dissociation would be a phenomenon known as anoikis (from the Greek word for "homeless"), in which cells detached from their basement membrane spontaneously undergo apoptosis. But live imaging showed that even when dissociated hESCs remained attached to the basement membrane, they blebbed and died. It appeared that the loss of lateral, not basal, connections was to blame, suggesting a role for cadherin-mediated cell-cell adhesion. They tested this notion by depleting calcium ions (cadherin function is calcium-dependent) and cadherin knockdown by RNAi in hESCs, and in both cases observed blebbing and apoptosis, suggesting that the loss of intercellular adhesion plays a triggering role in the apoptotic cascade. This jibes well with known differences between the cellular states of mouse and human ESCs; specifically, the similarity of hESCs to mouse epiblast cells.



Extensive blebbing in dissociated hESC. Nucleus, green; cell membrane, red.

Loss of cadherin function also induced the upregulation of the Rho signaling pathway, of which ROCK is a component. Previous work has shown that this pathway is subject to multiple upstream activators, so Ohgushi et al. performed an shRNA knockdown screen to identify responsible factors, and turned up a heretofore poorly characterized Rho-GEF family member known as Abr.

The involvement of Abr cements the importance of Rho signaling in the demise of dissociated hESCs. Importantly, Rho is frequently found to interact with another small G protein, Rac. Using a pulldown assay, Ohgushi looked at the control of this factor in dissociated hESCs, and found that, in contrast to Rho, Rac was significantly downregulated, strongly suggesting that Rho and Rac have reciprocal roles in this process. The take-home message appears to be that loss of cadherin-mediated cell-cell adhesion in dissociated hESCs upregulates Abr, leading to a Rho-high/Rac-low state, consequent elevation of ROCK, and the hyperactivation of myosin, causing blebbing and apoptotic death.

The developmental and evolutionary significance of this mechanism remains a stimulating open question. "In mouse, the cells of the ICM show no polarity, while those of the epiblast do, enabling them to adhere and form a sheet-like structure, and we have seen that dissociated mouse epiblast cells undergo the same apoptosis as do dissociated hESCs," says Sasai. "This dance of death induced by the hyperactivation of myosin is an intriguing cellular phenomenon in the development of a multicelled 'society' during early embryogenesis."

DNA methylation not required in extraembryonic tissues

Chisa MATSUOKA, Morito SAKAUE, Akiko YAMAGIWA

Genomic techniques have not only shown us the diversity of genetic mechanisms for establishing, maintaining and regulating the body's functions, they have also brought into clear focus the fact that life is not ruled by genetics alone. The study of heritable changes in phenotype independent of the underlying DNA is collectively referred to as epigenetics, and has begun to reveal the intricacy and fundamental importance of such processes as chromatin remodeling and the modification of genomic sequences by various proteins. DNA methylation is one such modification, in which methyl residues are added to and maintained at specific sites on the genome through the action of methyltransferases. These have been shown to be essential to the growth and survival of somatic cells but, interestingly, not to pluripotent embryonic stem cells (ESCs). The question of whether DNA methylation functions in any type of differentiated cells comes down to their role in extraembryonic tissues, which has never been fully determined.

To address this question, Morito Sakaue, Hiroshi Ohta, and colleagues in the Laboratories of Mammalian Epigenetic Studies (Masaki Okano, Team Leader), Genomic Reprogramming, and Pluripotent Stem Cell Studies studied extraembryonic development in mice lacking all three DNA methyltransferases known to have physiological function. In a report published in *Current Biology*, they showed that in these triple knockout (TKO) embryos, while differentiation into the embryo proper was affected, the development of extraembryonic stem cells and tissue was normal.

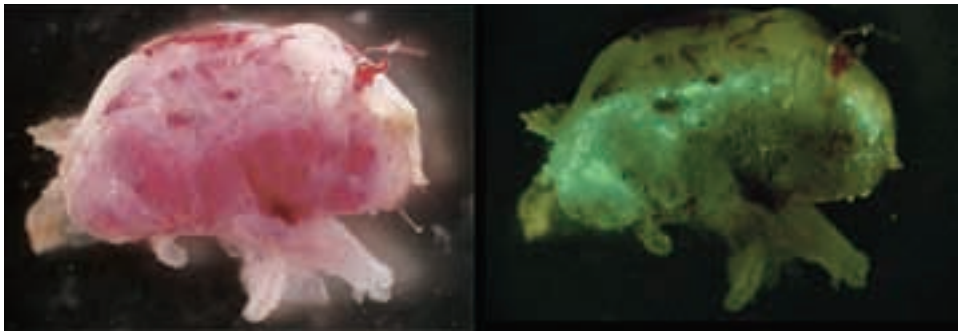
Sakaue and Ohta examined developmental abilities of mouse embryos deficient for active DNA methyltransferases, Dnmt1, Dnmt3a, and Dnmt3b, (TKO) by using nuclear transfer (NT). No abnormalities were seen in the development of NT embryos up to the blastocyst stage, even in the absence of all three Dnmts. To examine subsequent stages, the team made aggregates of TKO-NT and wildtype embryos (DNMT mutations are known to be lethal to embryos), and found that few mutant cells were detected in the embryo proper, but they contributed

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

to extraembryonic tissues in the chimeras. So, while DNA methylation is not necessary for the earliest differentiation event that separates the extraembryonic tissue from the embryo itself or for further differentiation in the extraembryonic tissues, it is required for cell survival within the embryo.

Their next question was, Why? On observing the TKO embryos, they saw signs that somatic differentiation had begun to take place, but that the cells did not survive. Sakaue and Ohta used retinoic acid to steer mutant ESCs toward a neuroectodermal fate, and found that, just as in wildtype, the cells expressed the appropriate genetic markers but that they were subject to extensive apoptotic cell death, possibly linked to the DNA damage response. When they turned to the trophectoderm, however, they did not find unusual levels of apoptosis. When they used Gata4 to induce a second extraembryonic lineage, known as primitive endoderm, these too showed no significant differences from wildtype, suggesting that DNA methylation is not required in extraembryonic tissues in the mouse.

As a final test of the role of DNA methyltransferases, Ohta and Sakaue made trophectoderm stem (TS) cells from triple knockout blastocysts made by nuclear transfer. They found that, like the differentiated extraembryonic cells, the TS cells showed normal behavior and viability, and could proliferate for dozens of passages in culture. Using genetic inducers, they further showed that these stem cells were capable of differentiating into all of the expected cell types, and even contributing to placenta in vivo. Despite these similarities, however, the mutant TS cells showed differences in gene expression revealed by microarray, suggesting that DNA methylation does have a regulatory function in TS cells.



$Dnmt1^{-/-}Dnmt3a^{-/-}Dnmt3b^{-/-}$ (TKO) cells (green) contribute to placenta in a chimeric TKO NT/wildtype embryo. Left, a bright field image; right, a dark field image with green fluorescence.

"In somatic cells, epigenetic marks on chromosomes support the stable maintenance of cellular properties over the long term, while cellular properties are reprogrammed during embryogenesis, and are associated with the reorganization of epigenetic marks; that is, cells have 'stable' and 'flexible' modes in their epigenetic regulation" says Okano. "Mechanisms that tolerate growth without DNA methylation in ES and TS cells may underlie the epigenetic flexibility of these cell types and early embryos."

2010 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.

Beginners' course in human ES cell culture

The Division for Human Stem cell Technology held a number of courses for first-time research users of human embryonic stem cells. The instruction focuses on freezing and thawing, generation of feeder cells, and techniques for confirming pluripotency. All steps were taught using human induced pluripotent stem cells (iPSCs), kindly provided by the Kyoto University Center for iPS Cell Research and Application (CiRA). Students who complete this course are eligible to apply for permission to use human ESCs under the guidelines established by the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Summer school for area high school students

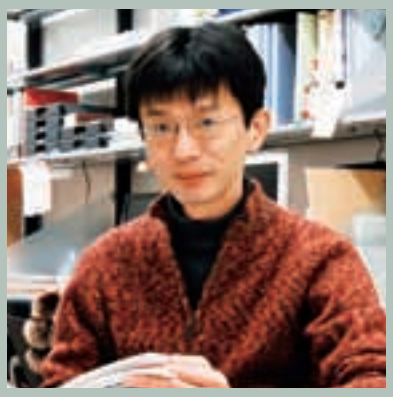
The CDB held its annual summer school program for area high school students on August 24 and 26. The one-day program of lectures and demonstration experiments focused on *C. elegans* research. The lecture, by Team Leader Hitoshi Sawa, explained why these roundworms are useful in developmental biology, what types of research they can be used in, and how they have contributed to Nobel Prize-winning science. The students next visited the Sawa lab before trying their hand at simple biology techniques, such as observing roundworms under a microscope and collecting them for further study. The next compared mutant phenotypes against wildtype animals, and collected eggs and watched the first few rounds of zygotic cell division.

Developmental methods for high school biology teachers

The RIKEN Center for Developmental Biology held its third recurrent course in developmental biology for teachers of high school biology on October 2 and 3. Held in conjunction with the Japanese Society of Developmental Biologists every year in the fall, the course is intended to give teachers an opportunity to learn hands-on techniques in embryology. This year's program, which focused on chicken development, featured a lecture by Prof. Sadao Yasugi of Kyoto Sangyo University on the history of embryology studies in chicken, and an overview of the current state of the science in areas such as body axis formation and organogenesis. Folowing the talk, Yasuo Ishii, a lecturer at the same university, led a two-day training course in which teachers learned how to extract, culture, and observe chick embryos, and to prepare them for microscopic examination. This was complemented by a demonstration of similar techniques in the Japanese quail by Yoshina Usui, a teacher at Higashi Suma High School. All aspects of the training course were designed to enable teachers to apply the subject matter and techniques in their own classrooms.

Egg of hagfish, *Epatretus burgeri*, containing pharyngula-stage embryo

Germline Development



Akira NAKAMURA Ph.D.

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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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Publications

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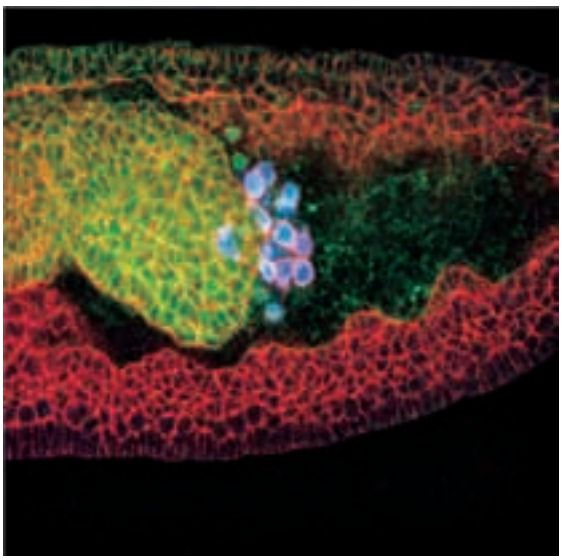
Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the *Drosophila* fruit fly, the formation and differentiation of germ cells is controlled 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.



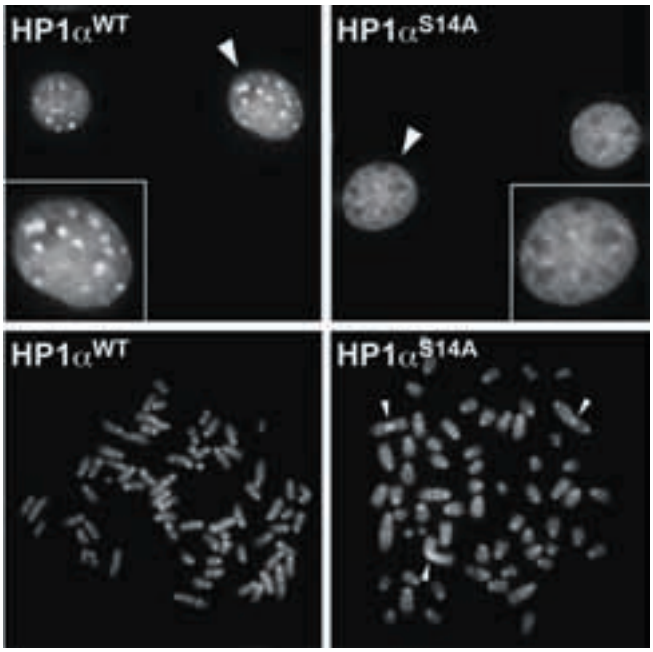
Migrating gem cells (blue) in stage 10 *Drosophila* embryo.

Chromatin Dynamics

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 interrelated, 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.



Disruption of HP1α N-terminal phosphorylation leads to defects in its heterochromatic localization (upper panels) and increased chromosomal instability (lower panels).

Jun-ichi NAKAYAMA Ph.D.

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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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Publications

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Stem Cell Biology

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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, and returned to Kyoto in 1993, as professor at the Kyoto University Faculty of Medicine. He was appointed CDB group director in 2000.

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Publications

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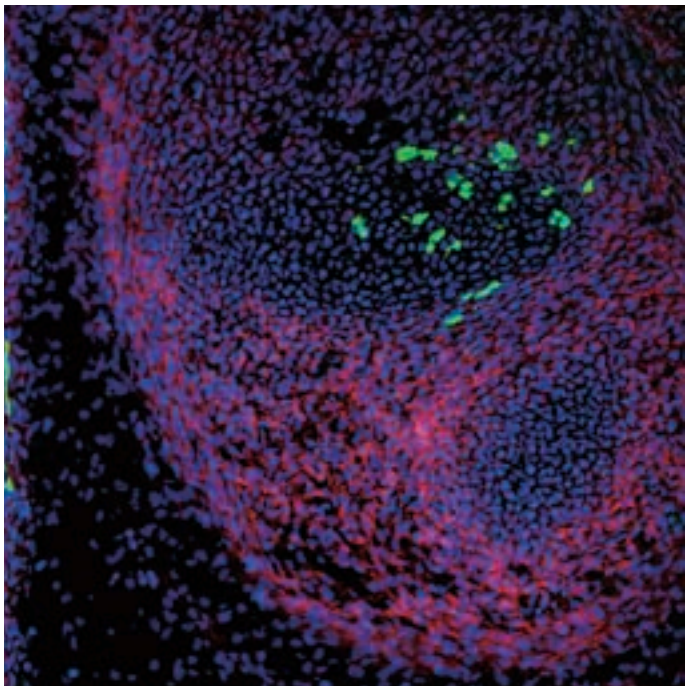
The stem cell system can be thought of as a means by which organisms maintain cell renewal. All forms of life require the endless recycling of both materials and energy, making it impossible for either organisms or individual cells to live independent of their environments. In bodies made up of many 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.



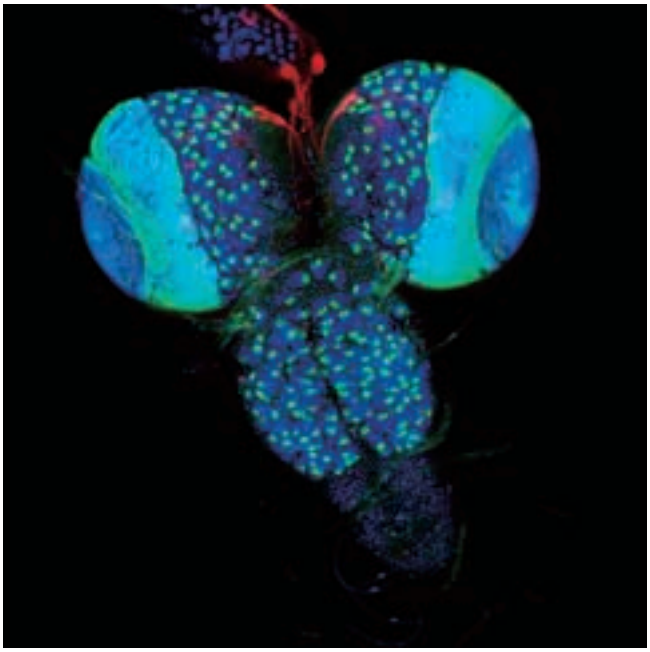
PDGFRα^{+} mesenchymal stem cells derived from neuroepithelial cells in 14.5 embryo

Growth Control Signaling

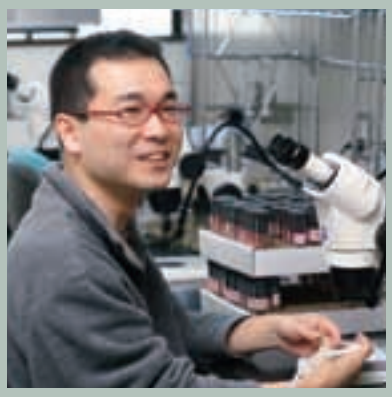
The processes of animal development, including organ size and body size, are genetically predetermined, but these processes are also influenced by environmental factors such as nutrition and temperature. The close link between cell and tissue growth control and environmental cues ensures that developmental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

Cell proliferation and differentiation in each tissue and organ are kept under strict regulation both spatially and temporally. Research has revealed the nature of spatial signals, such as growth factors and morphogens, but the way in which these signals direct cell and tissue growth over time remains poorly understood. For example, how do signals sent to quiescent cells direct them to enter the cell cycle and begin proliferating at appropriate developmental stages; and how do they know when to exit the cell cycle and/or undergo differentiation? In addition to the intrinsic gene expression programs, 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 sensory 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 cell/tissue size; and 3) how do endocrine signals interact with metabolic and growth regulators? We will combine biochemical and genetic approaches, along with quantitative and qualitative imaging and cell-biological analysis, to identify and characterize the relevant signal transduction pathways.



Drosophila larval brain showing proliferating cells (green) and insulin-producing cells (red)



Takashi NISHIMURA Ph.D.

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Takashi Nishimura earned 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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Publications

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Pluripotent Stem Cell Studies

Hitoshi NIWA M.D., Ph.D.

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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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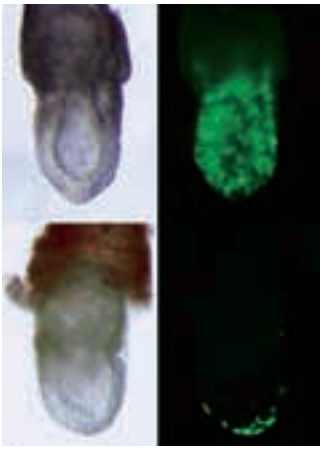
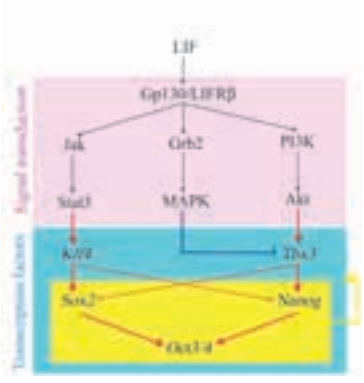
Pluripotency is a term used to describe the ability of certain types of stem cells that are capable of giving rise to at least one type of cell from all three germ layers—endoderm, mesoderm and ectoderm. Stem cells are also characterized by their ability to produce copies of themselves with similar differentiative 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 4 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.

In October 2009, the Laboratory for Pluripotent Cell Studies was re-designated as a Project Lab in the Center Director's Strategic Program for Stem Cell research, and renamed the Laboratory for Pluripotent Stem Cell Studies.



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).

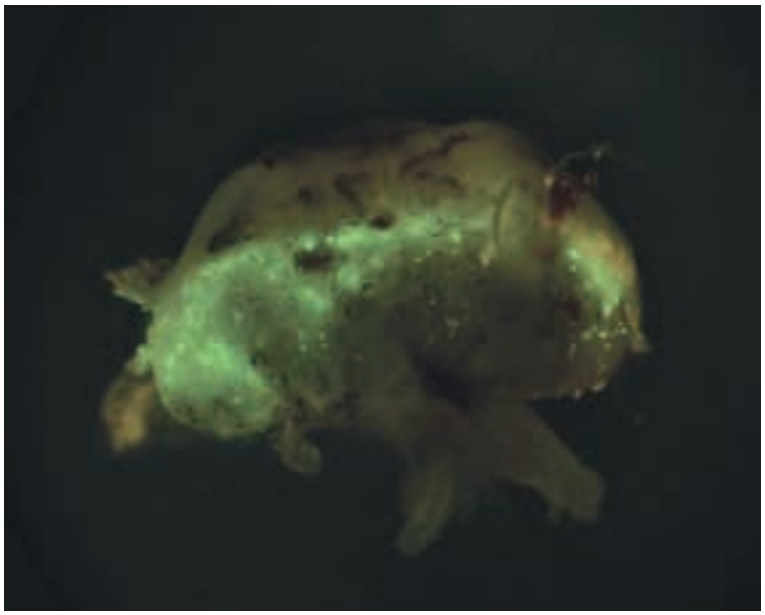


Schematic model of transcription factor networks for pluripotent stem cells, trophectoderm and primitive endoderm

Mammalian Epigenetic Studies

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 team 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.

Masaki OKANO Ph.D.

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Masaki Okano received his baccalaureate and master's degrees from Kyoto University, and his doctorate in biochemistry 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, serving as an instructor in medicine at Harvard for the last two years of that period. He was appointed team leader at the RIKEN Center for Developmental Biology in 2001, and returned to work in Japan full-time in 2002.

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Publications

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Mammalian Molecular Embryology

Tony PERRY Ph.D.

Tony Perry received his B.Sc. in Microbiology from the Department of Bacteriology at the University of Bristol, England and his doctorate from the University of Liverpool four years later. From 1989 he became a postdoctoral fellow and temporary lecturer working on epididymal sperm maturation at the University of Bristol and in 1996 won a European Molecular Biology Travel Fellowship to study oocyte activation. Dr. Perry developed a novel method of transgenesis which he primarily worked on at the Rockefeller University. In June 2002 he took his present position as Team Leader of the Laboratory of Mammalian Molecular Embryology at the RIKEN CDB, where he started to work on the mechanisms of key cytoplasmic events at fertilization in mammals, including chromatin remodeling and meiotic exit. He was appointed Reader at the University of Bath in 2009.

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Publications

Perry A C and Verlhac M H. Second meiotic arrest and exit in frogs and mice. *EMBO Rep* 9. 246-51 (2008)

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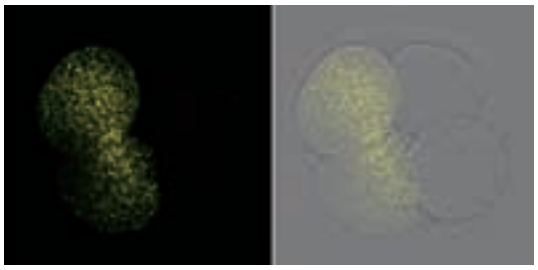
There can be few, if any, cells as specialized as sperm and egg; they appear unique (you can even see a mammalian oocyte with the naked eye) and don't typically divide by themselves. Yet when they combine at fertilization, the single cell they generate is transformed within hours to produce a totipotent cell: one which is completely unspecialized, in that from it all cell types develop to produce an entire individual.

Our laboratory combines molecular and cell biology with micromanipulation of mouse gametes and embryos to study the nucleo-cytoplasmic events that occur immediately after sperm-egg union (oocyte activation) and their developmental consequences. One long-standing question concerns why oocytes don't begin to divide by themselves in the absence of a sperm. To address this, our group developed a novel approach that revealed that the removal of the protein Emi2 from oocytes caused them to resume the cell cycle as if the oocytes had been activated by a sperm. We then extended this to show that Emi2 works through Cdc20 and that both parthenogenetic activation and fertilization require Cdc20. This may be the first formal demonstration that the signaling required for parthenogenesis and fertilization has molecular components common to both; this is significant given the application of parthenogenetic activation in nuclear transfer and other current research.

During meiotic resumption, sperm chromatin undergoes almost complete remodeling; its nucleoproteins, which are mostly protamines, are removed and supplanted by maternal histones which are subsequently modified. We study and manipulate this overall process and have found that chromatin remodeling varies depending on the provenance of the nucleus; the fate of somatic cell chromatin is different from chromatin associated with a sperm-derived genome, even when both are in the same oocyte. Chromatin remodeling is not necessarily of critical importance; gross hyper-acetylation during fertilization has only a limited effect, if any, on development. We are analyzing the developmental significance of different modes of epigenetic remodeling and how they are regulated.

We are also interested in additional interactions between sperm head components and the oocyte cytoplasm, in a bid to discover what happens during fertilization and the earliest moments of the new embryo. It would be useful to attribute molecular identities to the proteins involved in these interactions and characterize them functionally. This task is a daunting one, as the sperm contributes >500 distinct nuclear and cytoplasmic protein species at fertilization, and yet detecting them in newly fertilized oocytes and embryos requires exquisitely sensitive methods. With a greater understanding of any sperm contribution to development, our lab hopes to gain insights into the processes by which embryonic stem cells are formed and carcinogenesis is initiated.



Cytoplasm from a mouse egg expressing a mitochondrially-targeted green fluorescent protein was transplanted into one cell of a two-cell embryo; the paired images show the same embryo after cell division, on the left with laser illumination only, and merged with DIC illumination on the right.

Mammalian Germ Cell Biology

All of the diverse cell types in the body can be broadly classed as either somatic or germline cells. In contrast to somatic cells, which work to maintain the constant, stable form and function of an individual organism's body, germ cells provide the faithfully-replicated information needed to establish subsequent generations of individuals. In order to fulfill this role, these cells need to exhibit certain unique properties, including the ability to revert (generally through fusion with another germline cell) to a state of developmental totipotency and maintain that totipotent state until the start of ontogeny, the ability to undergo epigenetic reprogramming, and to divide meiotically.

Research in our laboratory is geared to elucidating the developmental bases of germline function at the molecular levels from the very earliest stages of germline development. Specifically, our subjects of interest are the molecular mechanisms (a signaling as well as transcriptional principle) involved in the formation of mouse primordial germ cells (PGCs), the cellular progenitors of sperm and oocytes, the genome-wide epigenetic reprogramming that takes place immediately following PGC establishment, the development and application of technologies to enable the analysis of transcriptional regulatory networks at the single-cell level, and ultimately, reconstitution of germ cell development in vitro.

Our studies have so far shown that germ cell specification in mice integrates three key events: repression of the somatic program, reacquisition of potential pluripotency, and genome-wide epigenetic reprogramming. We have identified a PR-domain-containing protein, Blimp1 (also known as Prdm1), as a critical factor for PGC specification. Using a highly representative single-cell microarray technology that we developed, we identified complex but highly ordered genome-wide transcription dynamics associated with PGC specification. This analysis not only demonstrated a dominant role of Blimp1 for the repression of the genes normally down-regulated in PGCs relative to their somatic neighbors, but also revealed the presence of gene expression programs initiating independently from Blimp1. Among such programs, we identified Prdm14, another PR-domain-containing protein, as a key regulator for the reacquisition of potential pluripotency and genome-wide epigenetic reprogramming. The launch of the germ cell lineage in mice, therefore, is orchestrated by two independently acquired PR-domain-containing transcriptional regulators, Blimp1 and Prdm14. Furthermore, we have uncovered a signaling principle for germ cell fate specification. Such studies may provide fundamental information on the reconstruction of the germ cell lineage from pluripotent stem cells in vitro.

Sperm differentiated from induced primordial germ cells from the epiblasts engineered to express GFP (left) are fertilization-competent and can be used to generate normal offspring (right; green shows GFP fluorescence).

Mitinori SAITOU M.D., Ph.D.

Mitinori Saitou received his M.D. from the Kyoto University Faculty of Medicine in 1995, and received his Ph.D. in 1999 for his study of the structure and function of tight junctions under Shoichiro Tsukita in the Kyoto University Graduate School of Medicine. He then moved to the Wellcome Trust/Cancer Research Campaign Institute (present name: Wellcome Trust/Cancer Research UK Gurdon Institute), where he worked as a postdoctoral research associate in Azim Surani's laboratory, focusing on the origin of the germ line in the mouse. He was appointed team leader at the CDB in 2003, and received a three-year grant from the Japan Science and Technology Corporation (JST) PRESTO program that same year, and was affiliated to the Kyoto University Graduate School of Biostudies as associate professor in 2004. He moved to the Kyoto University Graduate School of Medicine as professor in 2010.

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Publications

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Organogenesis and Neurogenesis

Yoshiki SASAI M.D., Ph.D.

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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, Genesis,* and *Developmental Dynamics.*

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Publications

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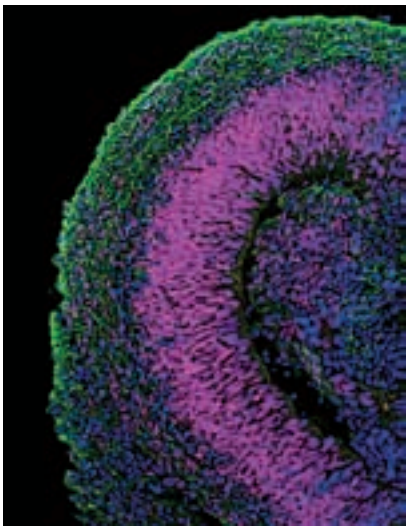
The complexity of the fully formed brain defies description, yet this organ arises from a nondescript clump of cells in the embryo. The specification of the dorsal-ventral axis is significant in neural development in that the central nervous system forms on the dorsal side of the body in all vertebrate 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.

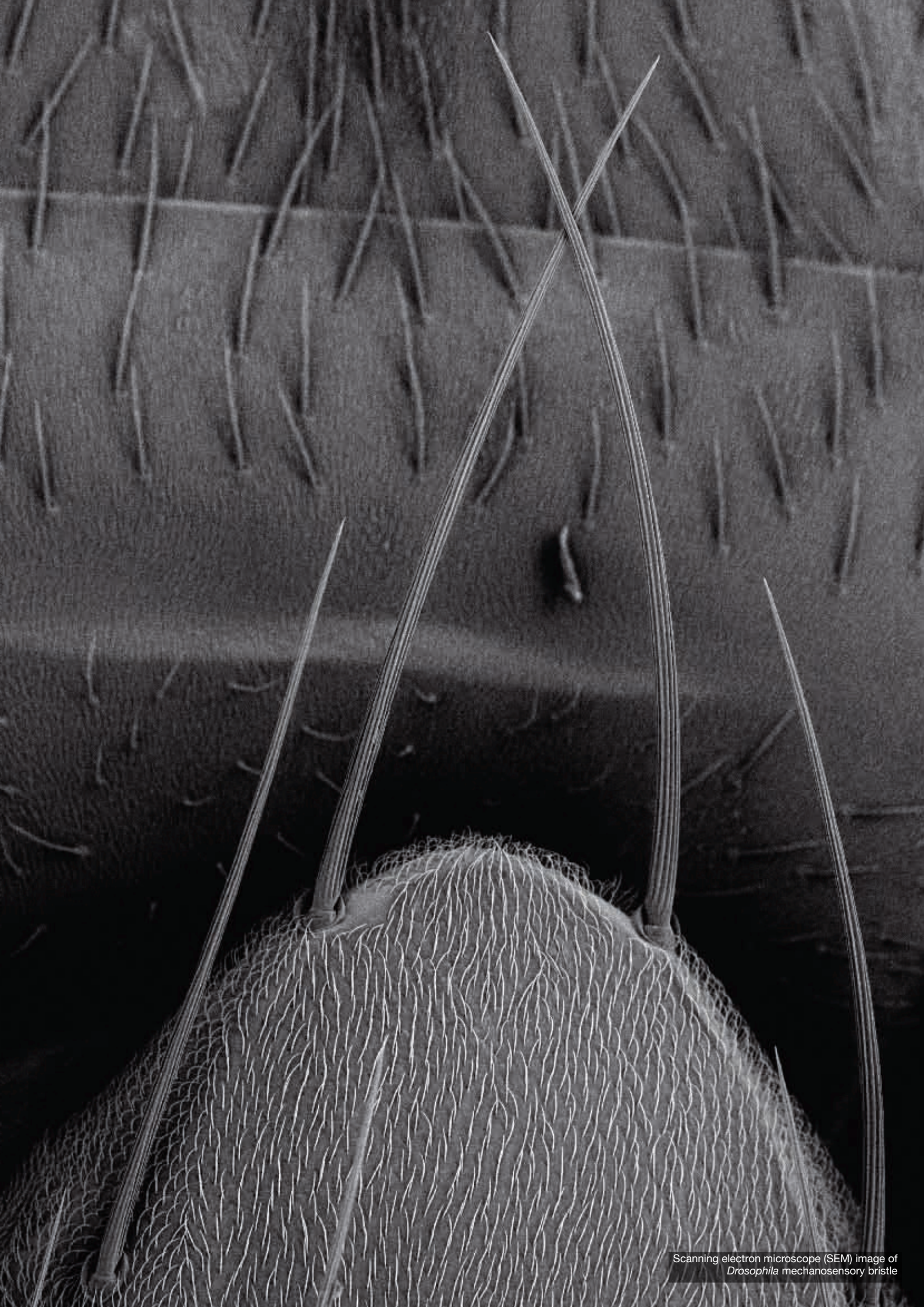
Using the African clawed frog, *Xenopus laevis,* as a model in molecular embryological studies, our group is engaged in clarifying the structure and extent of the signaling networks involved in setting up the dorsal-ventral axis and determining neural fate in the ectoderm. These studies focus on molecules 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 primate embryonic stem (ES) cells to differentiate into a range of specialized neuronal types. The application of similar techniques to human ES cells represents a field of study that, although still at an early stage, shows immense clinical promise.

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



Self-formation of layered cortical tissue in three-dimensional culture of human ES cells (red: the telencephalic marker Bf1)



Scanning electron microscope (SEM) image of *Drosophila* mechanosensory bristle

Orientation of asymmetry by external factors

Yukinobu ARATA

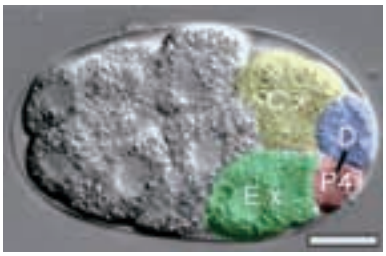
The diversity of cell types in the animal body is the result processes such as asymmetric cell division, in which a mother cell divides mitotically to give rise to two daughters of different character. The axis of asymmetric cell division is critical for determining the future positions of the differentiated progeny in developing embryos. These processes have been intensively studied in models such as the developing nervous system in the fruit fly and in the earliest stages of roundworm embryogenesis. Despite the importance of these phenomena and the scientific attention paid to them, a number of fundamental questions remain. Particularly controversial among these is the problem of whether various events that occur during asymmetric division are regulated by extrinsic or intrinsic mechanisms.

Yukinobu Arata and others in the Laboratory for Cell Fate Decision (Hitoshi Sawa, Team Leader), working in collaboration with scientists from the University of North Carolina at Chapel Hill, addressed this question in fine detail with respect to some of the earliest cell divisions in the *C. elegans* embryo. In a report published in the journal *Development*, they showed that while the asymmetric distribution of the protein PAR-2 occurs in a cell-autonomous manner, its localization to the point of cell-cell contact relies on external signals mediated by the transmembrane protein MES-1 and tyrosine kinase SRC-1.

Arata and colleagues focused on a set of germline precursor cells (P0 through P4) that arise in successive rounds of asymmetric cell division in the very early *C. elegans* embryo, and their interactions with neighboring cells. One important characteristic of the P cell lineage is that the axis of the P3 and P4 polarity is in reversed orientation to that of the P0 and P1 cells. This polarity reversal allows the P2 cell to give rise to a P3 daughter in contact with the endodermal precursor E, and for P3 to generate a P4 cell that is also in contact with an endodermal precur-

Arata Y, et al. Extracellular control of PAR protein localization during asymmetric cell division in the C. elegans embryo. *Development* 137.3337-45 (2010)

sor, E.x. PAR polarity proteins are known to localize to the points of contact between the germline and endodermal precursor cells, suggesting extracellular signals are at work. However, some previous studies had suggested that the regulation of the polarity reversal that takes place between the P2 and P3 divisions is cell-intrinsic.



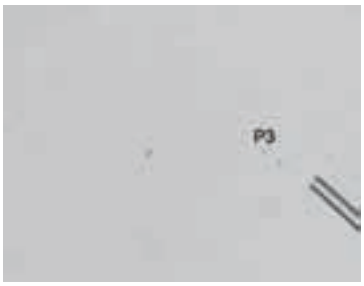
16-cell stage of *C. elegans* embryo

The team sought to gain a better understanding of this process by isolating individual P-lineage cells from developing embryos in vitro using glass micropipettes. They were surprised to find that the P4 cells deriving from isolates were frequently in inverted position, suggesting that the P3 division axis might rely on extracellular cues. To test this possibility, Arata altered the contact site by isolating cells and re-attaching germline precursors with either of the endodermal precursor cells and watched for effects on the division axis. The results showed that endodermal precursor cells exercise a clear influence on P cell disposition, indicating the axis of division is determined by extracellular signals. The daughters of P2 and 3 cells attached to control cells, however, showed highly variable division axes, highlighting the necessity of external inputs in axis orientation.

They used a similar strategy to look at protein distribution and centrosome positioning. In P cells exposed to both endodermal precursor and control cells, asymmetric distribution of PAR proteins occurred, but interestingly the localization of these proteins to the point of cell-cell contact failed to take place in P cells attached to control cells. Simultaneously, the centrosome in P cells in contact with endodermal lineage cells consistently located adjacent to the site of contact between cells, but when control cells were used, the centrosome location was variable, suggesting that external cues from the endodermal cells determine the orientation of asymmetric cell division by regulating both PAR protein distribution and centrosome orientation.

The molecules responsible remained unknown but, based on previous reports of the role of two factors—MES-1 and SRC-1—in establishing differential daughter cell sizes, the team investigated the possibility that they might be involved in axis determination as well.

In embryos with losses of function in *mes-1* and *src-1* they found that the PAR-2 protein was asymmetrically distributed as usual, but that it often failed to accumulate at the cell-cell contact site indicating that these genes shows clear defects in axis orientation. And lastly, to determine whether MES-1 and SRC-1 were required in the P lineage cell or its endodermal neighbor, they tested various combinations of chimeric pairs of P and endodermal cells using cells isolated from mutants and wild type embryos, and found that while the transmembrane protein MES-1 plays an essential part on both sides of the cell-cell junction, the tyrosine kinase SRC-1 is needed only in the signal-receiving P cell. This is the first experimental demonstration in which cell positions were manipulated to directly test the role of cell signaling on directional control of PAR protein localization during asymmetric cell division, work that may shed new light on the directional control of cell polarization in many other developmental processes.



Cells in the embryo were isolated and manipulated using a glass micropipette.

"I observed cell divisions of cells isolated by micropipette from developing embryos in vitro, and I have to say that I never got tired of watching them; they're quite beautiful," says Arata. "This study began when I noticed that the progeny of P2 and P3 were sometimes in positions opposite to their normal ones. I wanted to find out why."

Purkinje neurons from ES cells

Keiko MUGURUMA

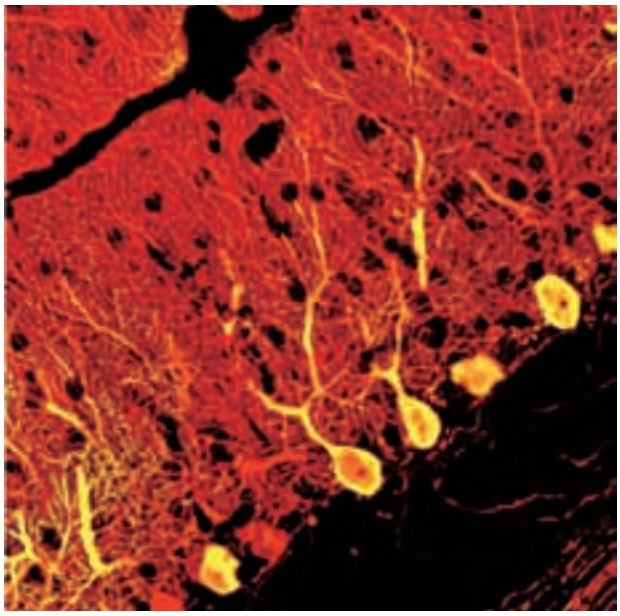
The cerebellum is one of the largest regions of the mammalian brain, and is responsible for controlling motor and other bodily functions. These functions are enabled by the activity of a distinct group of large and highly arborized cells known as Purkinje neurons, which integrate multiple inputs from other parts of the brain through their many dendrites and send inhibitory signals to cerebellar nuclei involved in motor coordination. The loss of Purkinje cell function is associated with a severe form of ataxia (loss of motor function), making this cell type one of tremendous potential both for the study of disease and the development of future clinical applications. Attempts to generate Purkinje neurons from stem cell sources at high efficiencies in vitro, however, have proven difficult.

Keiko Muguruma and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director), working in collaboration with scientists from the KAN Institute and Kyoto University, achieved a breakthrough in Purkinje cell induction, selectively deriving these neurons from mouse embryonic stem (ES) cells. In a report published in *Nature Neuroscience*, the group showed that, by recapitulating aspects of the cerebellar developmental environment, they were able to generate functional Purkinje cells in quantity.

The work began using a technique for neuronal induction using serum-free culture of embryoid body-like aggregates (SFEBq) previously developed by the group and applied to the differentiation of numerous neural cell types. In order to adapt this protocol to Purkinje induction, they looked to how these neurons are triggered to develop in the embryo itself. Purkinje cell precursors are induced by signals from a region in the posterior midbrain known as the isthmic organizer. By simulating this structure in cultured cells, Muguruma sought to give rise secondarily to Purkinje neurons.

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

Using a trial and error approach, she tested various transcription factors at different timepoints and in different orders, before finally arriving at a method for inducing cerebellar tissue. This involves SFEBq-culturing mouse ES cells for 1–2 days before adding the caudalizing factors insulin and FGF2, yielding an isthmic organizer-like tissue after four days. This region secretes inductive factors, which over time lead to the differentiation of cerebellar precursors at a very high efficiency of around 80%. Subsequent addition of an inhibitor of the ventralizing factor hedgehog to these precursors at day 13 gives rise to Purkinje neurons at a rate of around 30% of all cells by day 19. Subjecting this population to cell sorting by flow cytometry allows for even greater purities, of up to about 90%.



ES cell-derived Purkinje cells (yellow) integrate into mouse cerebellum

The Purkinje neurons derived through this approach were similar in morphology and surface marker expression, but would they function in the same way as their embryonic equivalents? Muguruma introduced ES-derived Purkinje cells into the cerebella of day 16 mouse embryos to find out. Allowing the transplanted embryos to develop to term, she found that their cerebellar tissues contained many such cells, testifying to the power of the new technique. The transplanted Purkinje neurons not only integrated into the tissue, but also formed functional neural circuits, and exhibited normal electrophysiology and neuronal inputs.

“It is still early days,” says Sasai, “but if we are able to derive populations of human cerebellar neurons in a similar fashion, these might be of great benefit to the study and treatment of diseases such as spinocerebellar ataxia in the future.”

Del1 quells Wnt in head development

Akira TAKAI

In amphibians, the anterior-posterior axis develops headfirst, with forebrain representing the default fate and more caudal tissues emerging secondarily through the action of multiple posteriorizing factors. These factors play an instructive role in specifying the fates that targeted regions adopt, but also work to suppress the development of anterior structures. It is this combination of inhibition and induction that sets up the gradient of tissue identities along the A-P axis. Of the many known factors of this kind, Wnt stands out for its potent caudalizing effect, but what prevents such posterior factors from transforming anterior structures as well?

That question has been answered in part through a study by Akira Takai and others in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director), which showed that the secreted protein Del1 inhibits Wnt signaling in embryonic head regions in the African clawed frog, *Xenopus laevis*. Published in the journal *Development*, the report describes how this extracellular matrix protein staves off its posteriorizing effects through interaction with a downstream component of the canonical Wnt pathway.

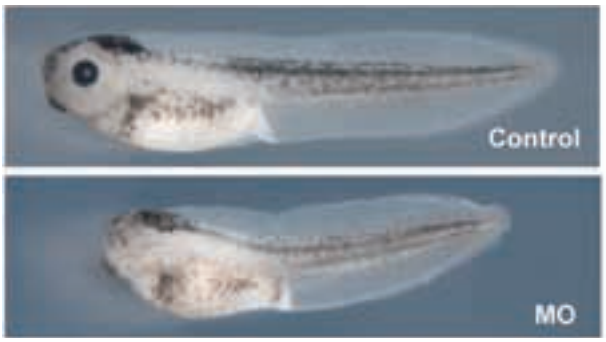
Del1 was already well known to the group from previous work in which they had shown that the protein inhibited BMP, a major ventralizing factor that works in the establishment of the dorsal-ventral axis. So they were intrigued when they found that injection of *Del1* mRNA into a different part of the early embryo resulted in a phenotype with an oversized head and eyes. To validate that finding, they tested for *Del1* effect in artificially induced secondary axes, which can be triggered by injection of anti-BMP factors, such as *Chd*. Such axes rarely develop anterior structures, but when *Del1* was co-injected with *Chd*, eye tissue formed, indicating that misexpression of *Del1* promotes anteriorization. The converse was also true—loss of *Del1* function (induced by morpholino) caused embryos to develop smaller than normal heads.

The group next looked at the structure of the Del1 protein in an effort to identify the domain responsible for its anteriorizing activity. Wildtype Del1 has two EGF-like and two Discoidin domains. By selectively deleting individual domains and testing their effects on animal cap embryos (a useful model of amphibian development), they pinpointed the anteriorizing function to the Discoidin domains.

Now that they had the responsible domain, Takai et al. sought to identify where it fit into the larger picture in

Takai A, et al. Anterior neural development requires Del1, a matrix-associated protein that attenuates canonical Wnt signaling via the Ror2 pathway. *Development* 137. 3293-302 (2010)

molecular terms. Knowing that Wnt is an important inducer of posterior fates, they tested for *Del1* effects in Wnt-posteriorized embryos. They found that co-injection of *Del1* was sufficient to rescue embryos from this phenotype, while co-injection of other anti-BMP factors did not, excluding the possibility that *Del1*'s effect was due to its established role as a BMP antagonist. And, importantly, *Del1* did not affect phenotypes induced by other posteriorizing factors, such as FGF or Nodal, indicating that its effect in this context is specific to the Wnt pathway.

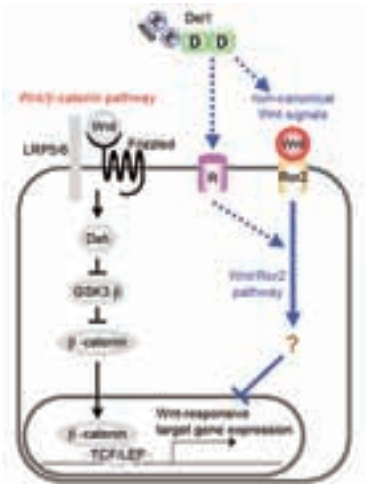


Del1 function is required for head development (wildtype at top; Del1 morphant at bottom).

The group performed a TOPflash assay, which uses luciferase to visualize ß-catenin-mediated transcriptional activation, to watch how Del1 might be interacting with the canonical Wnt pathway (in which ß-catenin is a key component). Their initial results suggested that *Del1* works downstream of ß-catenin, which was reconfirmed in immunostaining experiments that showed that ß-catenin localized to the nucleus even in the presence of *Del1*. The mechanism, however, remained unclear.

The non-canonical Wnt signaling pathway protein Ror2 seemed a promising candidate, as it had previously been shown to have a downstream inhibitory effect on Wnt. A preliminary test of this notion, in which *Ror2* was inhibited in an embryo in which *Del1* was overexpressed, showed a weakening of the *Del1*+ phenotype, suggesting possible interactivity. In a clever test of this relationship, the group added a morpholino that inhibited Ror2 to embryos that had been induced by Del1, and found that the depletion of Ror2 significantly reduced the usual anteriorization.

"We found it intriguing that Del1, a matrix protein secreted from the anterior neural region, functions as a barrier to protect the region from caudalizing Wnt signals," says Sasai. "You might say that Del1 acts as a kind of shield in the maintenance of regional identity."



Model schematic of how Del1 inhibits Wnt/ß-catenin in a Ror2-dependent manner.

New anti-BMP mechanism unveiled

Rieko YAKURA, Toshihiro ARAMAKI

Powerful molecular players frequently require major powerful inhibitors to keep them in check. Bone morphogenetic protein, or BMP, is one such factor, playing central roles in multiple developmental processes; its suppression in appropriate contexts is likewise a critical component of many embryogenetic programs. The induction and patterning of neural tissues provides a good example of this to-and-fro. BMP, which is highly expressed on the embryo's ventral side, is suppressed by inhibitors secreted from the Spemann organizer, leading to neural induction. BMP signaling is also involved in the subsequent pattern formation in this neural tissue. But, while it is clear that the suppression of the BMP pathway at given sites and time-points is crucial to the patterning of the early embryo, just how this is accomplished is imperfectly understood.

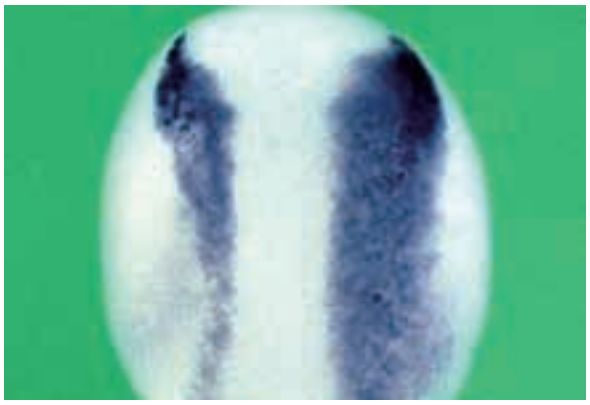
Toshihiro Aramaki and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director) shed light on this process through the lens of a new controller of BMP signaling. In a report published in *Developmental Cell*, the group identified the membrane protein Jiraiya as a specific inhibitor of a subunit of the BMP receptor in the African clawed frog, *Xenopus laevis*.

Jiraiya first caught the researchers' eye when it was captured in a screen for genes downstream of the BMP inhibitor Chordin. Its dorsal expression pattern recalled a character, named Jiraiya, who appears mounted on an amphibian in a famous kaubuki play, thus earning it its colorful name. The protein is first expressed in the dorsal ectoderm of the early gastrula, shifting subsequently to the dorsal aspect of the neural tube. When Aramaki knocked down its function using a morpholino antisense oligonucleotide, he saw an increase in the scope of expression of lateral neural plate marker genes in the treated embryos, accompanied by upregulation of activated

Aramaki T, et al. Jiraiya attenuates BMP signaling by interfering with type II BMP receptors in neuroectodermal patterning. *Dev Cell* 19.547-61 (2010)

Smad1, a factor downstream in the BMP pathway, suggesting that this newly identified protein worked to limit dorsal neuralization.

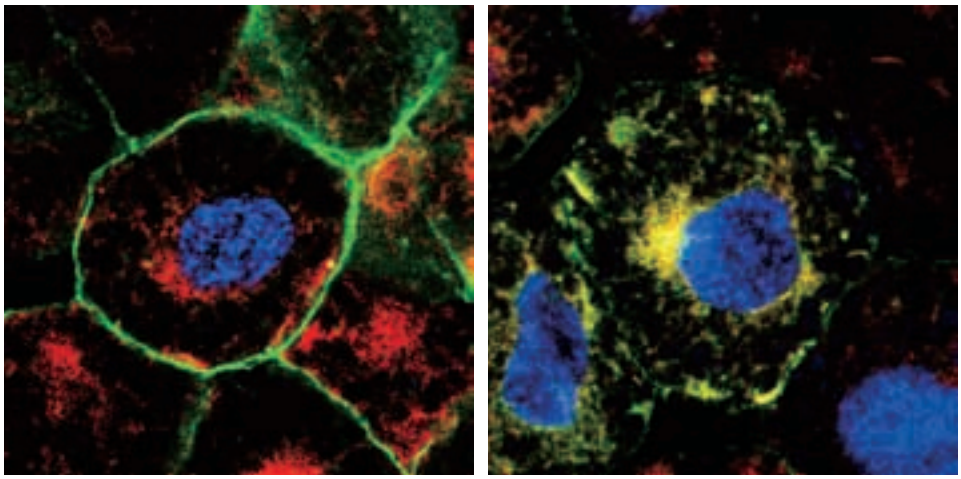
To get a clearer sense of the mechanism, the group used an animal cap assay of embryonic cells to test the effect of Jiraiya overexpression. They found that Jiraiya interferes with the function of BMPRII, one of two subunits of the BMP receptor. The suppressor effect was specific to BMP; no inhibition was seen, for example, of a second member of the TGF-ß family, activin. The question was: how does Jiraiya achieve this? Immunostaining revealed that Jiraiya tended to accumulate more in the cytoplasm than at the plasma membrane, suggesting it might work intracellularly to block the function of the membrane-bound BMPRII, which was supported by the failure of this receptor component to integrate into the cell membrane in cells overexpressing Jiraiya.



Expansion of lateral neural plate marker gene expression (right) following Jiraiya knockdown

They next looked for the site of interaction between these proteins by creating a series of mutant proteins from which various domains were deleted. They found that when BMPRII lost its C-terminal, it became much less sensitive to Jiraiya's quelling effect. Compellingly, the BMPRII tail domain contains a highly conserved 6-amino snippet not found in any other TGF-ß receptor family member, suggesting a locus for the specificity of the BMPRII-Jiraiya interaction. The finding represents the first identification of a receptor-level inhibitor of BMP.

"We hope to be able to tease out Jiraiya's mechanism of action in vivo," says Sasai. "It will be especially interesting to work out how Jiraiya functions in mammals, and specifically whether it inhibits the BMP pathway there as well."



BMPRII (green) accumulates at plasma membrane in control cells (left), but is sequestered cytoplasmically in Jiraiya-overexpressing cells (right), resulting in much lower expression of this receptor subunit in the membrane.

Stage-dependent outcomes of symmetric stem cell division

Fumio MATSUZAKI, Atsushi KITAJIMA

Asymmetric cell division, in which a single mother cell divides to give rise daughters with different properties such as gene expression and morphology, is a fundamental mechanism in the generation of the many types of cells in animal bodies. This phenomenon has been extensively studied in the nervous system of the fruit fly *Drosophila melanogaster*, in which specific protein factors are allocated to the basal side of dividing neuronal progenitors, steering the cell that inherits these basal determinants to a ganglion mother cell (GMC) fate, while its mitotic sibling becomes a larger neuroblast. Past studies have shown that daughter cell characteristics such as cell size and protein localization are under the control of independent molecular mechanisms. But the downstream consequences of the loss of asymmetry in these features remain something of an open question.

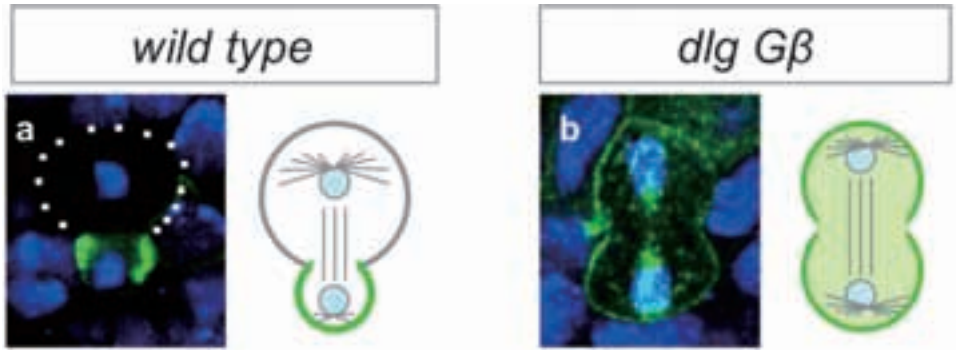
Working with mutants for a pair of genes linked to the maintenance of asymmetric mitosis, Atsushi Kitajima and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director), in collaboration with the National Institute of Genetics, observed how the loss of these functions leads to abnormal progeny. The study, published in *Developmental Biology*, revealed that, in embryos of these mutants, the daughter cells that would normally form neuroblasts instead differentiate into neurons.

Kitajima began by generating flies carrying mutations for *Gβ13* (which is associated with maintaining differential sizes in the daughters of neural progenitors) and *dlg* (an oncogene linked with controlling the asymmetric intracellular localization of fate determinants). As expected, neuroblast progeny in these cells showed abnormal symmetry in cell size and equal distribution of determinant proteins. The group next monitored the fate of cells born from the mutant neuroblasts in embryos, and determined that while the newborn daughters bore the hallmarks of neuroblasts and GMCs, the stem cell nature of the progeny neuroblasts was not maintained, as most ultimately differentiated into neurons.

Asymmetric cell division is seen not only at embryonic stages, but in larval flies as well. Using a conditional knockout to interfere with *Gβ13* and *dlg* function in specific tissues and observe the lineage effects. Interestingly, Kitajima et al. found that unlike in the embryo, the double-mutant cells failed to differentiate, and proliferated abnormally instead in a manner similar to that seen in mutants of the basal determinant Prospero (itself an oncogene). The authors speculate that the larger size

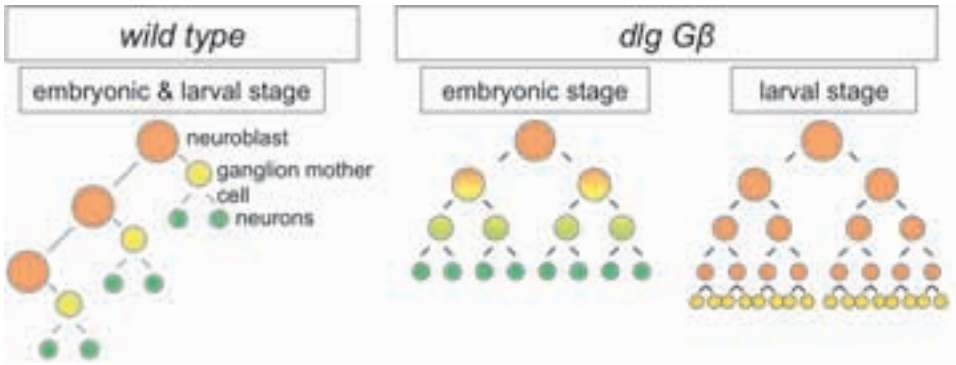
Kitajima A, et al. Progenitor properties of symmetrically dividing Drosophila neuroblasts during embryonic and larval development. *Dev Biol* 347. 9-23 (2010)

of the mutant progeny may prevent a sufficient amount of Prospero from being allocated to a single daughter (the presumptive GMC), leading to misregulation of cell proliferation in larval neural tissue, a surmise that was borne out by the finding that overexpression of Prospero in the double-mutants rescued the phenotype.



In asymmetrically dividing wildtype cells (right), Miranda (green) is sequestered to only one of the daughter cells. In *dlg-Gβ* mutants, Miranda is distributed to both daughters.

"It is intriguing how symmetrically dividing stem cells behave so differently in embryonic and larval contexts," comments Matsuzaki. "We see how the same combination of mutations can lead to terminal differentiation in one milieu, but tumor-like over-proliferation in another. In the future, we hope to determine whether this holds true in other species as well."



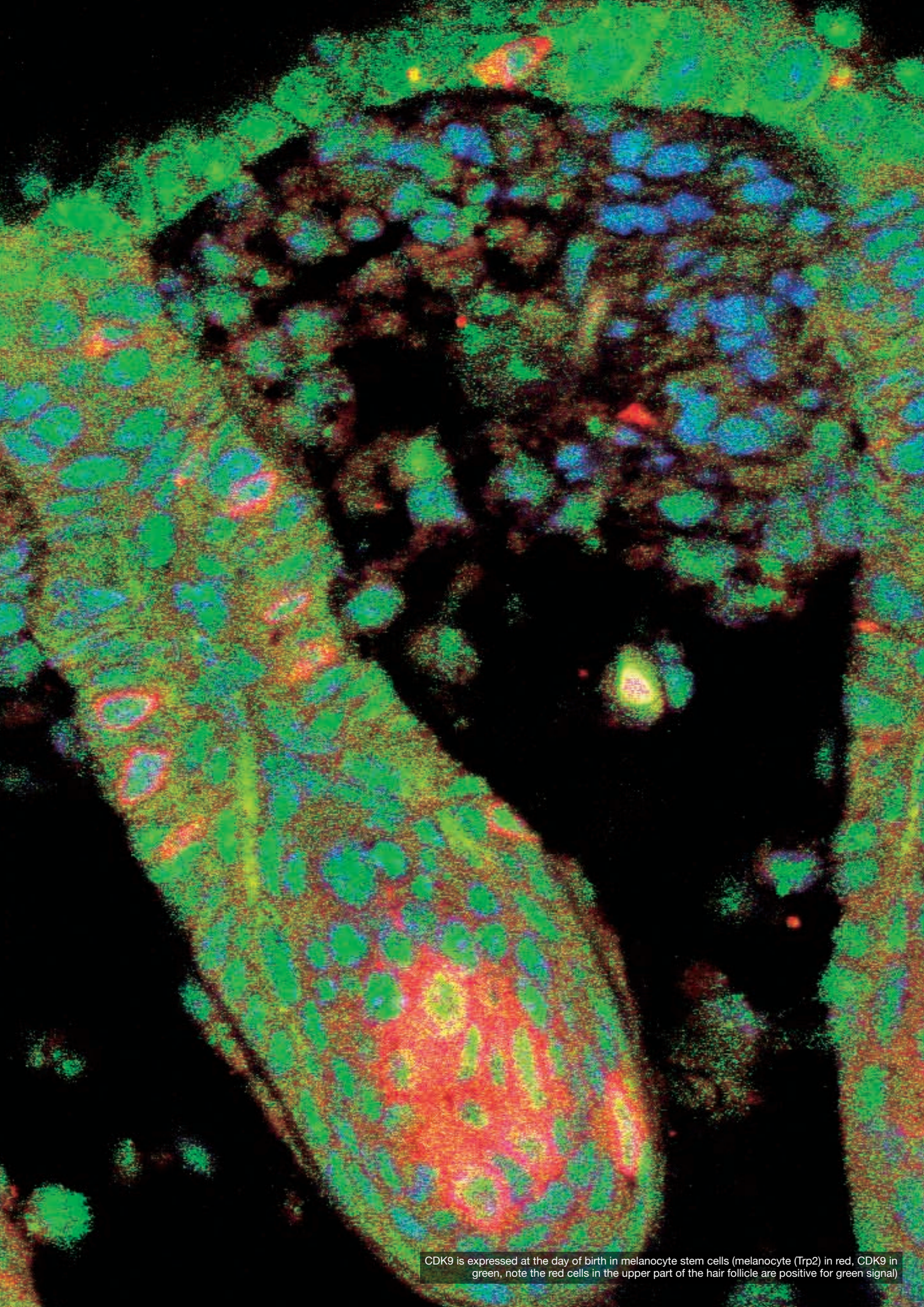
Cartoon scheme showing differences in the differentiation and proliferation of progeny cells born from wildtype, as well as *dlg-Gβ* embryonic and adult-stage neuronal progenitor cells.

2010 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
Teruhiko Wakayama	Team Leader	Genomic Reprogramming	MEXT Prize	Ministry of Education, Culture, Sports, Science and Technology
Jun-ichi Nakayama	Team Leader	Chromatin Dynamics	MEXT Prize	Ministry of Education, Culture, Sports, Science and Technology
Naoki Irie	Research Scientist	Evolutionary Morphogenesis	2010 Poster Prize	Society of Evolutionary Studies, Japan
Takao Suzuki	Research Scientist	Evolutionary Morphogenesis	2010 Poster Prize	Society of Evolutionary Studies, Japan
Chong Li	Junior Research Associate	Genomic Reprogramming	Best Poster Prize	International Society for Transgenic Technologies
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Osaka Science Prize	Osaka Science and Technology Foundation
Shigeru Kuratani	Group Director	Evolutionary Morphogenesis	Hyogo Science Prize	Hyogo Prefecture
Yukari Terashita	Student Trainee	Genomic Reprogramming	Award for excellent poster presentation	Asia Reproductive Biotechnology Society
Teruhiko Wakayama	Team Leader	Genomic Reprogramming	Yamazaki Teiichi Prize	Foundation for Promotion of Material Science and Technology of Japan



CDK9 is expressed at the day of birth in melanocyte stem cells (melanocyte (Trp2) in red, CDK9 in green, note the red cells in the upper part of the hair follicle are positive for green signal)

Embryonic Induction

Hiroshi SASAKI Ph.D.

http://www.cdb.riken.go.jp/en/sasaki

Hiroshi Sasaki received his B. Sc. in zoology from the University of Tokyo, and his master's and Ph.D. in developmental biology from the same institution. He worked as a research associate in Atsushi Kuroiwa's group at Tohoku University from 1990 to 1992, and in Brigid Hogan's lab at Vanderbilt University from 1992 to 1994. He returned to Japan to take a position as assistant professor at Osaka University beginning in 1995, where he remained until his appointment as team leader at the RIKEN in 2002.

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Publications

Nishioka N, et al. The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev Cell* 16. 398-410 (2009)

Yamamoto S, et al. Cthrc1 selectively activates the planar cell polarity pathway of Wnt signaling by stabilizing the Wnt-receptor complex. *Dev Cell* 15. 23-36 (2008)

Ota M and Sasaki H. Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling. *Development* 135. 4059-69 (2008)

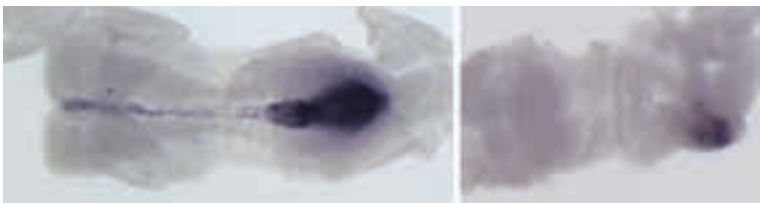
Sawada A, et al. Redundant roles of Tead1 and Tead2 in notochord development and the regulation of cell proliferation and survival. *Mol Cell* Biol 28. 3177-89 (2008)

Nishioka N, et al. Tead4 is required for specification of trophectoderm in pre-implantation mouse embryos. *Mech Dev* 125. 270-83 (2008)

Sawada A, et al. Tead proteins activate the Foxa2 enhancer in the node in cooperation with a second factor. *Development* 132. 4719-29 (2005)

During vertebrate embryogenesis, simply structured regions of undifferentiated cells organize and cooperate to give rise to the anterior-posterior and dorsal-ventral body axes, the neural tube and other embryonic structures deriving from the three germ layers, following developmental patterns that can be collectively summarized as the adult body plan. The embryo is a hive of activity, including cell differentiation, proliferation and migration, strictly regulated in both space and time by molecular signals emitted by special regions of the embryo referred to as “signaling centers.” These centers produce diffusible proteins that serve to regulate embryonic development, guiding neighboring cell communities to take up the roles they will play in the adult animal, or steering them away from inappropriate fates.

Research in our laboratory focuses on these signaling centers, especially those of the mouse, as a model for studying the regulation of embryogenetic processes. We focus particularly on the node and notochord, which are of central importance in the formation of the early embryo. We focus on the control of the expression of the Foxa2 transcription factor in the formation and maintenance of signaling centers, as well as search for new factors involved in the control of embryonic development by such centers. We have recently revealed that members of the Tead family of transcription factors are important not only for signaling center formation, but also in a broad range of processes in mouse development, including cell proliferation and differentiation in pre- and post-implantation embryos. Our lab is now analyzing the roles of Tead family proteins and their regulation by cell-cell contact information as a new approach to the study of early mouse development.



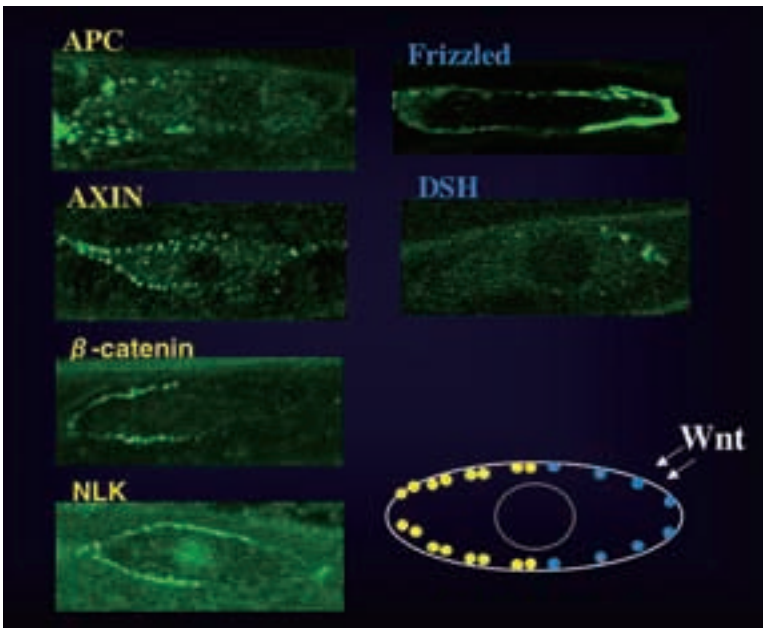
Day 8.5 mouse embryos showing that the notochord and node (signaling centers regulating trunk/tail development; stained purple on the left) is absent in the *Tead1;Tead2* double mutant (right; remaining staining is in the primitive streak). *Tead1;Tead2* double mutants also have defects in cell proliferation and apoptosis.

Cell Fate Decision

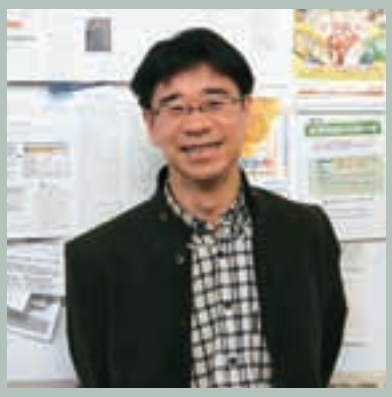
The development of multicellular organisms involves a single cell (such as a fertilized egg) which gives rise to diverse progeny through successive cell divisions. The process of cell division in which the resulting daughter cells are of different characters or "fates" is known as asymmetric cell division. One example of this form of cell division is seen in stem cells, which can produce one copy of themselves and one more specialized (differentiated) cell in a single division. Asymmetric cell division is central to the generation of cellular diversity, but because in higher organisms a given cell's "family relations" (known as cell lineage) are frequently unclear, it can be difficult to study the process in detail.

In the nematode, *C. elegans*, however, thanks to the transparency of the worm's body it is possible to track the division and other activity of individual cells in the living animal using only a low-magnification light microscope. In our laboratory, we take advantage of this ability to monitor developmental processes in real time, a method which we use in combination with genetic studies to investigate the mechanisms that underlie asymmetric division. We have determined that nearly every asymmetric cell division in *C. elegans* is mediated by β-catenins acting in the Wnt signaling pathway. This pathway works to polarize cells fated to undergo asymmetric division; we seek to gain a better understanding of how it achieves this by studying the intracellular localization of the Wnt pathway's molecular components.

We have also discovered numerous mutant *C. elegans* phenotypes in which the asymmetric division of specific cells is disturbed, allowing us to identify more than 50 genes involved in this process. By investigating the function of these genes, we hope to clarify the biological mechanisms of asymmetric cell division even further.



Asymmetric cortical localization of the Wnt pathway components



Hitoshi SAWA Ph.D.

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Hitoshi Sawa obtained his baccalaureate, master's and doctoral degrees from Kyoto University in the period from 1982 to 1991. He worked as a postdoctoral fellow at the California Institute of Technology on a Human Frontier Science Program grant during the period from 1991 to 1994, then at the Massachusetts Institute of Technology from 1994 to 1997 under support from the Howard Hughes Medical Institute. He returned to Japan in 1997 to continue his work at Osaka University, first as a postdoc, then as researcher funded by the Japan Science and Technology Corporation PRESTO program. He took his current position at the RIKEN CDB in 2001. He was appointed team leader at the RIKEN CDB in 2001. He was appointed professor in the National Institute of Genetics in 2010.

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Publications

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Early Embryogenesis

Guojun SHENG Ph.D.

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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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Publications

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

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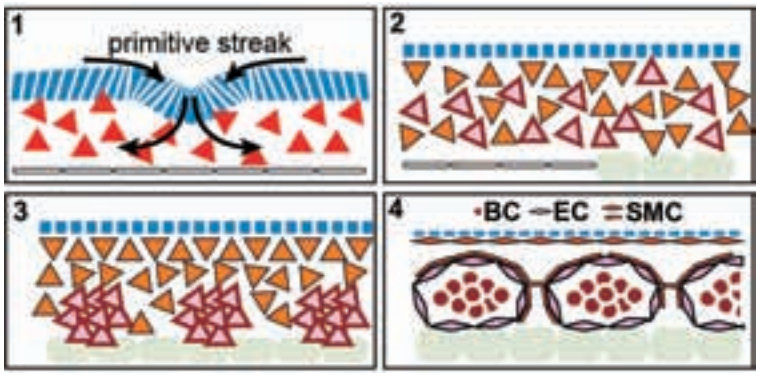
Our lab studies how mesoderm cells form and differentiate during early vertebrate development, using the chick as the main model organism. Our research currently focuses on two aspects: 1) the formation and early regionalization of mesoderm cells during gastrulation; 2) differentiation of ventral-most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



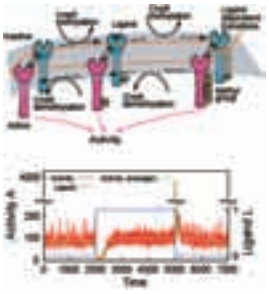
The circulatory system of early chicken embryos (E5 shown here) is complex and contains embryonic, allantoic, and yolk sac sub-systems. The diversity of blood cells during this period is revealed by the transcriptomic analysis of non-red blood cells in circulation.

Physical Biology

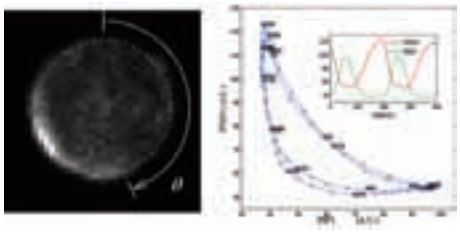
Advances in measurement technologies have afforded us glimpses into dynamic functional processes, such as morphogenesis and information processing, in cells and tissues involved in development and regeneration. The truly organic dynamism of the biological phenomena exhibited by living cells, individually 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 molecule 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.



Simulated stochastic behavior of signal transduction system that is responsible for response and adaptation in bacterial chemotaxis.



Spontaneous formation of localized signal of Phosphatidylinositol 3,4,5-trisphosphate (PIP3) and the averaged dynamics of PIP3 and PTEN.

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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Publications

Arai Y, et al. Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. *Proc Natl Acad Sci USA* 107. 12399-404 (2010)

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Shibata T and Fujimoto K. Noisy signal amplification in ultrasensitive signal transduction. *Proc Natl Acad Sci USA* 102. 331-6 (2005)

Developmental Genomics

Asako SUGIMOTO Ph.D.

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Asako Sugimoto received her B. Sc. from the Department of Biophysics and Biochemistry in the University of Tokyo, School of Science in 1987, and her doctorate from the same institution in 1992. She worked as a postdoctoral fellow in Joel Rothman's laboratory in the University of Wisconsin-Madison from 1992 to 1996, before returning to Japan to assume an assistant professorship at the University of Tokyo. She remained in that position until 2002, pursuing concurrent work as a Japan Science and Technology Corporation PRESTO researcher from 1997 to 2000. She was appointed team leader at the RIKEN CDB in 2001. In April 2010, she was appointed professor at Graduate School of Life Sciences, Tohoku University.

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Publications

Hanazawa M, et al. PGL proteins self associate and bind RNPs to mediate germ granule assembly in *C. elegans. J Cell Biol (2011) in press*

Terasawa M., et al. *Caenorhabditis elegans* ortholog of the p24/p22 subunit, DNC-3, is essential for the formation of the dynactin complex by bridging DNC-1/p150 (Glued) and DNC-2/dynamitin. *Genes Cells* 15.1145-57 (2010)

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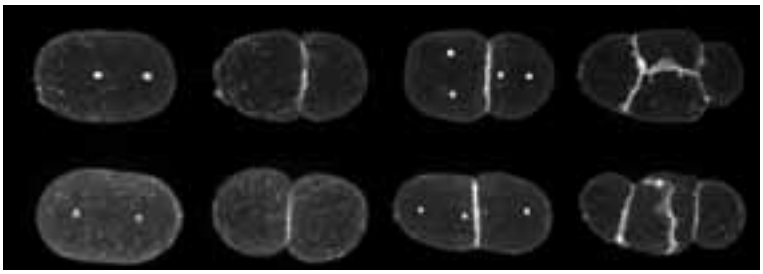
Fertilized eggs give rise to complex animal structures through strict coordination of cell divisions, cell fate determination and differentiation. To generate two daughter cells with distinct cell fates from a single cell division (called “asymmetric cell division”), the mother cell is polarized before dividing and cytoplasmic components are asymmetrically segregated. A mitotic spindle is formed and positioned along the polarized axis, and the cell division produces two daughter cells that contain distinct cell contents.

Our laboratory aims to understand the gene/protein networks controlling these dynamic cellular processes using the nematode *Caenorhabditis elegans* embryos as an experimental model. This organism provides an extremely useful system for studying such processes at the whole genome level, as its genome has been fully sequenced and the lineage of each cell in its body is known. Using high-resolution live microscopy to trace dynamic behaviors of proteins in combination with gene knockdown by RNAi, we are investigating the gene networks that control these processes.

Our current research focuses on understanding the spatio-temporal regulatory mechanisms of mitotic spindle formation. We revealed that two genetically separate pathways for microtubule assembly, one involving γ-tubulin and the other requiring aurora-A kinase, are responsible for the formation of mitotic spindles in early *C. elegans* embryos. We are investigating how these pathways spatially and temporally coordinate to form mitotic spindles.

In addition to studying mitotic spindle formation, we are also studying two other phenomena which are crucial for cell fate determination in early embryogenesis of *C. elegans*. The first is how mitotic spindles are positioned within the cell according to the established cell polarity. The second is how germ granules (large complexes of mRNAs and proteins, believed to be the “germ cell determinants”) are assembled and segregated specifically into the germ lineage. Through these studies, we hope to develop a more detailed picture of the regulation of dynamic cellular processes by gene networks.



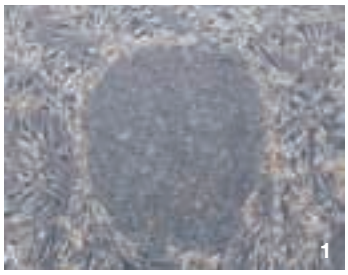
The first two rounds of cell divisions in *C. elegans* embryos. Centrosomes and cell membrane are labeled with GFP. Top: wildtype. Bottom: par-3 mutant, showing a defect in the cell division axis.

Retinal Regeneration

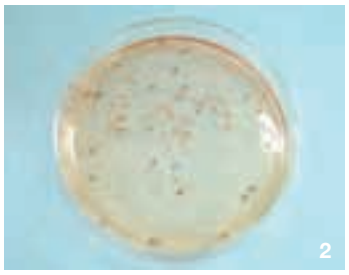
The retina has been called the “most approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that 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.

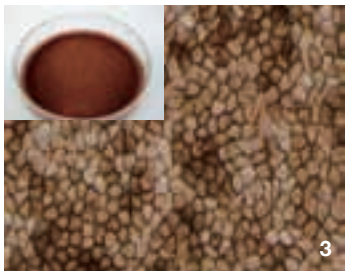
Therapeutic applications cannot be developed from basic research alone; the clinical approach—a thorough understanding of the medical condition to be treated—is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily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



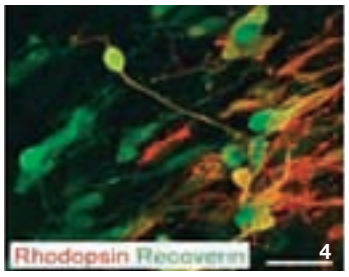
Human iPS cells



Retinal pigment epithelial cells differentiated from iPS cells



Purified retinal pigment epithelial cells



Photoreceptor cells differentiated from iPS cells

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 came back 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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Jin ZB, Okamoto S, Mandai M and Takahashi M. Induced Pluripotent Stem Cells for Retinal Degenerative Diseases: A New Perspective on the Challenges, *J Genetics* 88:4, 417-424 (2009)

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

Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph.D.

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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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Publications

Ito S and Takeichi M Dendrites of cerebellar granule cells correctly recognize their target axons for synaptogenesis in vitro. *Proc Natl Acad Sci U S A* 106. 12782-7 (2009)

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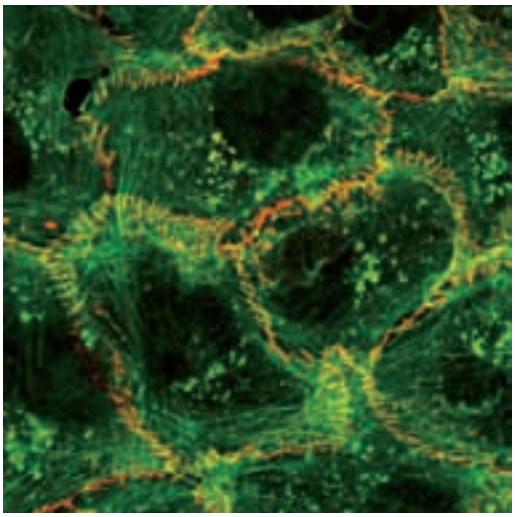
Animal cells organize into tissues with complex architecture. Our lab is exploring the molecular mechanisms by which individual cells assemble into a tissue-specific multicellular structure, such as epithelial sheets and neural networks. We are also interested in the molecular basis of how the tissue 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.

Cell-cell adhesion is an active process, which can be affected by a number of cell-internal and external factors. This dynamic nature of cell-cell adhesion is implicated in various processes of cell assembly, such as the tightening or loosening of cell contacts and changing of cell assembly patterns. We are conducting cell biological analyses to unravel the principal mechanisms that can regulate cell-cell adhesion. A growing body of evidence suggests that cadherins cooperate with the cytoskeletal and/or motile machineries, such as actin filaments or microtubules, in modulating cell assembly. We are therefore studying the mechanisms underlying the crosstalk between cadherins and such cytoskeletal systems. These studies include analysis of the functions of microtubule minus end-associated proteins and kinesin motors, as well as actin dynamics regulators, in cell-cell contact regulation.

A second area of interest to our lab is to learn how the cell-cell adhesion machinery contributes to morphogenetic cell behavior. We are analyzing the roles of cadherin in cell movement, in order to explore the mechanisms underlying the contact-dependent regulation of cell locomotion. At the tissue level, using the embryonic brain as a model system, our team is attempting to determine how cadherin and associated molecules control the remodeling of neuroepithelial layers, such as neural tube closure and the migration of neuronal progenitor cells. We are also investigating the roles of protocadherins, members of the cadherin superfamily, deficiencies of which have been implicated in neuronal defects in brain development. Through these studies, we expect to gain deeper mechanistic insights into the ways by which cells build elaborate structures of the animal body.



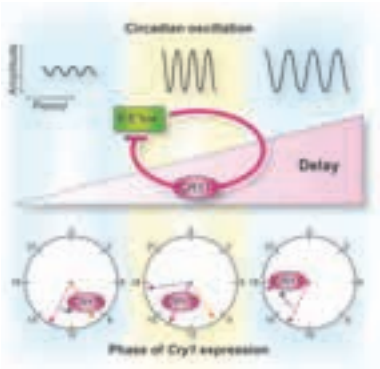
Double-immunostaining for F-actin (green) and Kusabira Orange-tagged E-cadherin (red) introduced into A431D cells. In these cells, E-cadherin dynamically moves along cortical actin filaments, resulting in the unique distributions shown here.

Systems Biology

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

Over the last several years, the Laboratory for Systems Biology (LSB) has worked to establish Experimental Systems Biology at the Molecular-to-Cellular level and apply them to system-level questions of complex and dynamic biological systems, such as the mammalian circadian clock. In October 2009, our laboratory was re-designated as a Project Lab in the Center Director's Strategic Program for Systems Biology research to promote the challenging tasks. Based on the achievements over the last 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 a organism. To this aim, we attempt to invent and combine several key technologies ranging from (i) a rapid engineering of the genome of ES cells, (ii) generation of "100% chimera" animals for F0 phenotyping, and (iii) phenotype analysis of the 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 disorders.



Delayed Feedback repression: The essence of the clock
Cryptochrome1 (Cry1), an essential mammalian clock component, serves as a strong repressor of morning-time transcription and displays a delayed expression in the evening. Ukai-Tadenuma and Yamada et al. revealed that the delayed expression of Cry1 is realized by a combinatorial transcriptional regulation via day-time and night-time cis-elements, which can be simply recapitulated by a phase-vector model. They also revealed that delay in feedback repression is required for mammalian clock function, and that the prolonged delay in feedback repression slows circadian oscillation. These results suggest that the delayed-feedback repression is a design principle of the transcriptional circuit in mammalian circadian clocks.

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

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

Hiroki R. Ueda received his M.D. from the Faculty of Medicine of the University of Tokyo in 2000, and received his Ph.D. from the University of Tokyo Graduate School of Medicine in March 2004. While an undergraduate, he worked as a research assistant on a biological simulation system project at Sony Computer Science Laboratories. As a graduate student he went on to work as a researcher (2000) and then as a group leader (2002), at Yamanouchi Pharmaceuticals, 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 Systems Biology under CDB's Center Director's Strategic Program.

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Ukai-Tadenuma M, et al. Delay in Feedback Repression by Cryptochrome 1 Is Required for Circadian Clock Function. *Cell* 144.268-281 (2011)

Masumoto K. H, et al. Acute induction of Eya3 by late-night light stimulation triggers TSHbeta expression in photoperiodism. *Curr Biol* 20.2199-206 (2010)

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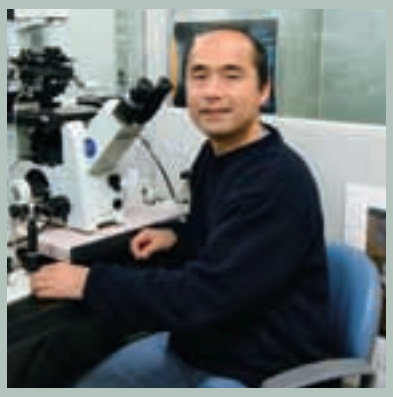
Genomic Reprogramming



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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Ono T., et al. Inhibition of class IIb histone deacetylase significantly improves cloning efficiency in mice. *Biol Reprod* 83.929-37 (2010)

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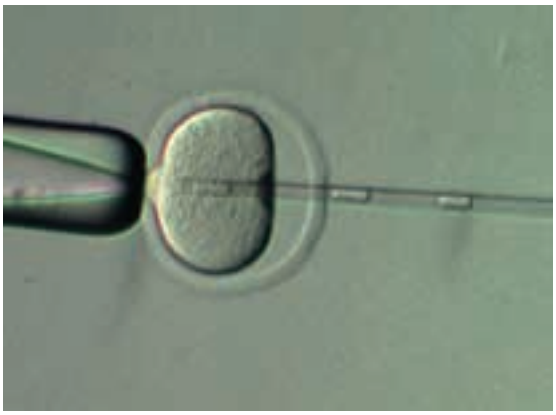
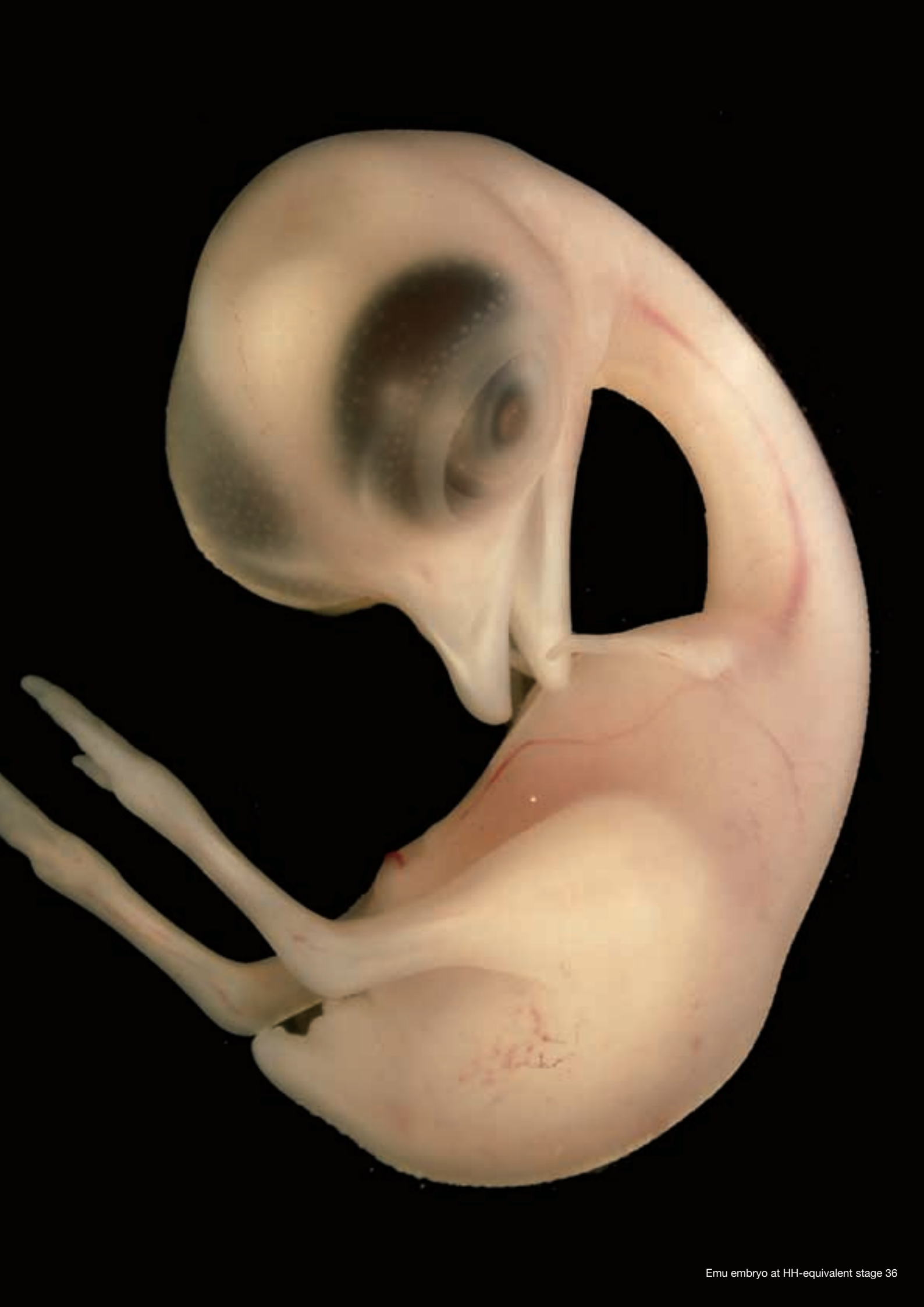
A limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual cells 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, thereby 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



Emu embryo at HH-equivalent stage 36

Transcriptional off-switch in stem cells

Rasmus FRETER

Stem cells are known mainly for their ability to self-renew and to give rise to cells of different types, but many have a third important characteristic as well: they can shut down, becoming “quiescent.” This is particularly crucial for stem cells that function in the adult body, as they need to be able to maintain tissue homeostasis by dividing to produce progeny cells in precisely the right numbers to maintain integrity without over-proliferating. This dormant state has been studied mainly in term of cell cycle regulation, cell metabolism, and interactions with the stem cell niche. More recent evidence suggests that transcriptional activity is also suppressed in quiescent stem cells, but the mechanisms by which this slowdown is achieved have yet to be identified.

A study by Rasmus Freter and others in the Laboratory for Stem Cell Biology (Shin-Ichi Nishikawa, Group Director) revealed the mechanistic basis for stem cells’ transcriptional slumber. Published in the journal *Stem Cells*, this new work reveals that adult stem cells globally suppress the phosphorylation of a specific amino acid in the tail-end of the crucial enzyme, RNA polymerase II (RNApII), in mouse.

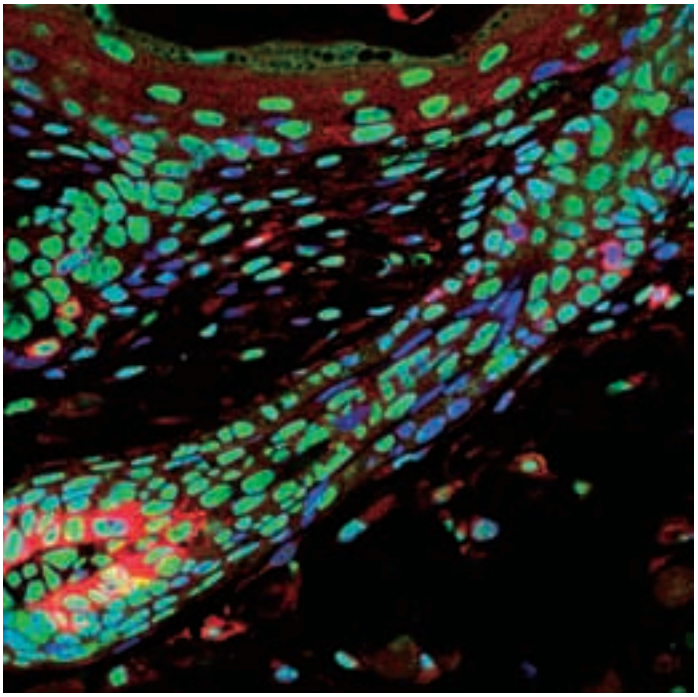
Freter began the study knowing that mRNA transcription is markedly reduced in quiescent melanocyte stem cells. Looking for the underlying mechanism, he tested for the phosphorylation of serine 2 in the C-terminal domain of RNApII, which is known to trigger elongation, a necessary step in the synthesis of mRNA. While melanocytes and migrating progenitors stained positive for phosphorylated ser2, this was downregulated in stem cells that had settled into their niche in the hair follicle.

Quiescence is typically induced when a stem cell is deprived of survival factors, which in the case of melanocyte stem cells include SCF/c-Kit. To determine whether a relationship existed between this stem cell factor and

Freter R, et al. Adult stem cells exhibit global suppression of RNA polymerase II serine-2 phosphorylation. *Stem Cells* 28.1571-80 (2010)

phosphorylation of this amino acid, the group used a transgenic mouse in which SCF is constitutively overexpressed. They found that the melanocyte stem cells in adult animals lacked phosphorylated ser2, but interestingly, that this was present at the time of birth. Apparently a short-lived signal caused dephosphorylation in the immediate postnatal period, but was later overridden by the constitutive effect of SCF in the mutant animals.

Looking for clues behind the dephosphorylation in the transgenic adults, Freter analyzed the expression of the cyclin-dependent kinase complex p-TEFb, which is composed of CDK9 and a cyclin partner, and is known to trigger the phosphorylation of ser2. He found that while CDK9 was unperturbed in differentiated cells, it was specifically downregulated in melanocyte stem cells, presumably as a result of the global transcriptional suppression that takes place in these cells. This supposition was bolstered by the finding that conditional knock-in of CDK9 did not induce phosphorylation in these melanoblasts. The group further found that the inhibition of CDK9, used here as a readout of the phosphorylation status of ser2, increased the survival of cultured cells during growth factor starvation by suppression of cell death pathways.

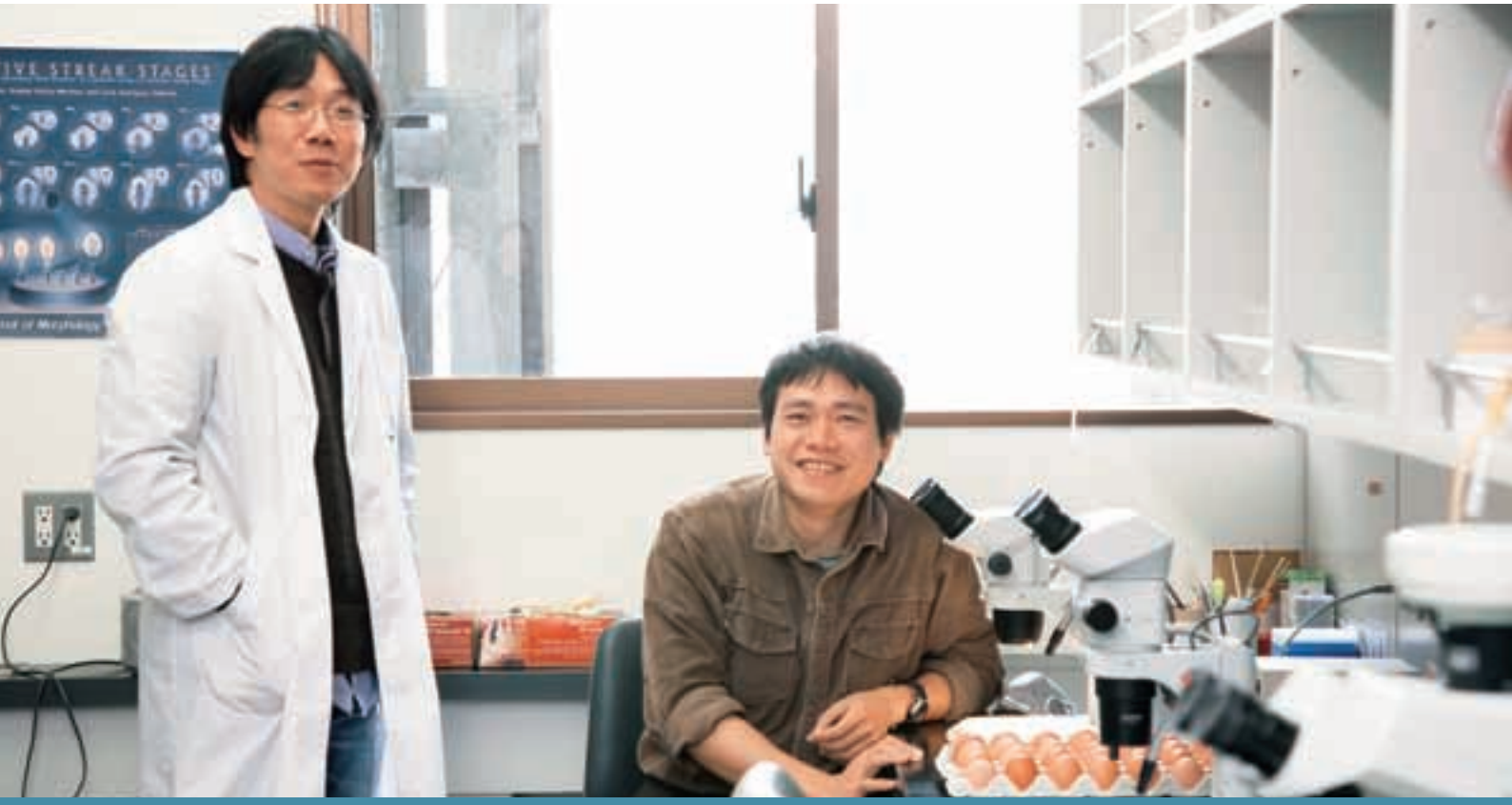


Absence of mRNA transcription elongation in adult melanocyte stem cells (melanocytes (Trp2) in red, and mRNA transcription (CTD-Ser2-p) in green).

To determine whether the role of ser2 phosphorylation in the C-terminal domain was common to quiescent stem cells in other tissues, the group examined a range of other stem cell types, including satellite cells in muscle, spermatogonia stem cells and blood-producing hematopoietic stem cells, in addition to the melanocyte and keratinocyte stem cells from the hair system. They found that in all cases, the inactive stem cells showed lower ser2 phosphorylation than that seen in actively cycling cells, suggesting that global transcriptional suppression may be a general feature of stem cell quiescence.

“Downregulation of CTD Ser2 phosphorylation is beneficial for cell survival when cells are confronted by stress, and thus maintains the stem cell compartment throughout the life of the organism,” says Freter. “The detection of transcriptional quiescent cells in organs, or even cancer tissues, may therefore be useful in the identification of cells with stem cell-like properties."

New twist on ex ovo culture in bird



Hiroki NAGAI, Meng-Chi LIN

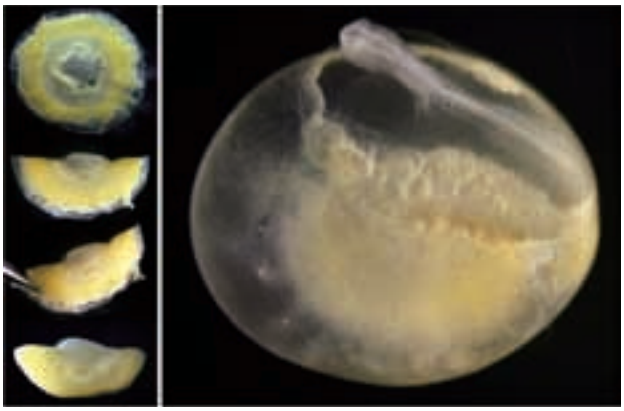
Birds, such as the chicken, provide an excellent model for the study of many developmental processes, and remain one of the most commonly studied species in classical embryology. The earliest stages of avian embryonic development are not amenable, however, to manipulation within the egg, necessitating methods for embryo culture. A number of techniques for sustaining the growth of chicken embryos ex ovo have been developed, but are limited by cumbersome set-up and relatively short survival times. Methodological advances might therefore expand the utility of ex ovo approaches and enable studies of later occurring developmental phenomena.

Hiroki Nagai and colleagues in the Laboratory for Early Embryogenesis (Guojun Sheng, Team Leader) have done just that with a new twist on ex ovo chick embryo culture, which they have christened the modified Cornish pasty (MC) method, as it is based on a previous technique that involves folding the explanted embryo in two, similar to the preparation of the well-known British folded pastry. The new method, described in the journal *Genesis*, is simpler to perform than its predecessors and sustains embryos to later stages of development.

The protocol involves removing the embryo from the egg during the primitive streak phase of development, between stages 3 and 4 on the Hamburger-Hamilton (HH) scale, folding it along an axis parallel to the primitive streak so that it takes on a half-moon shape, and transferring it to culture medium. Interestingly, within 24 hours most of the embryos "inflate," apparently due to active transport of fluid, and rise to the surface of the medium. This fluid transport is important, as it maintains epiblast tension, which is crucial for early development. The majority of embryos maintained under these conditions grew at normal rates for the first day of culture, but later slowed slightly relative to normal development.

Nagai H, et al. A modified cornish pasty method for ex ovo culture of the chick embryo. *Genesis* (2010)

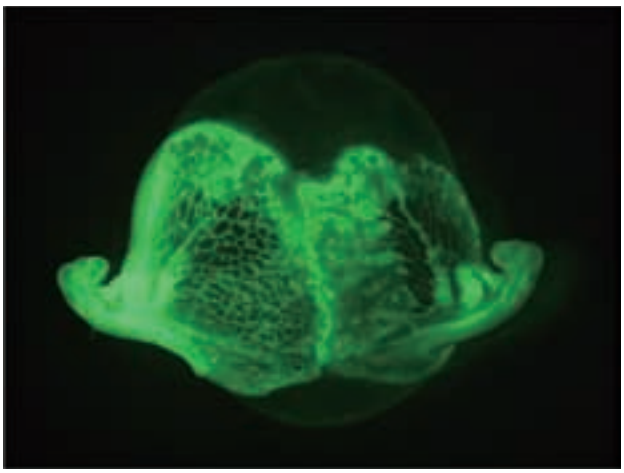
Importantly, about half of all MC cultured embryos survived with normal morphology until HH18, an advance over previous methods, which could only sustain growth until HH13–15. This survival gain is significant in that a number of features of vascular, head, and limb formation only begin to appear at around stage 18. In a number of the MC embryos, growth of the head region continued beyond HH18, although limb development was arrested, meaning that the new method might prove useful in ex ovo studies of even later stages of head development.



The MC culture is prepared by folding the streak stage embryo and trimming the margins (left). After one day in culture, the embryo inflates into a balloon shape, with the embryo proper positioned on top and growing healthily.

To confirm that their new method could be used in complement with other manipulations, Nagai et al. tested several embryological techniques in the folded explants. They found that embryos electroporated with GFP grew normally and were stained as expected with fluorescent signals. They next tried a trickier experiment, known as parabiosis, in which a pair of embryos is co-cultured in close contact with each other, resulting in twin embryos with shared blood circulation. Using a slightly modified version of their new method, they found that they could generate parabiotic pairs of chicks, quails, and even combinatorial parabiosis involving a chick-quail pairing. Such experimental systems are useful in the study of blood development.

"The chick is a wonderful model organism for developmental biology studies, but the modified Cornish pasty culture, or the dumpling culture as I like to call it, shows that we still don't understand the embryo well, as no one would have predicted that twinning and parabiosis can be created this way," says Sheng. "Our next goal is to use this technique to test some of the hypotheses regarding the origin of definitive hematopoietic stem cells."



A pair of chick/chick twins, after 2-day culture with the MC method. The left-side embryo has been injected intracardially with a fluorescent dye, and vascular fusion of the twins is highlighted by fluorescent signals in the vasculature of the right-side embryo shortly after injection.

X inactivation a toss-up in ES cells

Kazuhiro MURAKAMI

Cells have evolved a variety of strategies for ensuring that they get just the right doses of gene products. X inactivation, in which one of the two X chromosomes in female mammals is epigenetically silenced, is one of the best known and most intensively studied of these mechanisms. One of this process's more interesting features is that cell lineage determines whether inactivation occurs in a randomized or deterministic fashion. In somatic lineages differentiated in post-implantation embryo, X inactivation can take place in either the paternally or maternally supplied chromosome, while in extraembryonic tissues segregated from pluripotent stem cells in pre-implantation embryo, the paternal X is always inactivated. But has this transition already been made in the pluripotent stem cells of inner cell mass, which represents both the first fruit of differentiation and the starting point for all somatic lineages?

Kazuhiro Murakami in the Laboratory for Pluripotent Stem Cell Studies (Hitoshi Niwa, Project Leader) and colleagues used a pair of modified mouse embryonic stem (ES) cell lines to answer that question, and found that when the pluripotent cells were forced to differentiate into extraembryonic lineages, X inactivation in their progeny cells took place randomly. Their findings, published in *Development*, help to better constrain the timing of this crucial epigenetic event.

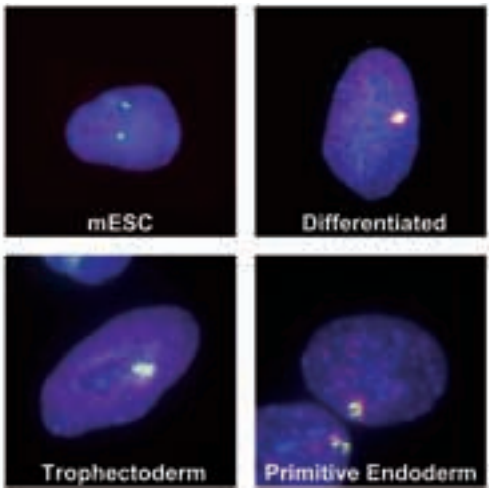
The team selected two lines of female mouse ES cell lines in which the paternal and maternal X chromosomes could be readily distinguished, due to genomic polymorphism or fluorescent labeling. ES cells such as these do not normally give rise to extraembryonic lineages, such as trophectoderm or primitive endoderm, but previous work by the Niwa lab had shown that forced expression of Cdx2 or Gata6 could steer ES cells down those respective pathways. Murakami used this combination of cells and transcription factors to generate ES-derived extraembryonic lineages in which the pattern of X inactivation could be visualized.

X inactivation is associated with the epigenetic silencing by trimethylation of histone 2 lysine 27 (H3K27) as well as the coating with Xist RNA, so the team first confirmed that their induced trophectoderm and primitive ectoderm cells had indeed undergone silencing, and found that, in contrast to undifferentiated ES cells in which inactivation has not occurred, the extraembryonic cells showed clear Xist-H3K27 association at a single locus.

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)

The question was: did this happen only in the maternal X, or randomly to either the paternally or maternally donated chromosome?

Murakami et al. first used a PCR-restriction fragment length polymorphism (RFLP) assay to detect transcripts specific to each genomic donor. While both sets of transcripts are expected to appear in undifferentiated cells, only the maternal transcripts appear in normal extraembryonic tissues, due to imprinted X inactivation. However, in the induced trophectoderm and primitive endoderm (in which X inactivation clearly occurs), both paternal and maternal X chromosome contributions remained, suggesting that the process occurred randomly.

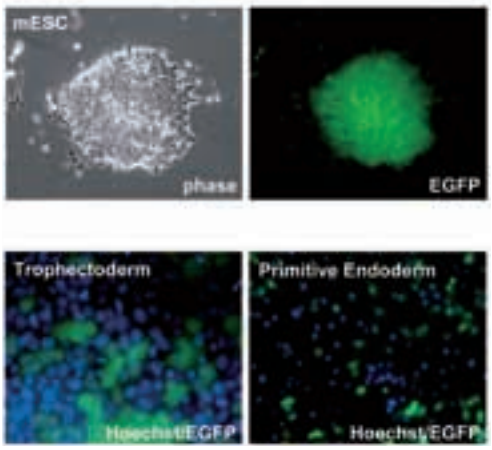


Xist RNA/H3K27me3 immuno-FISH in induced extra-embryonic cells. The spreading of Xist RNAs was detected by Xist exon probes (green). The H3K27me3 signal was detected by Anti-H3K27me3 antibody (red).

This finding was supported by results of parallel experiments using a second ES cell line in which the paternal X chromosome was labeled by EGFP. If imprinted X inactivation took place in the induced extraembryonic cells, the fluorescent signal would be lost, but in both primitive endoderm and trophectoderm, the cells glowed, testifying to the stochastic nature of the chromosomal silencing.

To make a final confirmation that the X inactivation imprint is already lost in the inner cell mass, the team, in collaboration with the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) used the ES cells expressing EGFP on its paternal X chromosome to generate cloned blastocysts. As anticipated by the results from their in vitro differentiation studies, X inactivation in the trophectoderm of these blastocysts occurred randomly, in contrast to the usual imprinting seen in the same tissue in control embryos. The imprinting that leads to selective X inactivation, it seems, is completely lost in ES cells.

"The precise developmental time-point of the transition in the X chromsome's epigenetic state from imprinted to random X inactivation was unknown, but we were able to show that the early epiblast from which ES cells are derived completes the transition event by erasing all epigenetic modifications for imprinted X inactivation," says Niwa. "The epigenetic state of ES cells may be a sort of tabula rasa."



Phase-contrast and GFP fluorescence images of B142b-geoEG Cdx2ER and Gata6GR ES cell lines. The active state of the paternal X chromosome was detected by GFP fluorescence. Nuclei were counterstained by Hoechst 33342 (blue).

Better cloning through chemistry

Tetsuo ONO

Cloning of animals by nuclear transfer remains an art as much as it is a science. Mouse cloning efficiency has lingered in the low single digits since the first success in 1998, at which time only 1–2% of all attempts was capable of generating cloned offspring. In the intervening years, that number has only risen by a few percentage points, and even when using the best available techniques, a cloning success rate of above 5% is considered high. A number of recent advances in cloning technology have involved the use of chemicals that interfere with the action of epigenetic regulators known as histone deacetylases (HDACs). But the reason why these inhibitors promote cloning success is unknown.

Tetsuo Ono and others in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) have now identified a pair of new HDAC inhibitors that improve the rate of full-term development in cloned mice. These new cloning boosters share a subset of characteristics with previously identified molecules of similar function, which may point the way to a better mechanistic understanding of how these new ingredients work.

There are multiple types of HDAC, which are categorized into five classes (I, IIa, IIb, III and IV), and are expressed nearly ubiquitously, functioning as regulators of gene expression. Their inhibitors play an equally important role in fine-tuning their regulatory effects, but can also have cytotoxic effects, suggesting that the selection of inhibitor can have a major effect on whether it helps or harms the cloning process.

To determine which works best in raising the efficiency of cloning and genomic reprogramming, Ono tested a number of HDACi proteins, with specificities for different classes of HDAC. These included well-established HDAC inhibitors, such as trichostatin A and scriptaid, as well as two new molecules, suberoylanilide hydroxamic

Ono T, et al. Inhibition of class IIb histone deacetylase significantly improves cloning efficiency in mice. *Biol Reprod* 83.929-37 (2010)

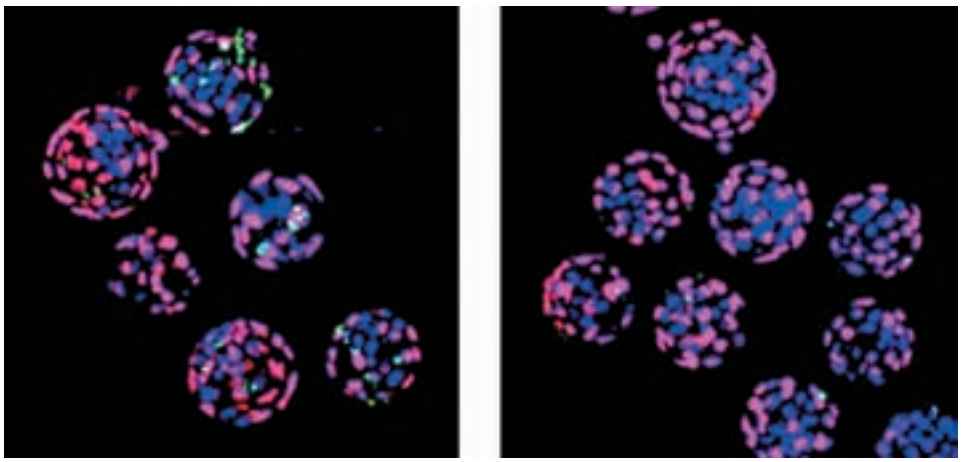
acid (SAHA) and oxamflatin—all of which inhibit HDAC classes I, IIa and IIb. The effects of valproic acid, an inhibitor of HDAC I and IIa, but importantly not of IIb, were also examined.

The team treated cloned embryos with varying concentrations of these HDAC and followed their development through hallmark stages such as blastocyst formation and full-term development. They found that at all stages, the embryos treated with trichostatin A or the new factors SAHA and oxamflatin outperformed those treated with valproic acid and untreated controls. The new factors yielded particularly strong improvements in cloning efficiency, with 9.4% of SAHA treated embryos and up to 7.5% of the oxamflatin-treated group reaching full term.

Immunostaining of the cloned blastocysts revealed that the SAHA and oxamflatin-treated embryos had significantly higher numbers of cells in the inner cell mass (the population of cells that gives rise to the embryo proper), suggesting that these HDAC inhibitors might enhance the reprogramming of somatic cell nuclei. TUNEL assays, which reveal apoptotic activity, showed that the SAHA and oxamflatin blastocysts had fewer cells undergoing programmed cell death, while those treated with valproic acid were more apoptotic.

As a final functional test, Ono et al. generated embryonic stem cell lines from the cloned blastocysts (ntES cell lines) treated with the various factors, and found that HDACi-treated clones were two to three time more likely to be able to give rise to a pluripotent stem cell line.

“We wanted to know why only cloned embryos require HDACi treatment to encourage genomic reprogramming,” says Wakayama. “What we found was that perhaps the oocyte cytoplasm reprograms the somatic cell nucleus too strongly. By inhibiting a particular oocyte HDAC, we could achieve more appropriate reprogramming of donor nuclei, resulting in a higher cloning success rate.”



Incidence of apoptosis (TUNEL assay) in cloned blastocysts treated with SAHA (right) and VPA (left). The apoptotic cells (green spots) were significantly decreased in SAHA treated embryos.

The first light of day heralds spring's coming

Koh-hei MASUMOTO, Maki UKAI-TADENUMA

Many organisms in both the plant and animal kingdoms rely on length-of-day cues to detect seasonal change, and modulate their physiological activities to suit the time of year. This photoperiodism is involved in sexual maturation as well as hibernation and migratory cycles in animals, and has been implicated in seasonal affective disorder in humans. Previous research has shown that, in quail, an increase in day length induced the expression of the thyroid stimulating hormone, ß subunit (TSHß), which is a crucial regulator of the bird's physiological function. But just how information about the length of daylight is transduced to *TSHß* has remained a mystery.

Now, Koh-hei Masumoto and colleagues in the Laboratory for Systems Biology (Hiroki R. Ueda, Project Leader) show in a unique mouse model that the transcription factor *Eya3* not only induces *TSHß* expression but does so in response to light stimuli delivered early in the daily cycle. Their results, which appear in *Current Biology*, reveal an interesting mechanism behind the seasonal control of *TSHß* expression.

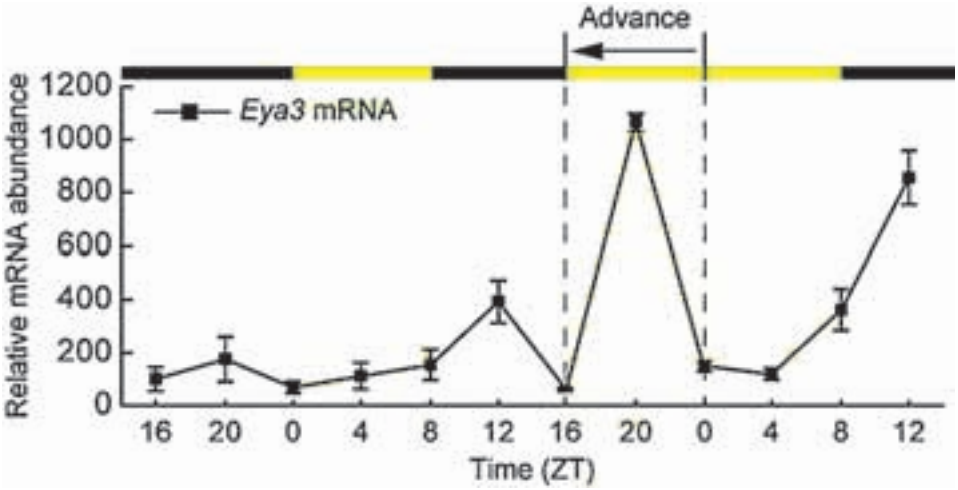
Most of the mouse strains commonly used in research do not exhibit photoperiodism, but, it was recently reported that melatonin-proficient CBA/N mice show increased expression of *TSHß* in the pars tuberalis region of the brain in response to longer daylight, just as in the quail. Identification of this mouse model was an important first step, as it meant that the lab could bring the wealth of accumulated knowledge of mouse physiology and advanced genetic techniques, to bear on the problem.

Masumoto started by conducting a whole-genome microarray analysis on pars tuberalis samples from mice that had been raised in either long-day (16 hours of light, 8 hours dark), or short-day (8h light, 16h dark) conditions. He found 246 genes (including *TSHß*) strongly expressed in the long-day samples, and 57 that were upregulated in the short-day mouse brain. Wanting to determine what would happen to the induction of *TSHß* when mice were shifted between short-day and long-day regimes, Masumoto found that while switching the lights off eight hours later (in a sense, delaying night) had no effect on *TSHß*, the opposite adjustment, speeding the arrival of morning's light by eight hours, caused a rapid increase in the mRNA's expression.

But it still wasn't clear what linked its expression to changes in the onset of day, so the team did a second round of DNA microarray testing, looking for genes that

Masumoto KH, et al. Acute induction of *Eya3* by late-night light stimulation triggers *TSH*ß expression in photoperiodism. *Curr Biol* 20.2199-206 (2010)

began to be expressed in advance of the switched-on *TSH*ß. They found a total of 34, and homed in on a favorite candidate, *Eya3*, by virtue of, among other things, its known role as a transcriptional factor. *Eya* family factors work by forming complexes with *Six* family proteins, enabling them to regulate target genes. Looking back to the pars tuberalis from the brains of long-day mice, Masumoto et al. found that both *Eya3* and *Six1* were strongly expressed. Testing this association in an in vitro reporter assay, they confirmed that the co-expression of these two factors led to activation of the *TSH*ß promoter. Further, this activation is enhanced by *Tef* and *Hlf*. Although *Eya3*'s expression under long-day conditions has been known previously from work in birds and sheep, this current work from the Ueda lab points both to its broad conservation and its mechanism of action.



Shifting the start of the light period forward by 8 hours for mice raised in short-day conditions results in upregulation of *Eya3*, which in turn induces *TSH*ß expression.

"In natural settings, of course, the progression toward a longer day is more gradual, so we think that the induction of *TSH*ß by *Eya3* should also play out over a longer time," says Ueda. "But in this study we have shown that it is possible to induce *Eya3* in a short period by manipulating exposure to light, which should be useful for future research into photoperiodism. We will also be very interested in working out how information about day length is relayed to this transcription factor, and why it is that morning light plays such a key role in determining photoperiod."

Animal Resource and Genetic Engineering

Mutant mice are an important resource used in biological and medical research. The quality and efficacy of research is greatly dependent on how easily mutant mice can be generated, propagated, and housed, but researchers today tend not to engage in routine generation of mutant mice. This laboratory's major function is to develop mutant mice for research in the fields of developmental biology and regenerative science, and to maintain the CDB's experimental rodent resources.

Shinichi AIZAWA Ph.D.

Staff

Unit Leader
Shinichi AIZAWA

Research Scientist
Shuichi KANI
Go SHIOI
Hirotaka TAO

Visiting Scientist
Toshihiko FUJIMORI

Technical Staff
Takaya ABE
Kana BANDO
Naoko HATAMOTO
Michiko HIGASHIKAWA
Mari KANEKO
Hiroshi KIYONARI
Taketsugu MITSU
Makiko OGAWA
Naoko OHSHIMA
Mayo SHIGETA
Megumi WATASE

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 to 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 level.

CS7 BL/6 Es(HK4)-derived offspring

Staff

Unit Leader
Kazuki NAKAO

Attending Veterinarian
Naoko KAGIYAMA

Technical Staff
Kenichi INOUE
Yuki KANEKO
Takuya KAWADA
Hiroshi KIYONARI
Miho SATO
Mayo SHIGETA
Aki SHIRAISHI
Norie TANAKA
Tomoko TOKUNAGA
Sachi YAKAWA
Sayaka YOSHIMURA

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 the 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.

Mice maintained by the Animal Resource Unit

Publications

Kiyonari H, Kaneko M, Abe S and Aizawa S. Three inhibitors of FGF receptor, ERK, and GSK3 establishes germline-competent embryonic stem cells of C57BL/6N mouse strain with high efficiency and stability. *Genesis* 48.317-27 (2010)

Nakao K. and Inoue K. Cryopreservation for broader production of transgenic mice by DNA injection into zygotes. *Exp Anim* 59.225-9 (2010)

Uchiyama Y, Sakaguchi M,Terabayashi T, Inenaga T, Inoue S, Kobayashi C, Oshima N, Kiyonari H, Nakagata N, Sato Y, Sekiguchi K, Miki H, Araki E, Fujimura S, Tanaka S S, Nishinakamura R. Kif26b, a kinesin family gene, regulates adhesion of the embryonic kidney mesenchyme. *Proc Natl Acad Sci USA* 107.9240-5 (2010)

Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Sudo K, Okumura K, Saito H, Nakae S. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci USA* 107.18581-6 (2010)

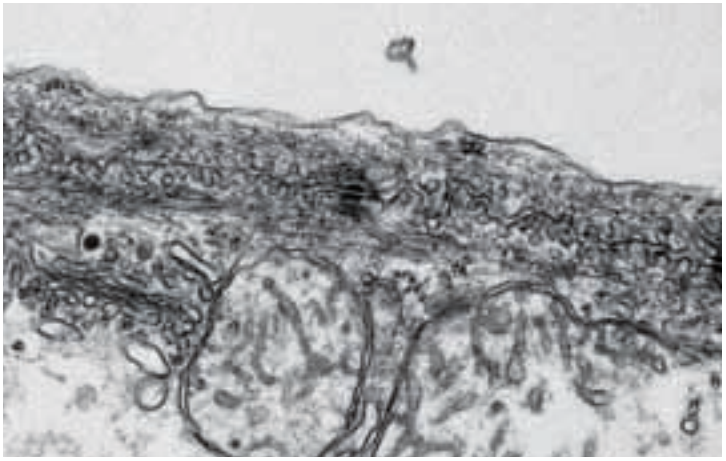
Electron Microscope

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.

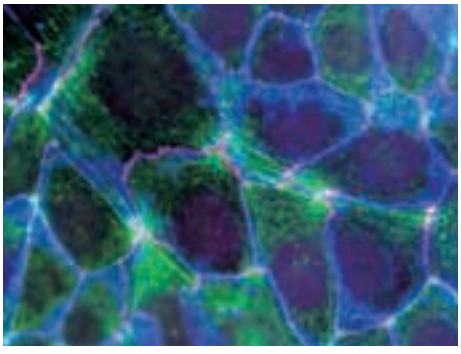
Shigenobu YONEMURA Ph.D.

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

Laboratory Head
Shigenobu YONEMURA

Technical Staff
Kisa KAKIGUCHI
Kazuyo MISAKI
Sachiko ONISHI
Makiko F. UWO

Visiting Scientist
Masatsune TSUJIOKA

Student Trainee
Tomoki FUJITA

Research Fellow
Ayuko SAKANE

Assistant
Mai SHIBATA

Publications

Yonemura S, et al. α-Catenin as a tension transducer that induces adherens junction development. *Nat Cell Biol* 12. 533-42 (2010)

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)

Iida A, et al. Metalloprotease-dependent onset of blood circulation in zebrafish. *Curr Biol* 20. 1110-6 (2010)

Yamamoto M, et al. Mib-Jag1-Notch signaling regulates patterning and structural roles of the notochord by controlling cell-fate decisions. *Development* 137. 2527-37 (2010)

Bio-imaging

The goal 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 MIMORI-KIYOSUE

Technical Staff
Tomoko HAMAJI

Part-time Staff
Satoko NAKAMURA

Agency Staff
Nobuyuki NAKANISHI
Yuka YAMAMOTO

Publications

Hotta A, Kawakatsu T, Nakatani T, Sato T, Matsui C, Sukezane T, Akagi T, Hamaji T, Grigoriev I, Akhmanova A, Takai Y and Mimori-Kiyosue Y. Laminin-based cell adhesion anchors microtubule plus ends to the epithelial cell basal cortex through LL5alpha/beta. *J Cell Biol* 189.901-17 (2010)

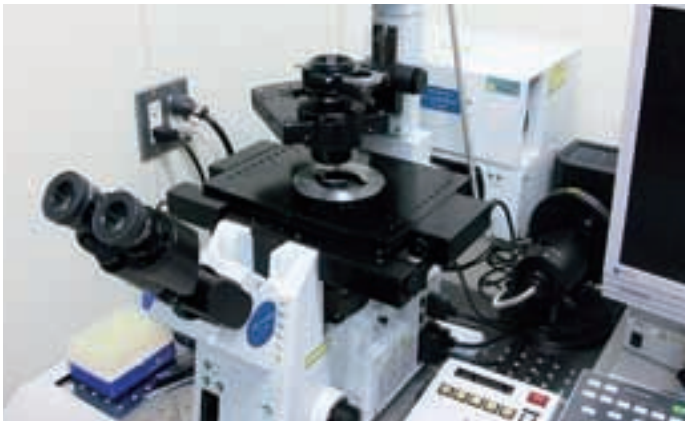
Optical Image Analysis Unit

Yuko MIMORI-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 gene analysis 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

Hiroshi TARUI Ph.D.

The Genome Resource and Analysis Unit aims to support a wide gamut of gene analysis research, ranging from DNA sequencing and gene expression analysis to storage and distribution of gene resources. Our facility can perform gene expression analysis and high-throughput gene screening using a DNA sequencing system that combines traditional DNA sequencers with the next-generation sequencer 454 sequencer, making it possible to apply it to a wider range of genomic experiments, We can also custom-make DNA microarray systems. Gene resources derived from a variety of species can also be stored and distributed to researchers according to their requests. By building upon existing technologies with the goal of creating new techniques and ideas, as well as providing solid support, we aim to respond flexibly to the needs of each research request.

Staff

Unit Leader
Hiroshi TARUI

Technical Staff
Tetsutaro HAYASHI
Kazu ITOMI
Yu LIN
Asuka MOMIYAMA
Kaori TATSUMI

Visiting Scientist
Osamu NISHIMURA

Part-Time Staff
Minako MOTOISHI

Assistant
Chiharu TANEGASHIMA

Publications

Yazawa S, et al. Planarian Hedgehog/Patched establishes anterior-posterior polarity by regulating Wnt signaling. *Proc Natl Acad Sci USA* 106. 22329-34 (2009)

Maki N, et al. Expression of stem cell pluripotency factors during regeneration in newts. *Dev Dyn* 238(6), 1613-6 (2009)

Egger B, et al. To be or not to be a flatworm: the acoel controversy. *PLoS One* 4(5), e5502 (2009)

Eto K, et al. Reduced expression of a RNA-binding protein by prolactin leads to translational silencing of programmed cell death protein 4 and apoptosis in newt spermatogonia. *J Biol Chem* (2009)

Functional Genomics Unit

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

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

Staff

Unit Leader
Hiroki R. UEDA

Special Postdoctoral Researcher
Yohei SASAGAWA

Research Specialist
Takeya KASUKAWA
Itoshi NIKAIDO

Visiting Scientist
Hiroki DANNO

Technical Staff
Junko NISHIO
Kenichiro UNO

Agency Staff
Chikako IMAI

Assistant
Ikuko TADA

Publications

Alev C, et al. Transcriptomic landscape of the primitive streak. *Development* 137. 2863-74 (2010)

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

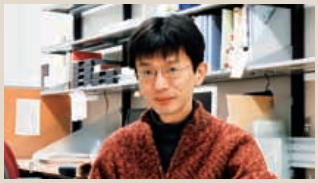
Isojima Y, et al. CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc Natl Acad Sci USA* 106. 15744-9 (2009)

Kawaguchi A, et al. Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis. *Development* 135. 3113-24 (2008)

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

Technical Staff
Kaori SHINMYOZU

Publications

Otani T, et al. IKKe regulates cell elongation through recycling endosome shuttling *Dev. Cell* 20.1-14 (2011)

Hayakawa T, et al. MRG15 binds directly to PALB2 and stimulates homology-directed repair of chromosomal breaks. *J Cell Sci* 123.1124-30 (2010)

Shirai A, et al. Methylation of ribosomal protein L42 regulates ribosomal function and stress-adapted cell growth. *J Biol Chem* 285.22448-60 (2010)

Terasawa M, et al. Caenorhabditis elegans ortholog of the p24/p22 subunit, DNC-3, is essential for the formation of the dynactin complex by bridging DNC-1/p150 (Glued) and DNC-2/dynamitin. *Genes Cells* 15.1145-57 (2010)

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.

Staff

Unit Leader
Yoshiki SASAI

Deputy Unit Leader
Masatoshi OHGUSHI

Research Specialist
Hiroyuki KITAJIMA

Research Scientist
Hidetaka SUGA

Collaborative Scientist
Tokushige NAKANO

Technical Staff
Michiru MATSUMURA-IMOTO
Maki MINAGUCHI
Eriko SAKAKURA

Company-Sponsored Research Trainee
Taisuke KADOSHIMA

Part-Time Staff
Yoshinori NAKAI

Publications

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

Nagase T, et al. Pericellular matrix of decidua-derived mesenchymal cells: a potent human-derived substrate for the maintenance culture of human ES cells. *Dev Dyn* 238 1118-1130 (2009)

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

Unit Leader
Yoshiki SASAI

Deputy Unit Leader
Mototsugu EIRAKU

Publications

Danjo T, et al. Subregional specification of embryonic stem cell-derived ventral telencephalic tissues by timed and combinatory treatment with extrinsic signals. *J Neurosci* 31.1919-33 (2011)

Eiraku M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 3.519-32 (2008)

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

Publications

Gold standards in the Diamond Age: The commodification of pluripotency. Sipp, D. *Cell Stem Cell* 5 360 – 363 (2009)

Sipp, D. Stem cells and regenerative medicine on the Asian horizon. *Regenerative Medicine* 4 911–918 (2009)

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.

Kobe night view. Photography: Shigekazu SHINYA

RIKEN Kobe Institute

The RIKEN Kobe 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).

The 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.

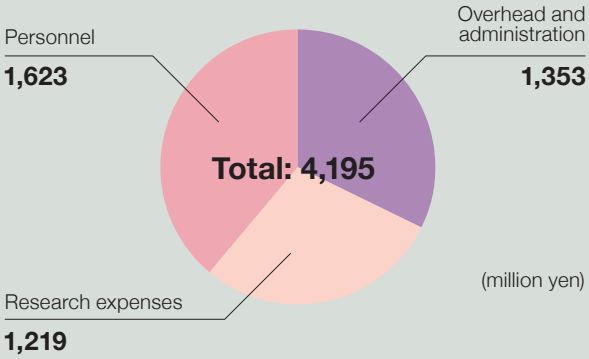
Research Promotion Division

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.

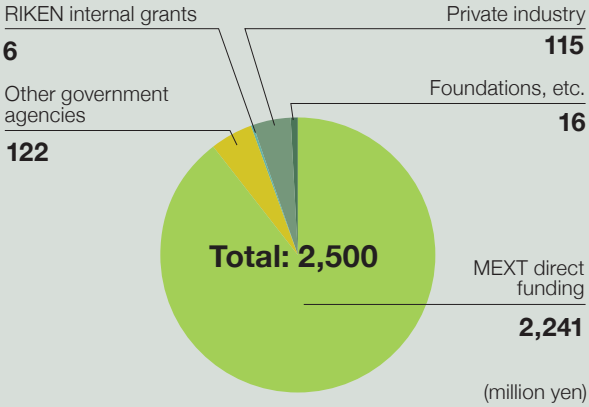
Safety Center

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.

2010 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, and other governmental and private sources. These external funds represent a significant component of the Center' s total funding every year, and were particularly significant in the fiscal year ending March 2010, representing an additional 2.5 billion yen in research, facilities, and operating funds.



2010 CDB Staff

Laboratory heads	28
Deputy unit leaders	2
Research scientists	118
Research associates	7
Technical staff	107
Assistants	24
Visiting scientists	82
Student trainees	44
Part-time staff	32
Research Promotion Division	52
Other	22
Total	518

2010 CDB Symposium

Frontiers in Organogenesis

March 23 – 25, 2010

The CDB hosted its eighth annual symposium, “Frontiers in Organogenesis” on March 23 – 25.
The three-day program featured a wide range of oral presentations organized into sessions focused on themes relating to the patterning, imaging, evolution, and dynamics of organogenic phenomena. In addition to the 25 talks, each day included a poster session in which more than 90 poster presenters discussed their work.

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

Session 1

Naoto Ueno (National Institute for Basic Biology, Japan)
Stefano Piccolo (University of Padova, Italy)
Masatoshi Takeichi (RIKEN CDB, Japan)

Session 2

Atsushi Miyawaki (RIKEN Brain Science Institute, Japan)
Scott E. Fraser (California Institute of Technology, USA)
Didier Stainier (University of California, San Francisco, USA)
Joachim Wittbrodt (University of Heidelberg, Germany)

Session 3

Jukka Jernvall (University of Helsinki, Finland)
Shigeru Kuratani (RIKEN CDB, Japan)
Neil H. Shubin (University of Chicago, USA)

Session 4

Ryoichiro Kageyama (Kyoto University, Japan)
Oliver Pourquié
(Howard Hughes Medical Institute,
Stowers Institute for Medical Research, USA)
Hiroyuki Takeda (The University of Tokyo, Japan)
Yukiko Gotoh (The University of Tokyo, Japan)
Yoshiki Sasai (RIKEN CDB, Japan)

Session 5

Benoit G. Bruneau
(Gladstone Institute of Cardiovascular Disease, USA)

Toshihiko Ogura (Tohoku University, Japan)
Elazar Zelzer (Weizmann Institute of Science, Israel)
Andrew McMahon (Harvard University, USA)

Session 6

Brigid L.M. Hogan (Duke University Medical Center, USA)
Mark A. Krasnow
(Duke University Medical Center, Stanford University School of Medicine and Howard Hughes Medical Institute, USA)

Christopher V.E. Wright (Vanderbilt University, USA)
Hideki Enomoto (RIKEN CDB, Japan)
Cliff Tabin (Harvard Medical School, USA)

2011 CDB Symposium

Epigenetic Landscape in Development and Disease

March 14–16, 2011

The ninth annual symposium "Epigenetic Landscape in Development and Disease" will be held on March 14 – 16, 2011 in the CDB Auditorium. We plan to discuss the question of to how extract an epigenetic landscape from the ever-growing set of data on epigenetic states. By facilitating communication between the leaders in these fields and promising younger scientists at a collegial forum, we hope to create a unique atmosphere that provides a comprehensive perspective on the current knowledge of the dynamics of organogenesis, leading to the generation of fresh perspectives and interactions.

Invited Speakers

C. David Allis
The Rockefeller University, USA

Adrian Bird
Wellcome Trust Centre for Cell Biology, UK

Joseph Ecker
The Salk Institute, USA

Klaus H. Hansen
University of Copenhagen, Denmark

Kristian Helin
University of Copenhagen, Denmark

Rudolf Jaenisch
Whitehead Institute for Biomedical Research and MIT, USA

Thomas Peter Jenuwein
Max-Planck Institute of Immunobiology, Germany

Tetsuji Kakutani
National Institute of Genetics, Japan

Minoru S.H. Ko
National Institute on Aging, NIH, USA

Avi Ma'ayan
Systems Biology Center New York, USA

Jun-ichi Nakayama
RIKEN CDB, Japan

Hitoshi Niwa
RIKEN CDB, Japan

Stuart H. Orkin
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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 500 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 2010.

date	title	speaker
01-07	Stoichiometry of the integrin adhesion complex in *Drosophila* tissues	Yoshiko INOUE
01-13	Regulation of VEGF-induced angiogenesis by receptor endocytosis	Masanori NAKAYAMA
01-15	Gamete activation and interactions required for reproductive success in *C. elegans.*	Andrew SINGSON
01-20	Using stem cells and reprogramming to study ALS	Kevin EGGAN
01-21	Mechanisms of musculoskeletal patterning and integration during craniofacial development	Rich SCHNEIDER
01-25	Chicken embryonic stem cells (cESC) as a non-mammalian embryonic stem cell model	Bertrand PAIN
01-25	Role of cell death for completion of organogenesis within developmental time window	Erina KURANAGA
01-26	Molecular and cellular mechanisms of *C. elegans* Q neuroblast development	Guangshuo OU
01-28	Primary contribution to zebrafish heart regeneration by a subpopulation of cardiomyocytes	Kazu KIKUCHI
02-15	Targeted capture and next-generation sequencing identifies TPRN, encoding TAPERIN, as the mutated gene in nonsyndromic human deafness DFNB79	Thomas FRIEDMAN
03-03	Primate pluripotent stem cells: Bridging gaps in scientific knowledge between mice and humans	Gerald SCHATTEN
03-04	Regulation of Rap2A by the ubiquitin ligase Nedd4-1 controls neurite development	Hiroshi KAWABE
03-15	FGF regulation of cortical patterning	John RUBENSTEIN
03-18	Cell polarity regulator Pard6b is essential for trophectoderm formation in the preimplantation mouse embryo	Vernadeth B. ALARCON
03-18	Molecular analyses of mouse mesoderm formation and axial elongation morphogenesis using embryonal carcinoma cells	Yusuke MARIKAWA
03-19	Translational control of the self-renewal versus differentiation decision in the germ line	Rafal CIOSK
03-26	Identification and analysis of novel Spemann/Mangold organizer genes	Heiko LICKERT
04-01	Therapeutic and research potential of human stem cells: How GE sees the future of stem cells	Stephen MINGER
04-02	TRIOBP: a novel actin bundler that generates resilient rootlets of inner ear hair cell stereocilia	Shin-ichiro KITAJIRI
05-07	Behind the scenes of scientific publishing	Katherine BROWN
05-13	Differential metabolomics for assessment of N-Acetyl-L-Cysteine pretreatment in strenuous exercise: A quantitative Model of oxidative stress inhibition for fatigue reduction	Philip Britz-McKIBBIN
05-17	Tumor cell dormancy and recurrence: a view from EMT	Hisataka SABE
05-18	Microtubules break symmetry in *C. elegans* zygotes by protecting PAR-2 from cortical exclusion by PKC-3 kinase	Fumio MOTEGI

date	title	speaker
06-17	Rules for enteric nervous system assembly: Biological and Mathematical Insights	Don NEWGREEN and Kerry LANDMAN
06-24	FGF signaling during avian gastrulation and mesoderm lineage diversification	Parker B. ANTIN
06-25	Wnt signals during stem cell self-renewal and tissue repair	Roel NUSSE
06-28	Developmental roles of Dchs1-Fat4 signaling in mice	Philippa FRANCIS-WEST
07-07	Wnts and Fgfs, via AKT and MAPK, direct discrete aspects of trigeminal placode development	Claire Ann CANNING
07-20	Exploring the creation of a tissue engineered trachea: Keeping our eyes towards the clinical world	Koji KOJIMA
07-29	Basement membrane movement breaks tissue boundaries during uterine-vulval attachment in *C.elegans*	Shinji IHARA
07-30	Evolution of a novel behavior mediated by the lateral line system adapts blind cavefish to life in darkness	Masato YOSHIZAWA
08-02	Chemotaxis in gradients: How a cell knows when to move and turn	Arthur MILLIUS
08-03	Dormancy in normal and malignant stem cells	Andreas TRUMPP
08-23	All paths lead to the thymus: A cross-species genetic analysis of Hoxa3 function.	Nancy MANLEY
08-23	Fate mapping studies reveal that adult microglia derive from primitive macrophages	Florent GINHOUX
08-23	Human artificial chromosome meets stem cells	Mitsuo OSHIMURA
09-01	Pattern formation in enteric nervous system development: Building a complex nervous system and human disease mechanisms	Robert O. HEUCKEROTH
09-29	Tracking stem cells at the single cell level: New tools for old questions	Timm SCHROEDER
10-04	Derivation of scalable vasculogenic precursors from human pluripotent stem cells during the onset of vasculogenesis	Ayelet DAR-OAKNIN
11-04	Integrin activation and cytoskeletal remodeling mediated by LIM domain proteins	Frieder SCHOECK
11-08	Cortical representation of olfactory bulb input revealed by retrograde mono-transsynaptic labeling	Kazunari MIYAMICHI
11-11	Asymmetric cell division and tumorigenesis in *Drosophila* neural stem cell lineages	Juergen A. KNOBLICH
11-15	Stepwise assembly of a tissue-specific network in the vertebrate brain	Manuel IRIMIA
12-01	DnaA and the timing of chromosome replication in *E. coli*: A systems biology modeling approach.	Marco Cosentino LAGOMARSINO
12-09	Regulation of endoplasmic reticulum stress in *Drosophila*	Hyung Don RYOO
12-13	Extensive regeneration ability in Amphioxus (*Branchiostoma lanceolatum*) provides insight into the evolution of chordate regeneration processes.	Ildikó SOMORJAI
12-21	Nuage: Birth and beyond of germline piRNA	Toshie KAI

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

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

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The RIKEN website provides a full, rich experience 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 is a publicly funded research organization established to conduct comprehensive research in science and technology, and to disseminate the results of its scientific research and technological developments. RIKEN carries out basic and applied research in a wide range of fields, including physics, chemistry, biology, medical science, and engineering.

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

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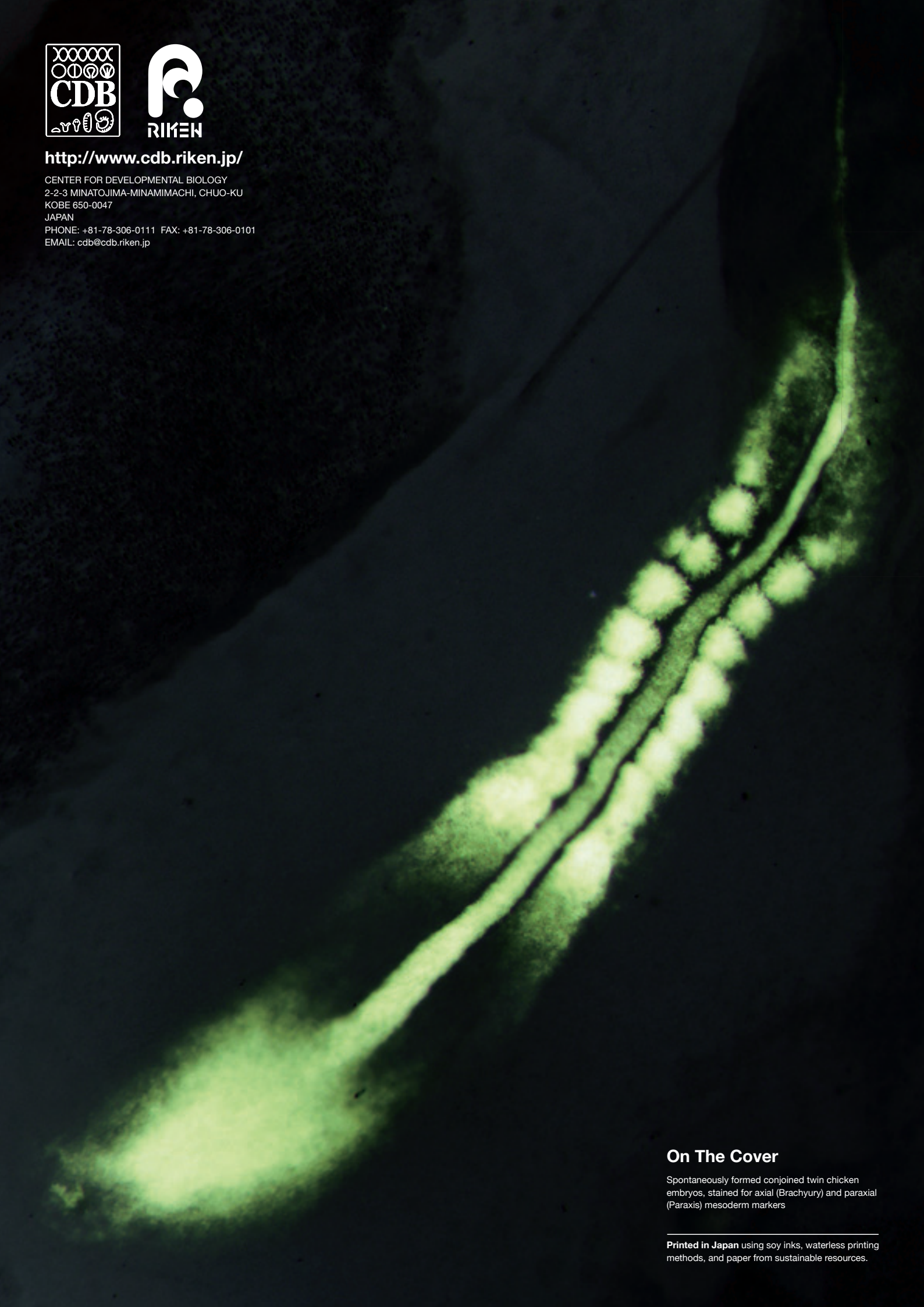

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On The Cover

Spontaneously formed conjoined twin chicken embryos, stained for axial (Brachyury) and paraxial (Paraxis) mesoderm markers

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