

RIKEN Center for Developmental Biology
2009 Annual Report



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**RIKEN Center for
Developmental Biology**

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The Center for Developmental Biology was launched in April 2000 under the auspices of the Millennium Project research initiative that was established to drive research in the fields of information technology, environmental science and the study of aging, areas of vital importance to both Japan and the world in the 21st century. 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 even more complex phenomena involved in organogenesis, and the

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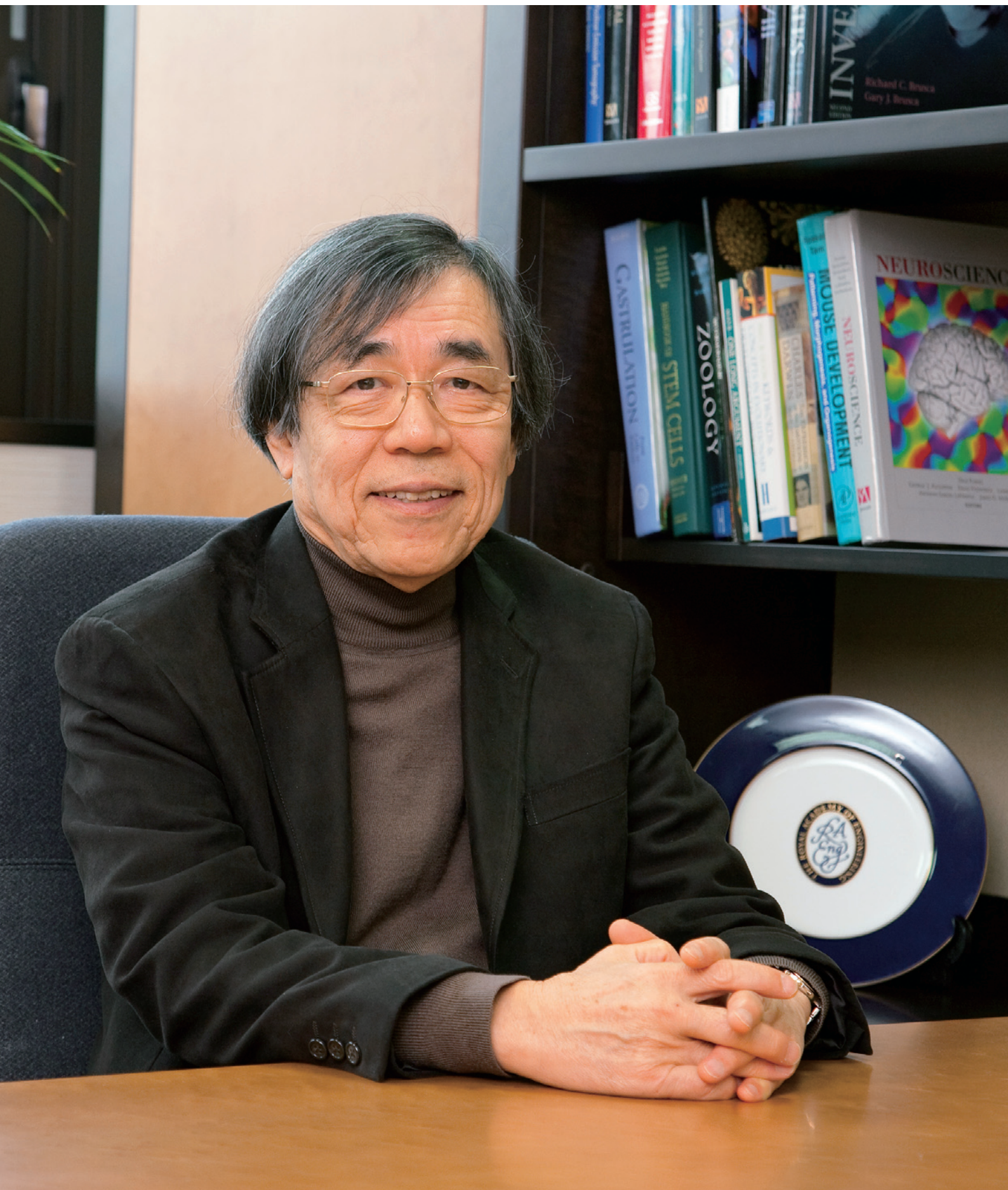
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biology of stem cells and regeneration. Scientists at the CDB conduct world-class studies in a truly international research environment, with ample core facilities and technical support and a critical mass of scientific talent and expertise. The CDB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





Message from the Center Director

The past year has seen the RIKEN Center for Developmental Biology continue in its tradition of advanced research into the mechanisms of development, regeneration, and stem cell biology, as well as take the first steps in the second decade since its establishment in 2000. The pace of change in these areas over the past ten years has truly been breathtaking, and we are proud as an institution to have been able to play at least some small part in pushing back the frontiers of our knowledge in these exciting fields of inquiry.

Research labs at the CDB made important strides on a number of fronts in 2009, publishing important new findings in areas ranging from the origins of the germline in mammals, to the genetic regulation of pluripotent stem cells, to the evolutionary advent of the turtle carapace. Studies of developmental phenomena in the nervous system were a particular highlight, generating novel insights into the formation of the olfactory system and the inner ear, as well as the molecular underpinnings of neuronal connectivity. Other studies focused on regulatory mechanisms that govern the circadian clock, and methods for measuring the state of these internal timekeepers.

Our mission continues to emphasize an unfettered, creative approach to fundamental research into the mechanisms of development, organogenesis and regeneration, but within that broad remit we also recognize the importance of concentrated focus on specific areas. To this end, we have established a pair of Strategic Programs within the CDB to allow for dedicated and sustained support for research into stem cells and systems biology. Research projects in these programs are expected not only to make significant contributions to individual scientific questions, but also to serve as platforms for spurring research advances and technology development across the entire field.

2009 was also a year of transitions, a welcome development as the Center grows and our scientists, many of whom entered as fledgling investigators, move on to the next phases of their careers. By the end of the 2009 fiscal year, nearly one-third of the original 30 CDB laboratories will have made the transition to a new home, and I am pleased to be able to say that all of our research leader alumni have gone on to head laboratories at major universities in Japan and abroad. Our younger research scientists have also done extremely well in their post-CDB appointments, gaining fellowships and faculty positions at research institutions throughout Europe, North America, and Asia. For our part, we welcomed one new research team and two new support units to the CDB in the past twelve months, and look forward to a new generation of research leaders and scientists to join us in the years to come.

Our commitment to outreach, dialogue, and international collaboration remains as strong as ever, and we conducted or participated in numerous programs designed to foster mutual understanding of scientific issues and developments between researchers and the public. We made special efforts to engage with the educational community, continuing in our summer school programs for local high school students and workshops for educators. We similarly continue to support the research community in Japan by offering practical courses in techniques for pluripotent stem cell culture and differentiation several times per year.

The excitement and anticipation surrounding the fields of development, organogenesis, stem cells and regenerative medicine remains undiminished as we enter the second decade of the new century, and we will continue to dedicate ourselves to bettering the understanding of how bodies come to be, how they maintain themselves, and how those that are damaged might one day be healed.

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 34 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 Institutional Review Board includes representatives from local academic, research, medical, and lay organizations, as well as CDB research leaders, and meets regularly to review and discuss programs and investigations with potential ethical, social, legal or public health and safety implications prior to their implementation.

Advisory Council

The CDB Advisory Council (DBAC) convenes regularly to review the performance and direction of the Center's research and governance, and submits reports of its findings and recommendations to aid in guiding future activities and decision-making. The ten-member council comprises top international scientists working in developmental biology, stem cells, and related fields.

- Austin Smith, DBAC Chair
University of Cambridge, UK
- Margaret Buckingham
Institut Pasteur, France
- Stephen Cohen
Temasek Life Science Laboratories,
Singapore
- Hiroshi Hamada
Osaka University, Japan
- Ryoichiro Kageyama
Kyoto University, Japan

- Haifan Lin
Yale University, USA
- Toshio Suda
Keio University, Japan
- Yoshimi Takai
Kobe University, Japan
- Patrick Tam
University of Sydney, Australia
- Christopher Wylie
Cincinnati Children's
Hospital Medical Center, USA

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.

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.

- **Pluripotent Cell Studies**

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- **Mammalian Epigenetic Studies**

Masaki OKANO Ph.D.

- **Mammalian Molecular Embryology**

Tony PERRY Ph.D.

- **Mammalian Germ Cell Biology**

Mitunori 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.

- **Systems Biology**

Hiroki R. UEDA 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

- **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.

Science Policy and Ethics Studies Unit

Douglas SIPP



Stem cell researchers gather in Kobe

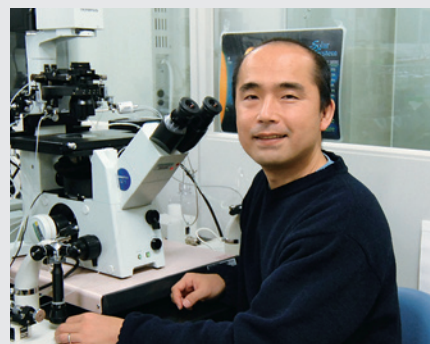
February 4

The CDB hosted a meeting on “Stem Cell Research in the Asia-Pacific Region” in collaboration with the Stem Cell Network: Asia-Pacific (SNAP) and the Japan Society for the Promotion of Science (JSPS), with additional financial support from Nature Publishing Group. Around 30 scientists from around the region gathered in Kobe for three days of talks on recent scientific advances and ethical, legal and social aspects of stem cell research.

Teru Wakayama wins JSPS Prize

March 9

Team Leader Teruhiko Wakayama (Laboratory for Genomic Reprogramming) was awarded the 5th JSPS Prize in recognition of his contributions to the study of animal reproduction through biotechnology. In 2008, Wakayama famously succeeded in generating a clone from a dead mouse that had been frozen for more than 15 years. A list of all awards won by CDB scientists appears on p. 54.



New Programs and Appointments

April 1

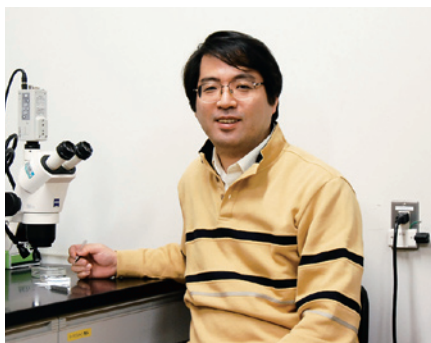
On the first day of the Japanese fiscal year, the CDB inaugurated its new Center Director's Strategic Program, to provide focused support for designated research fields; the first two fields selected were stem cell and developmental systems biology. Also on this day, Yuko Mimori-Kiyosue joined the Center as head of the new Optical Image Analysis Unit, which will provide support to CDB labs in microscopy techniques and technology management.

CDB Meeting on Sensory Development

April 13-15

At the height of the cherry blossom season, the CDB hosted a three-day meeting on “Common Themes and New Concepts in Sensory Formation.” More than 30 international speakers gathered to present and discuss their latest findings and enjoy Kobe's springtime clime. See a full list of CDB meetings from 2009 on p. 66.





CDB scientists win MEXT awards

April 14

Group Director Yoshiki Sasai was honored with a Science and Technology Prize from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) for his work in neural differentiation from pluripotent stem cells. That same day, Team Leader Mitunori Saitou was awarded with a MEXT Young Investigators Award in recognition of his research into mammalian germline specification.

CDB holds its 7th Open House

April 25

The CDB opened its doors to the public for a day of learning, interaction and fun, with activities, exhibits, games, talks and a science café event. Nearly 1,500 visitors came to find out more about the CDB's research and chat with scientists.



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Research article featured on Cell cover

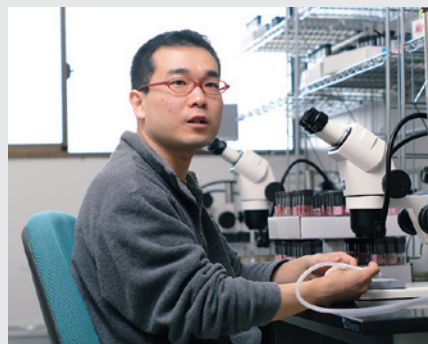
May 1

A major article on mammalian germline development published by the Laboratory for Mammalian Germ Cell Biology was featured on the cover the journal *Cell*. To learn more about this research, see the news highlight on p. 30.

Takashi Nishimura joins as new Team Leader

July 1

Takashi Nishimura joined the CDB as Team Leader of the Laboratory for Growth Control Signaling. Nishimura will focus on the regulation of tissue and organ growth by genetically encoded and extrinsic signals, using the fruit fly *Drosophila melanogaster* as a model system. Read more about his work on p. 41.



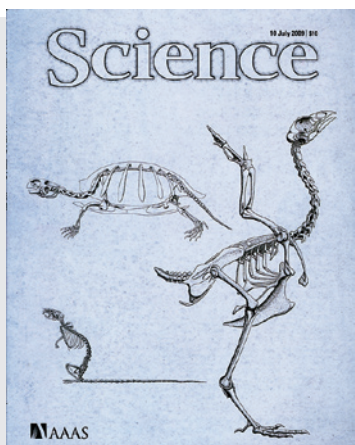


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CDB research makes cover of *Science*

July 15

The latest work on evolutionary innovation of the turtle carapace by the Laboratory for Evolutionary Morphology appeared on the cover of *Science* magazine on this day. See p. 50 for a full summary of this noteworthy research.

Summer school for high school students

August 4-6

The Center held two one-day summer school courses for local high school students, giving them a chance to learn more about the CDB's research and to try their hand at *Drosophila* experiments. For a complete list of courses held in 2009, turn to p. 36.



ISDB Meeting in Edinburgh

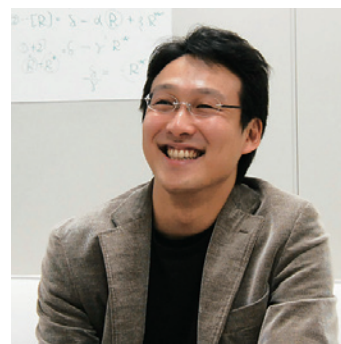
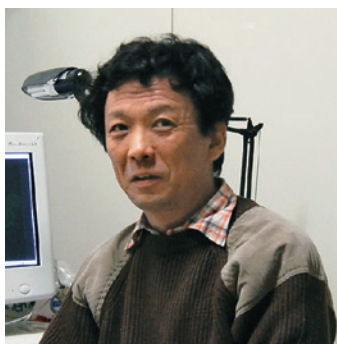
September 6-10

Many CDB scientists traveled to Edinburgh, UK to attend the 2009 Congress of the International Society of Developmental Biologists (ISDB). CDB Director Masatoshi Takeichi served as ISDB President from 2006 to 2009. In addition to providing administrative support to the society, the Center organized a space in the exhibitor's hall (shown).

Project Leaders appointed

October 1

Hitoshi Niwa and Hiroki R. Ueda became the first Project Leaders to head Center Director's Strategic Programs in Pluripotent Stem Cell Studies and Systems Biology. These projects will concentrate on priority areas of research determined by the CDB Center Director.





Annual retreat held in Sasayama

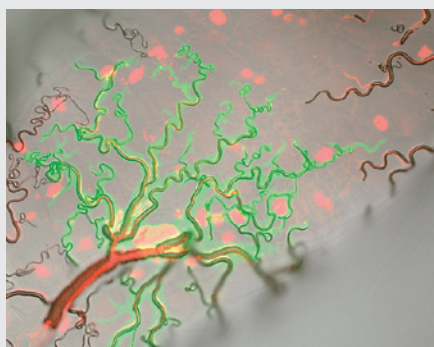
November 5-6

To promote interaction and exchange among research staff and students, the CDB holds a yearly getaway every fall. This year, the retreat was held for the first time in a meeting center in the mountains of western Japan. Lab heads, scientists, students and administrators convened for a day and a half of talks, posters, discussion and collegial exchange.

IBM Japan Science Prize

November 10

Hiroki R. Ueda, Project Leader of the Laboratory for Systems Biology, was awarded the IBM Japan 23rd Annual Science Prize, for his contributions to the field of systems biology through the large-scale analysis of biological data.



Excellence in imaging

November 18

Renowned microscope manufacturer Leica Microsystems awarded Shigeo Hayashi of the Laboratory for Morphogenetic Signaling its 2009 Grand Prize in scientific imaging. The image, showing a terminal branch of the *Drosophila* trachea, was previously featured on the cover of the 2004 CDB Annual Report.

Notch has a dHand in smooth muscle differentiation



Masahiro SHIN

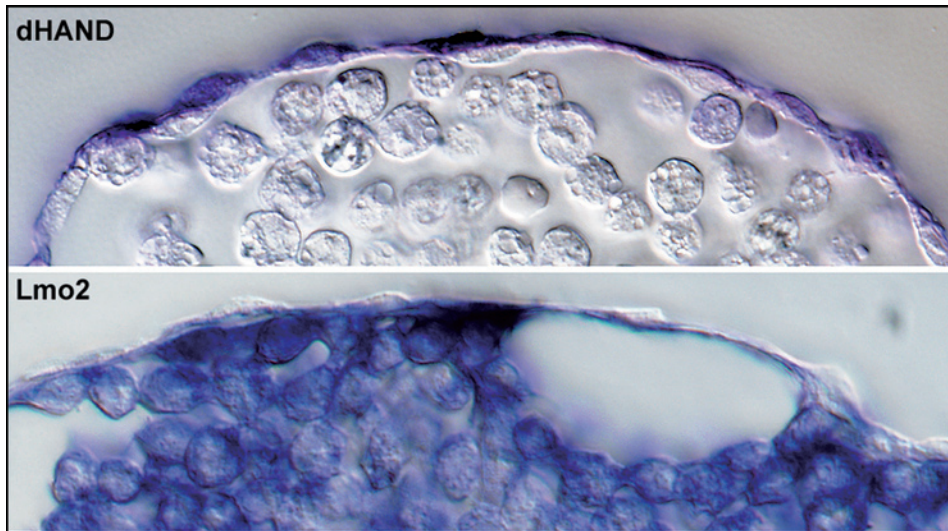
Not all development takes place inside the embryo. Many important functions, such as the formation of blood and blood vessels, are relegated to extraembryonic tissues in early developmental stages and reintroduced to the embryo proper subsequently. In the chicken embryo, the extraembryonic mesoderm gives rise to both blood and endothelial cells, but it has been never shown whether this represents its full differentiative gamut.

A study by Masahiro Shin and Hiroki Nagai in the Laboratory for Early Embryogenesis (Guojun Sheng; Team Leader) revealed that this same extraembryonic tissue is the earliest source of smooth muscle cells as well. In an article published in *Development*, the team identified the transcription factor dHand as a genetic marker of smooth muscle and showed that the segregation of this population from blood and endothelial progenitors is mediated by Notch signaling.

Notch has been shown to be involved in vascular remodeling and specification, but little was known about its role in ventral mesodermal differentiation (which gives rise to the population of cells that populate the extraembryonic mesoderm). To address this, Shin made a truncated Notch construct engineered such that the pathway was continuously switched on and introduced it into primitive streak-stage embryos to study the effects on cell fate. He found that a preponderance of the Notch-expressing cells took on a smooth muscle fate, with very few differentiating into blood/endothelial progenitors. In situ hybridization revealed that Notch is active in the posterior primitive streak, where ventral mesoderm is generated.

Conjecturing that Notch might act as a sorting mechanism to separate smooth muscle from blood and vascular progenitors, the team next sought a means of labeling smooth muscle, as no genetic markers for these cells had been identified. An in situ screen turned up the transcriptional factor dHand, which they found to be expressed in early smooth muscle cells as well as vascular smooth muscle layer and extraembryonic somatic mesoderm (also known to consist of smooth muscle cells) at later stages of development.

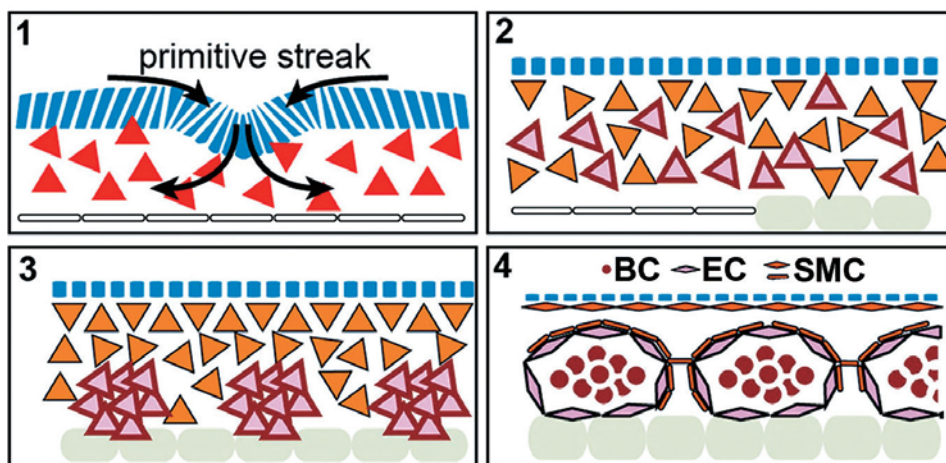
Blood and endothelial progenitors have their own specific marker in the gene *Scf*. When the team went back and looked at expression patterns in the constitutively Notch-active posterior streak, they found that cells expressing Notch also tended to express *dHand*, and not *Scf*.

**Figure 1**

dHAND marks the smooth muscle cells, which are tightly wrapped around vessel cells with developing blood cells inside the vessel lumen (both vessel and blood cells are marked by Lmo2).

Inhibiting the Notch pathway abolished this exclusion. Interestingly, they found that although Notch activation is associated with nearly exclusive smooth muscle cell contribution to the extraembryonic mesoderm, the absence of Notch has no effect on the induction or differentiation of these cells.

They next asked whether other signaling pathways known to work in the same vicinity might also be involved in smooth muscle specification. While BMP appeared to function primarily by ventralizing the mesoderm (leading to the induction of both smooth muscle and blood/endothelium), ectopic expression of Wnt strongly induced the smooth muscle marker *dHand*. Intriguingly, the signal responsible for inducing the blood/endothelial common progenitor has yet to be identified.

**Figure 2**

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.

"Perhaps many researchers aren't so interested in extraembryonic tissues, but a similar tug-of-war between muscles and vessels happens in cardiac development and in mesoderm stem cell differentiation in vitro," says Sheng. "So in that sense the molecular mechanisms uncovered in our studies may be of more general interest."

Endothelial cells give birth to blood



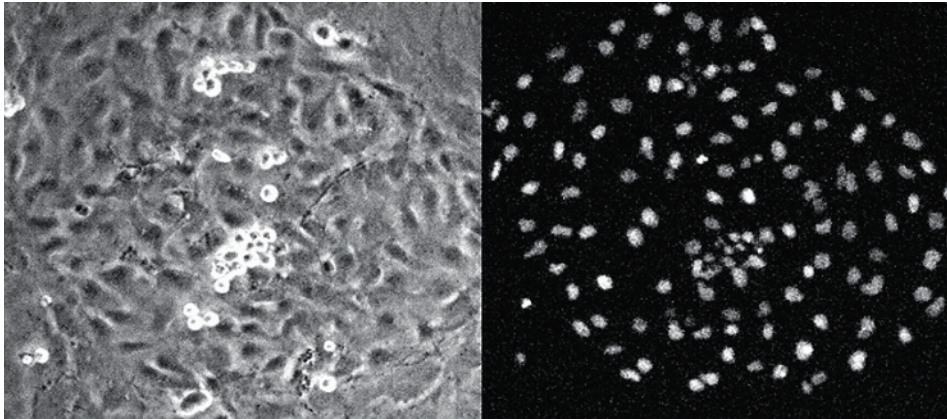
Timm SCHROEDER

An imaging technique developed through a collaboration between the CDB Laboratory for Stem Cell Biology (Shin-Ichi Nishikawa; Group Director) and Timm Schroeder, a former CDB research scientist who now heads the Independent Research Group for Hematopoiesis at the Helmholtz Zentrum München, German Research Center for Environmental Health, Institute for Stem Cell Research, yielded a compelling new insights into the origins of blood in the mammalian embryo. This represents the first joint publication between the two institutes, which established a research affiliation in 2005.

The debate over the ultimate source of embryonic hematopoiesis is fraught and longstanding, with competing hypotheses proposing that blood is generated from mesoderm, mesenchymal progenitors, endothelial precursors, or cells in the endothelium itself. But limits in the technologies used to visualize blood differentiation at the cellular level by observing only single genetic markers at single points in time have meant that the existence of hemogenic endothelial cells has remained in dispute.

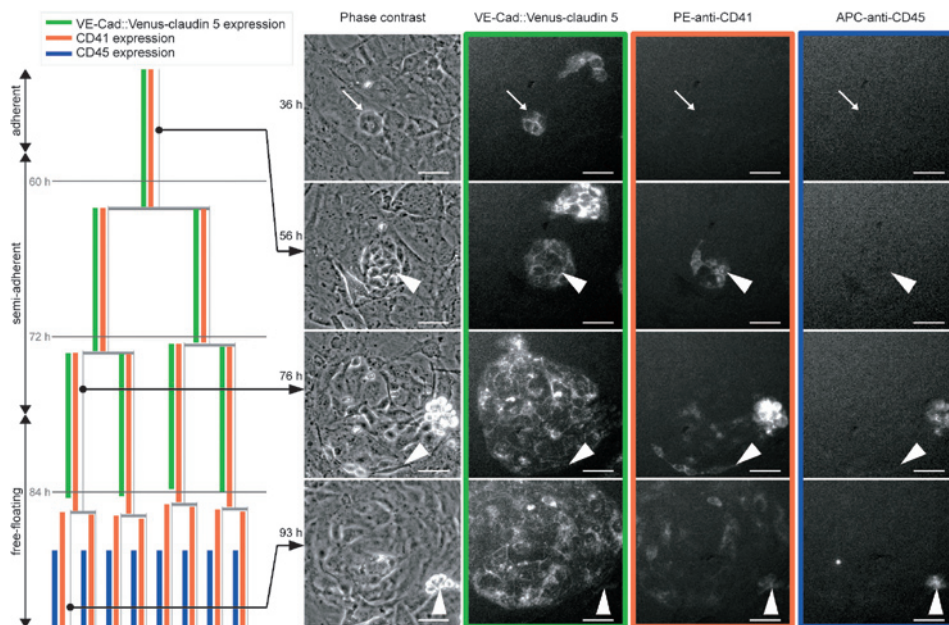
Schroeder set out to address these shortcomings by developing a bio-imaging approach that would enable him to watch well-characterized individual endothelial cells continuously during the differentiation process. Starting with mesodermal precursors (the cells from which endothelial cells originate) cultured under conditions known to induce endothelium, the team observed their morphology and behavior using computer-assisted time-lapse microscopy. From the total population, endothelial cells were isolated by selecting for the full set of their known characteristics, including sheet morphology, the ability to take up low-density lipoprotein, and the expression of VE-cadherin and the tight junction marker, claudin.

Similarly, blood cells were identified by their non-adherence, proliferation, expression of blood-specific marker proteins, and the absence of endothelial characteristics. Looking at more than 10,000 mesodermal colonies, the team finally narrowed their focus down to 144 that had endothelial sheet morphology and generated blood cells. In all of these, they found that adherent endothelial cells gave rise to blood cells that gradually detached completely from the colony. The free-floating blood cells expressed blood lineage markers in a similar sequence to that of emerging hematopoietic cells in vivo.

**Figure 1**

Hemogenic endothelial colony with blood cells generated by it, above. (Left: phase contrast; Right: Histone2B-VENUS labeled nuclei)

Importantly, the hemogenic endothelial cells failed to differentiate into either smooth muscle or cardiomyocytes, suggesting that they were not multipotent mesodermal cells. And they lacked the expression of CD45, a blood marker, further ruling out the possibility that they were bipotent blood-endothelial precursors. Indeed, the hemogenic cells gave rise solely to blood cells, while their neighboring sister cells generated only endothelial progeny. Mesodermal cells taken from mouse embryos showed similar hemogenic capacity, indicating that the findings were not merely an artifact of cell culture.

**Figure 2**

Molecular dynamics during endothelial-hematopoietic cell transition. Multiple molecular markers were simultaneously detected at the single cell level over days. Part of a haemogenic endothelial cell transition is shown. Haemogenic endothelial cells first express the endothelial marker VE-cadherin, but not the hematopoietic markers CD41 or CD45. Later, cells with endothelial morphology start expressing CD41, and develop into non-adherent blood cells as identified by CD45 expression. Hours after start of the movie and cells of pedigree (arrowheads) are indicated. Scale bars: 50 μ m.

Ins and outs of the early embryo



Noriyuki NISHIOKA

In the first few days of mammalian development, the embryo transforms from a clump of undifferentiated cells to a fluid-filled sphere called the blastocyst, with distinct inner and outer cell types. The interior cells, known as the inner cell mass (ICM), go on to form the embryo proper, while the external layer of cells, or trophectoderm, contributes to extraembryonic tissues. Previous work has shown that the ICM-trophectoderm balance is maintained by interdependent mutual inhibition between a pair of factors: *Cdx2*, which drives trophectodermal differentiation, and *Oct3/4*, which maintains the ICM in an undifferentiated state. But the mechanisms involved in tipping this balance to either side in even earlier embryonic regions have never been worked out.

A study by Noriyuki Nishioka and colleagues in the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) has revealed that Hippo signaling controls the expression of both *Cdx2* and *Oct3/4* in the early embryo. The report, published in *Developmental Cell*, describes an intricate interplay between factors in the embryo's inner and outer cells, in which the activation of the transcription factor *Tead4* in outer cells switches on *Cdx2* expression, while its suppression by the Hippo pathway in interior cells allows *Oct3/4* to dominate. The authors speculate that this may be triggered by differences in the cell adhesion environments in these two zones of the embryo.

Prior to the formation of the blastocyst, the cells in the embryo can only be distinguished from one another by their position in the exterior (surface) or interior of the embryo. It has been thought that positional information from the inner and/or outer cells might influence the contest between *Cdx2* and *Oct3/4* that defines the initial differentiation events in the blastocyst, but the mechanism to account for this remained elusive. There was, however, one tantalizing clue from previous work by the lab, which had showed that *Tead4* is required for *Cdx2* expression



and the formation of the trophectoderm. The mystery was that Tead4 is expressed in every cell of the embryo, not just the cells that differentiate into trophectoderm. Another member of the Sasaki team, Mitsunori Ota, added an important piece to the puzzle when, working on a separate line of inquiry, he found that in cultured cells and later-stage, post-implantation embryos the activity of Tead4 relied on a co-factor, Yap.

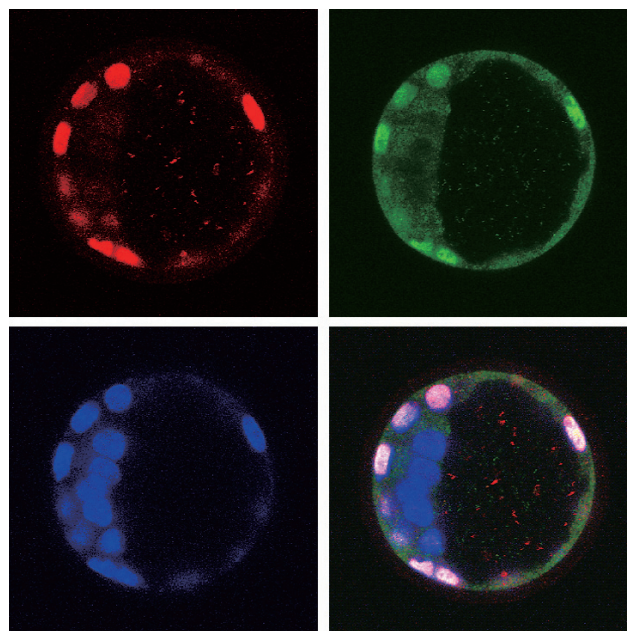
This prompted Nishioka to look for a possible role for Yap in the early embryo as well. Looking at the distribution of the Yap protein, they were interested to find that while Yap localizes to the nucleus in outer cells, it is cytoplasmic in the inner ones. Importantly, this nuclear localization in outer cells precedes the expression of Cdx2, which suggested that, in these cells, Yap might trigger Tead4, which then switches on Cdx2. Overexpression of Yap or activated Tead4 caused Cdx2 to be expressed aberrantly in inner cells, while the expression of a Yap protein engineered to lack its Tead-binding domain had no effect.

The question remained: What causes Yap to move to the nucleus only in outer cells? An experiment showed that a Hippo pathway member, Lats, phosphorylates Yap in cultured cells, causing it to remain in the cytoplasm. In the embryo, they found that phosphorylated Yap is only found in the inner cells, and that overexpression of Lats throughout the entire embryo causes a marked reduction of nuclear Yap and failure to form trophectoderm.

Thinking about what might account for the inner cell-specific activity of Lats, Ota showed that the activation of this factor causes Yap to stay in the cytoplasm. Armed with this knowledge of what happens in vitro, Nishioka and colleagues did a series of experiments in 8-cell embryos in which he either interfered with cell-cell adhesion or dissociated and rearranged the cells of the embryo, and found that cellular adhesion indeed does activate Hippo signaling, and that the adhesive environment determined by a cell's position affects the localization of Yap within the cell.

Other recent work from the Sasaki team had shown a role for Hippo signaling in the regulation of Tead, in a phenomenon known as contact inhibition of proliferation. In this latest work, they have demonstrated a similar link between cell contact and differentiation in the early mammalian embryo, the first evidence of how a cell's positional information determines its fate in this context.

"These new findings are an important step towards a better understanding of the mechanisms underlying differentiation along the inner-outer axis of the early embryo," says Sasaki. "But there have also been reports that cell polarity is important in pre-implantation differentiation, and there is much we still do not know, so we're looking forward to learning more about how such events are controlled during these critical early stages of development."



Expression of Yap (green) and Cdx2 (red) in outer cells of the blastocyst. Blue shows labeling of nuclei with DAPI. Merged image at bottom right.

2009 Events

In addition to its research activities, the CDB conducts events every year to engage more closely with the public, as well as to foster closer connections between labs and individual scientists. In 2009, the CDB held a number of successful public and closed events.



Open House

The RIKEN CDB held its annual Open House on April 25, welcoming more than 1,400 visitors from Kobe and the surrounding areas of Japan to learn about the Center's research and enjoy exhibits and activities for the curious of all ages. This event featured a talk by Shigeru Kuratani on the evolutionary developmental biology of the turtle shell, a special exhibit on green fluorescent protein to mark the 2008 Nobel Prize, and themed exhibits on germ cells and systems biology. A science café was held to allow people to meet and exchange views on questions from the leading edge of science, and a crafts corner, laboratory photo studio, and games for kids were all part of the fun.



CDB Retreat

The CDB held its institutional retreat at a new location in 2009, moving to the natural setting of a nature center in the mountains of Sasayama. At this yearly two-day meeting, CDB lab leaders, research scientists and student trainees gather in a closed meeting to encourage free and open discussion of preliminary findings and unpublished results, and to get to know each other better through conversation and time spent together away from the lab. This year's highlights included talks by two CDB alumni who left the Center to start their own labs, and musical and visual entertainment provided by members of the CDB staff.



A zebra finch embryo at approximately stage HH23 of development, with inset showing it intact with extraembryonic tissues and yolk.

Vertebrate Body Plan



Shinichi AIZAWA Ph.D.

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

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

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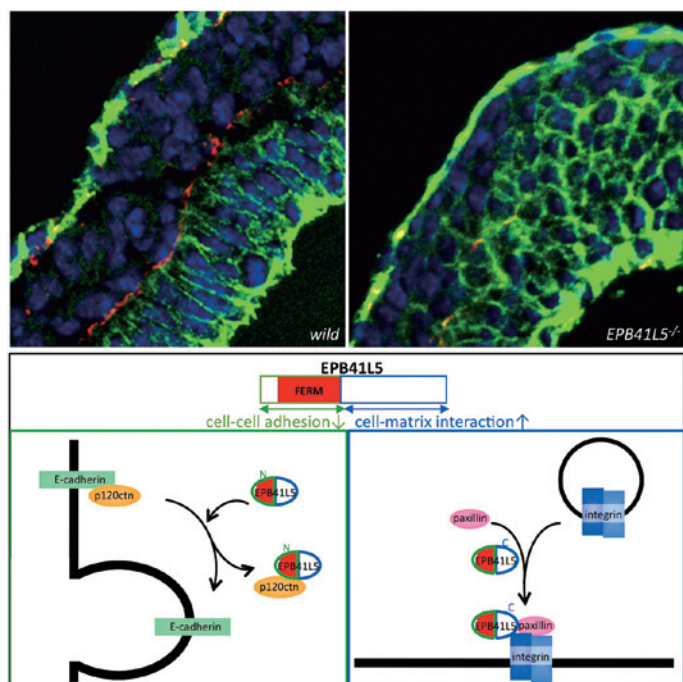
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All vertebrate species share certain features in the development of the brain, in that all vertebrate brains comprise four regions – telencephalon, diencephalon, mesencephalon and metencephalon (or cerebellum) – an organizational trait that is thought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



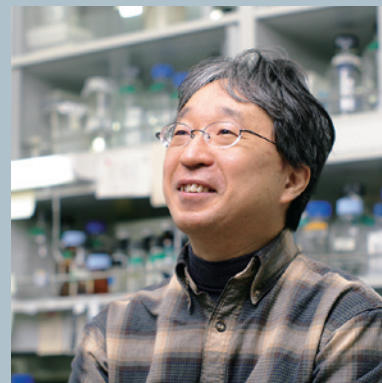
EPB41L5 regulates epithelial/mesenchymal transition post-transcriptionally; it binds to p120 catenin through its N-terminal FERM domain, thereby destabilizing E-cadherin toward endocytosis and to paxillin through its C-terminal, thereby activating integrin expression in cell surfaces or focal adhesion 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, GRF α 1-4. In vitro, these four receptors show differential affinities for specific GFLs. GFL signaling has been shown to affect neuronal growth, survival and migration as well as axon guidance, and defects in this signaling system have been implicated in a number of congenital health problems.

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



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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Burzynski G, et al. Genetic model system studies of the development of the enteric nervous system, gut motility and Hirschsprung's disease. *Neurogastroenterology & Motility*, 21, 113-27 (2009) Review.

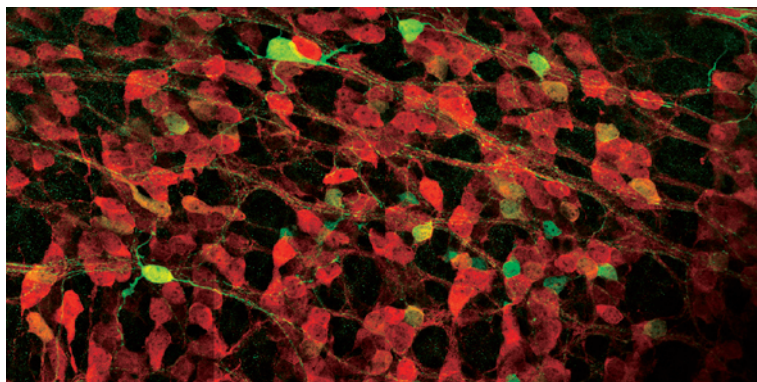
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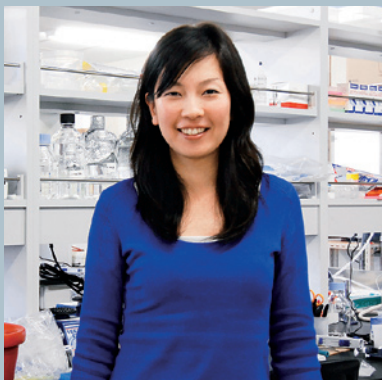
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Developing enteric nervous system (ENS) in which GDNF receptor RET was conditionally inactivated in a small population of ENS cells (mouse gut: embryonic day 14.5, Green: Ret-deficient cells, Red: enteric neurons).

Neocortical Development



Carina HANASHIMA Ph.D.

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

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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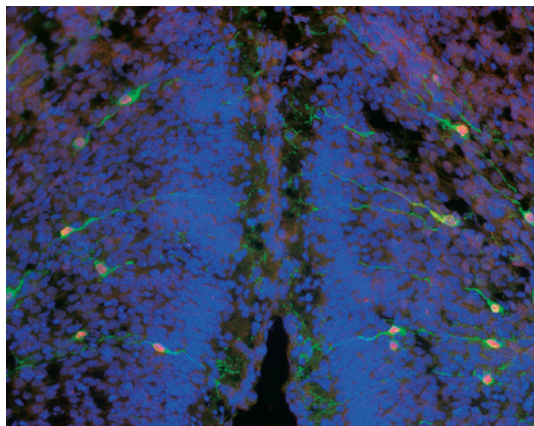
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The neocortex, by far the most complex structure of all vertebrate brain systems, is organized into cytoarchitectonic groups of neurons that process unique sensory inputs such as pressure, light and sound. Nonetheless this highly ordered structure 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 imply cortical intrinsic programs in which neuron fate is established by temporal changes in gene expression may be co-opted. However, in the mature cortex, cortical areas differ in their types and numbers of specific layer neurons along both the anterior-posterior (AP) and medial-lateral (ML) axes. We are exploring the extent to which intrinsic determinants control the specification of neuronal subtypes within discrete regions of the neocortex, as well as the extrinsic influences that refine the boundaries between functional areas of neocortex. To further these studies, we employ genetic manipulations in mice that will enable conditional loss of gene and cellular functions, recombination mediated cell-lineage tracing, and systematic approaches to identify novel molecules responsible for precise areal specification. Through these studies we wish to understand the mechanistic basis by which unique sensory perceptions and functional circuitries develop in the human neocortex.



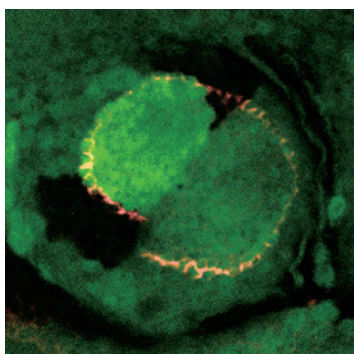
Recruitment of late-born neocortical neurons expressing membrane-targeted GFP (green) and nuclear lacZ (red) to the motor cortex.

Morphogenetic Signaling

The main research interest in my lab focuses on the mechanisms by which cell-cell and tissue-tissue interactions are modulated during embryonic morphogenesis. Our strategy is to identify intercellular signaling systems and intracellular transducers that control cell-cell and tissue-tissue interactions. 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.

The *Drosophila* tracheal system is 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. The branching patterns and cell fate are instructed by external cues, including FGF, WG, and Dpp. We are studying the roles of these signaling molecules in the specification and migration of tracheal branches, as well as the mechanisms that coordinate cell movement and cell adhesion. We additionally use 4D confocal imaging of GFP-labeled embryos to study the dynamism of cell and organelle movement in living organisms. Using combinations of GFP markers and transcriptional enhancers of cell-specific expression, we have been able to capture movements of tracheal cells at resolutions sufficient to image cytoskeletal organization and cell adhesion structures in single cells.

The development of appendages in *Drosophila* from primordial regions called imaginal discs is a second area of interest. During this process, subpopulations of cells in the imaginal discs segregate into distinct domains by coupling cell growth and differentiation to cell sorting, which provides us with an opportunity to study the regulation of cell affinity by positional information. Each limb primordium also coordinates its specific developmental pattern with other tissues, such as muscles, motor nerves and trachea, which are specified independently in other parts of the embryo. This understanding of mechanisms of limb specification and proximal-distal axis formation gained from work on *Drosophila*, however, must also be validated by comparative analyses in other species with simpler appendage structures. We focus on three species for these comparative studies: the bristletail, *Pedotontus unimaculatus*, the mayfly, *Ephemera japonica*, and the ragworm, *Perinereis nuntia*.



Homophilic cell adhesion molecules Capricious and Tartan are required for proper placement of segmental boundary in the leg. The boundary of pretarsal and tarsal 5 segment is marked with red signal, which normally appears as a ring. When the boundary is flanked by a group of cells lacking Capricious and Tartan (indicated by a loss of green signal) the position of the boundary was shifted interiorly and the boundary appeared uneven.



Shigeo HAYASHI Ph.D.

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Shigeo Hayashi received his B. Sc. in Biology from Kyoto University in 1982, and his Ph.D. in Biophysics from the same institution in 1987, for his work on lens-specific regulation of the chicken *delta crystallin* gene. Inspired by the discovery of the homeobox, he changed his research focus to the developmental genetics of *Drosophila* and spent three years as a post-doctoral research associate in Matthew Scott's lab at the University of Colorado before returning to Japan to work in the National Institute of Genetics. He became an associate professor at the same Institute in 1994, and professor in 1999. Also in 1999, he received the incentive prize of the Genetics Society of Japan for Genetic Studies on *Drosophila* Development. He was named group director of the Morphogenetic Signaling research group at the RIKEN CDB in May 2000. His current research interests are dynamic aspects of cell adhesion, cell migration and cell morphogenesis in *Drosophila*.

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Kato K and Hayashi S. Practical guide of live imaging for developmental biologists. *Dev Growth Differ* 50. 381-90 (2008)

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Vertebrate Axis Formation



Masahiko HIBI M.D., Ph.D.

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

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.

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Publications

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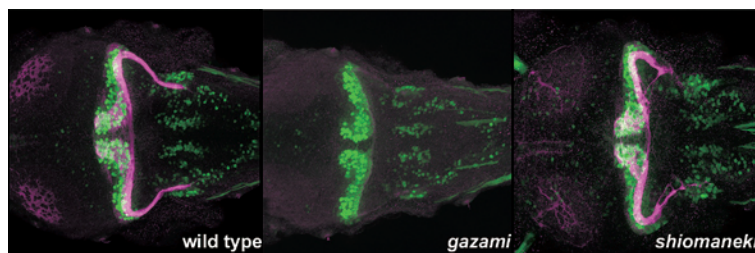
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During early vertebrate development, the embryonic dorso-ventral (DV) and anterior-posterior (AP) axes are established through a series of inductive signals that prefigure and determine the concerted movements and differentiation of a group of cells. 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 co-operates 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.

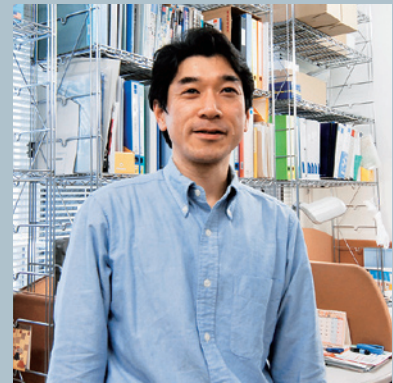


Development of cerebellar neurons in zebrafish larvae at 5 days post fertilization. Purkinje neurons (green) receive axons of granule cells (magenta) in wild type. Loss and abnormal extension of granule cell axons are observed in *gazami* and *shiomaneki* mutants, respectively.

Cell Lineage Modulation

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.



Toru KONDO Ph.D.

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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.

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Hide T, et al. Sox11 prevents tumorigenesis of glioma-initiating cells by inducing neuronal differentiation. *Cancer Res.* 69, 7953-7959 (2009)

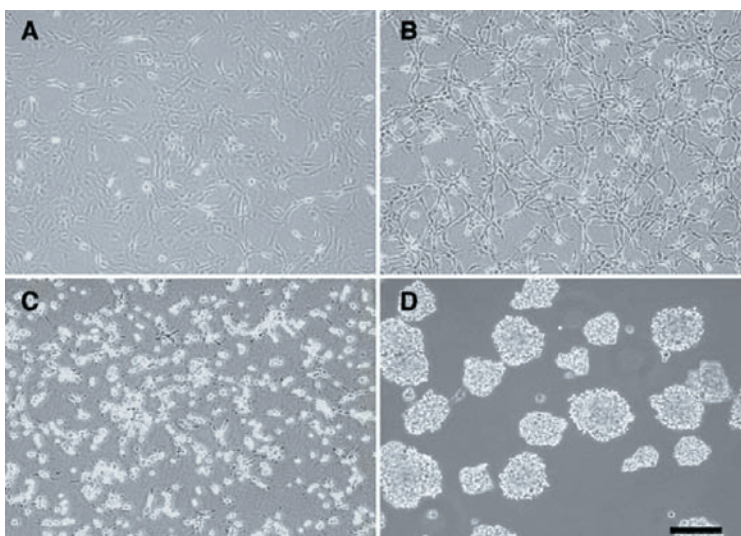
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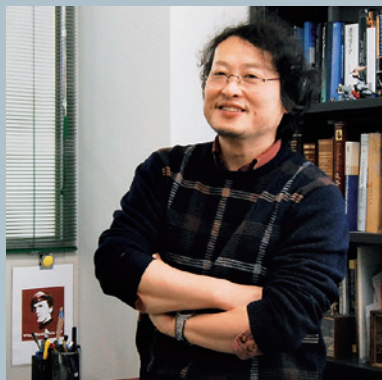
Kondo T. Making of glioma-initiating cells. *Inflammation and Regeneration* 28, 537-542 (2008)

Kondo T. Stem cell-like glioma cells. *Current Cancer Therapy Review* 4, 201-205 (2008)



Morphology of rat C6 glioma cells in different culture conditions, FCS(A), bFGF(B), PDGF(C) or PDGF+bFGF(D).

Evolutionary Morphology



Shigeru KURATANI Ph.D.

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

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

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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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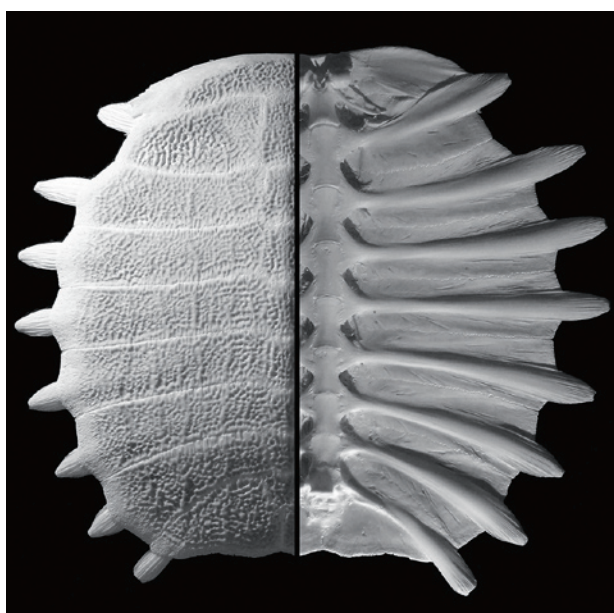
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By studying the evolutionary designs of diverse animal species, I hope to gain a deeper insight into the secrets behind the fabrication of morphological designs. Integrating the fields of evolutionary morphology and molecular genetics, our lab seeks 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.



Dorsal (left) and ventral (right) views of the carapace (dorsal half of the turtle shell) of Chinese soft-shelled turtle. The carapace is made of laterally expanded ribs.

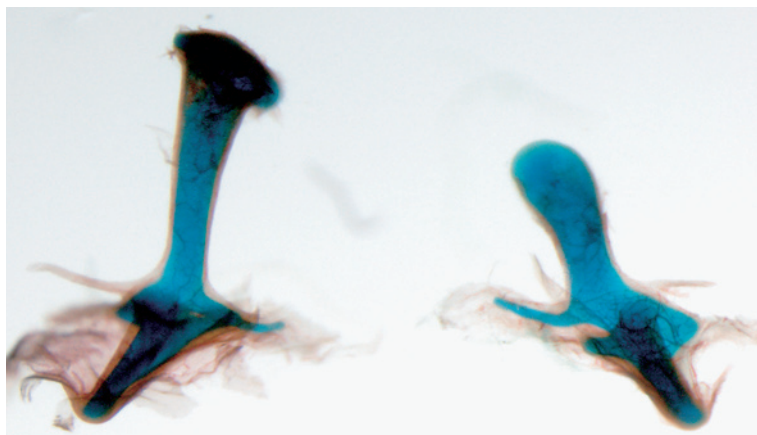
Sensory Development

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

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

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

We believe that an understanding of the embryological systems that control the development of the inner ear can be applied to the development of other organs 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.



The chick middle ear is made of one bone, the columella. Alcian blue highlights the cartilage of the columella. On the left is a control columella, the right columella is from an anotic embryo.



Raj LADHER Ph.D.

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

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

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Cell Asymmetry



Fumio MATSUZAKI Ph.D.

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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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Ogawa H, et al. Protein phosphatase 2A negatively regulates aPKC signaling by modulating phosphorylation of Par-6 in *Drosophila* neuroblast asymmetric divisions. *J. Cell Science* 122, 3242-3249 (2009)

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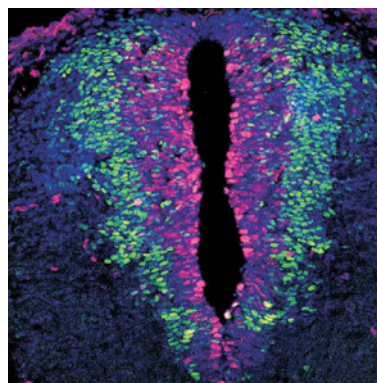
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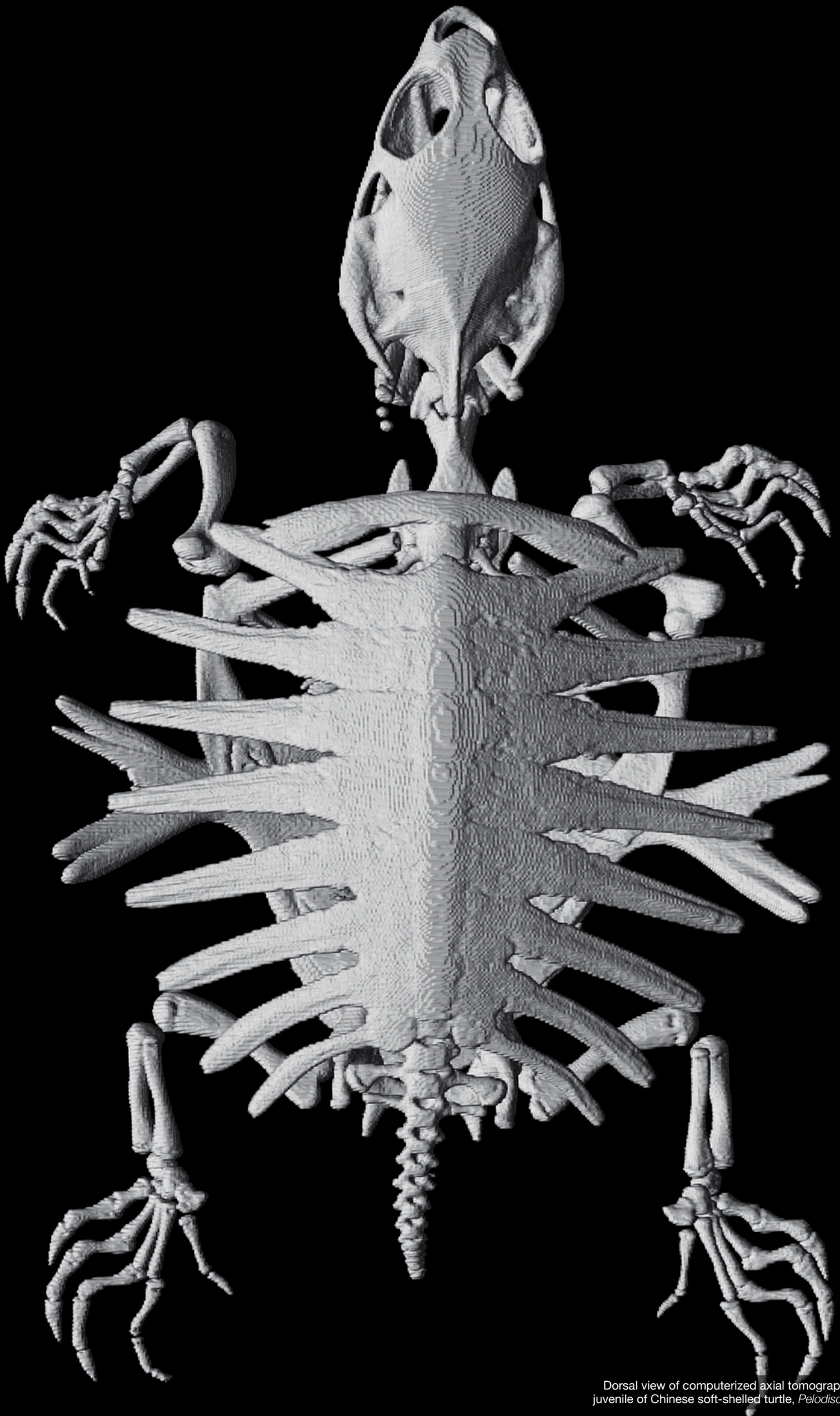
Our group seeks to explore the molecular mechanisms underlying the organization of cells into highly ordered structures in the developing brain, where neural stem cells generate a large number of neurons of different fates at appropriate points in time. Asymmetric cell division plays an essential role 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.

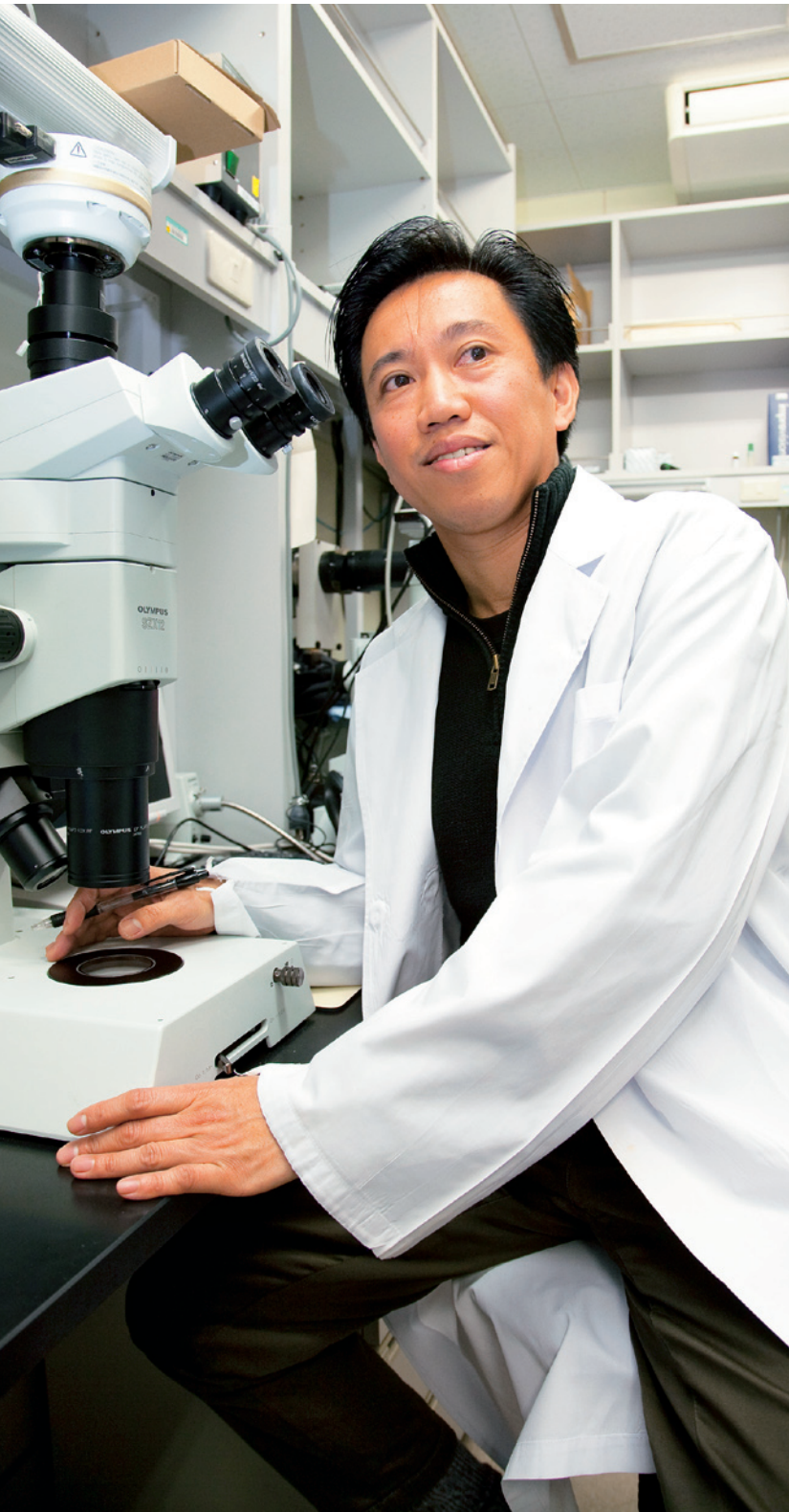


In the developing mouse spinal cord, Prospero (green) is transiently expressed in newborn neurons immediately after birth from mitotic neural progenitors (red).



Dorsal view of computerized axial tomography scanned juvenile of Chinese soft-shelled turtle, *Pelodiscus sinensis*.

Eye field refined by BMPs



Michael TERAOKA

In vertebrates, the eye develops from a single patch at the headward end of the neural plate, which later splits into two, allowing a pair of eyes to form from optical vesicles that bulge outward from the sides of the developing brain. The initial bisection of the eye field is achieved by signaling from the neighboring prechordal mesoderm, but how the subsequent shaping and fine-tuning of the resulting halved regions is directed is not well understood.

In a report published in *Developmental Biology*, Michael Teraoka and colleagues in the Laboratory for Sensory Development (Raj Ladher; Team Leader) described how a region of paraxial mesoderm works latterly to refine and restrict the eye field after its bisection in chick. The team found that signals from the rostral cephalic paraxial mesoderm help to limit the expression of eye field transcription factors through the action of the BMP signaling pathway.

Teraoka began with experiments in which he removed various tissue regions at developmental stages corresponding to eye formation. When he extirpated paraxial mesoderm near the head, he saw the optical vesicles subsequently became enlarged, due to an increase in the total numbers of cells in these structures. As no effect was seen on cell proliferation, he surmised that the increase in the numbers of optical vesicle cells might be due to a misassignment of cell fate instead.

Following this classic embryological approach, he next swapped rostral paraxial mesoderm from quails into regions of the embryonic chick head that do not normally feature mesoderm. He found that this affected eye development in the converse manner: the optic cups were reduced in size and the lens vesicles were small or absent. The transplantation also affected the expression of genes associated with eye development, such as *Pax6* and *crystallin*. Significantly, there was no effect on neural patterning in general, suggesting that the rostral paraxial mesoderm has an eye-specific role. This role, in turn, is specific to the rostral paraxial mesoderm as well; transplantation of other regions of quail mesoderm to the same part of the embryonic chick head had no effect on the development of the eye.

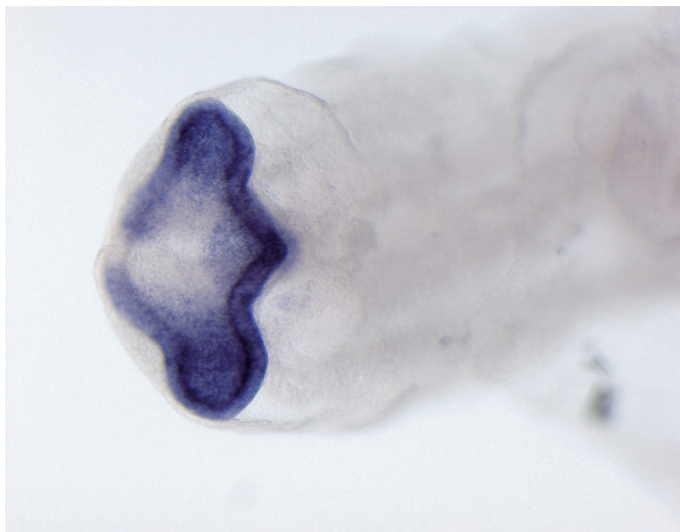


Seeking a possible molecular mechanism behind the rostral paraxial mesoderm's eye-inhibiting function, Teraoka focused on bone morphogenetic protein (BMP) signaling, which he found to be expressed in the paraxial mesoderm and, importantly, repressed in the axial mesoderm, which has no optic-inhibitory activity. He next implanted tiny beads soaked in BMP factors to test for effects on optic gene expression, and found that BMP4 caused reductions in several eye-related genes without affecting genes more generally involved in neural patterning. Knocking the BMP level down in vivo by shRNA had the opposite, pro-optic effect of increasing the size of the optic vesicles, which was mirrored by the antagonism of factors downstream in the BMP pathway as well. The team speculates that BMP's role may be mediated by its interplay with both canonical and non-canonical Wnt signaling, another pathway known to be important in eye development.

**Figure 1**

BMP4 soaked into a microgel bead (blue) inhibits the formation of the eye, by acting on a downstream optic determinant, *wnt8b*.

"I think that the most important aspect of this work is the finding that organ-generating fields are constantly being told what to do by their neighbors," says Ladher. "In this way the final shape and position is formed so that the organ can carry out its function."

**Figure 2**

BMP4 soaked into a microgel bead (blue) inhibits the formation of the eye, by acting on a downstream optic determinant, *Pax6*.

A complicated birth for the germ cell lineage



Yasuhide OHINATA

Segregating the germ cells used in reproduction from the rest of the body's (somatic) cells is a mission-critical task for the embryo for, more than any organ system, the germline is the body's *raison d'être*. In many species, the initial commitment of the germ lineage cells takes place early in development, and sets them off on a journey through somatic regions before they reach their final home in the gonads. This is true for mammals as well; in the mouse, the roots of the germline have been traced back to as early as the sixth day of development. But what triggers the expression of the first germline-specific genes has been a mystery.

A study by Yasuhide Ohinata and colleagues in the Laboratory for Germ Cell Biology (Mitinori Saitou; Team Leader) revealed signals even further upstream in the germ cell specification cascade, originating from the extra-embryonic ectoderm. Reported in *Cell*, the newly unearthed mechanism, which involves both the Bmp and Wnt pathways, was further shown to drive the high-efficiency differentiation of this tissue to the germ lineage *in vitro*, yielding viable germ cells (sperm) capable of fertilization following transplantation into testes.

The importance of Bmp signaling, including the Smad proteins downstream, from the extraembryonic ectoderm had been pointed at by earlier work from other labs, while Saitou's lab had previously identified *Blimp1*, *Prdm14* and *stella* as the earliest genetic factors in germline specification. Taking that as their starting point, Ohinata and colleagues asked how these two known factors might be connected. In the embryo, the germline specifying factors *Blimp1* and *Prdm14* are expressed in only a limited region of the epiblast proximal to the extraembryonic ectoderm, while *Bmp4* and *Bmp8* are uniformly expressed throughout that tissue. Loss-of-function studies revealed that germ cell specification is the result of an interaction between two signaling axes – one related to proximity to the extraembryonic ectoderm (involving the Bmps) and a second that represented posterior epiblast properties, which could be inhibited by an antagonistic signal from the anterior visceral endoderm (via a pathway mediated by *Smad2* and *FoxH1*).

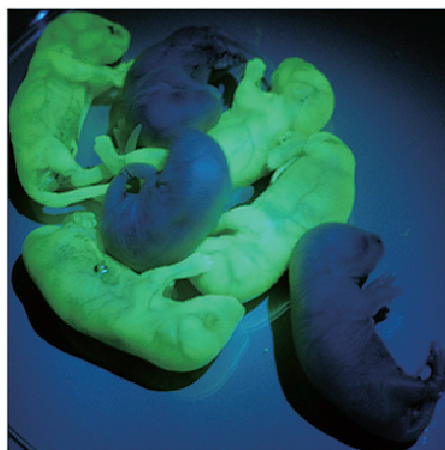
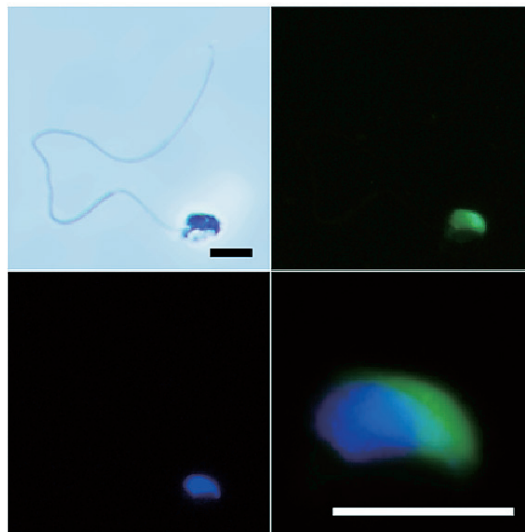


They next tried culturing these embryonic regions to see if their insights into the specification mechanism could be replicated *in vitro*. Using Day 6 epiblast and extraembryonic ectoderm, they showed that the addition of Bmp4 to the culture medium was sufficient to induce the expression of the germline-specifying factor *Blimp1* throughout the entire explanted region. Further studies using mutants for the Wnt factor *Wnt3*, which is involved in early embryonic patterning, showed that Bmp4's instructive function is Wnt-dependent.

Interestingly, a second Bmp factor, *Bmp8b*, which is known to be important for the generation of primordial germ cells, had no direct effect on germ cells themselves. Using a mouse model in which the *Bmp8b* gene had been knocked out, and further *in vitro* studies, Ohinata et al. determined that the role of this protein is to inhibit development of the anterior visceral endoderm, a tissue that has an inhibitory effect on Bmp4.

With this solid understanding of germ cell differentiation, Ohinata used the culture system the lab had developed to steer epiblasts to adopt this fate. The induced cells showed expression of *Blimp1* and other genetic and epigenetic hallmarks of germ cells. The real test of their character, though, came with their injection into the testes of sterile mice, which revealed that they were fully capable of further differentiation into viable sperm.

"In this study, we looked at the epiblast, which is composed of pluripotent cells that give rise to the embryo, so in that sense what we learned may also be applicable to other pluripotent cells, such as embryonic or induced pluripotent stem cells," says Saitou. "Although some ethical questions remain, it may be possible to apply this technique for germ cell differentiation in the future, which may have applications in the treatment of infertility in addition to its fundamental scientific interest."



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

A metabolomics approach to measuring circadian time

Our bodies, and those of all animals, have intrinsic mechanisms that ensure that their many internal systems, such as sleep and hormone secretion, follow the rhythms of the earthly day closely. Many diseases, such as those of the heart and vasculature, also show changes in the severity of symptoms at different times of day, and disturbances of the circadian rhythm can create great difficulties for individuals in their waking and sleeping lives. A technique for measuring the body's own time would open the prospects of a new "chronotherapy" approach to medicine. But we have yet to decipher the code of the body's clock.

A study by Yoichi Minami, Takeya Kasukawa and others in the Laboratory for Systems Biology (Hiroki R. Ueda, Team Leader) working in collaboration with colleagues at Keio University (Tsuruoka, Japan) has revealed a method for measuring bodily time by analyzing metabolic products in the blood. This new approach to reading circadian status is published in the *Proceedings of the National Academy of Sciences USA*.

Body time can be represented by a profile of "time-indicating metabolites" and can be accurately detected by "metabolite-timetable method". In the day (lower right) 'day-indicating metabolites' (orange) are at present at high levels, whereas 'night-indicating metabolites' (blue) are low. In the evening (upper left), the opposite is true.



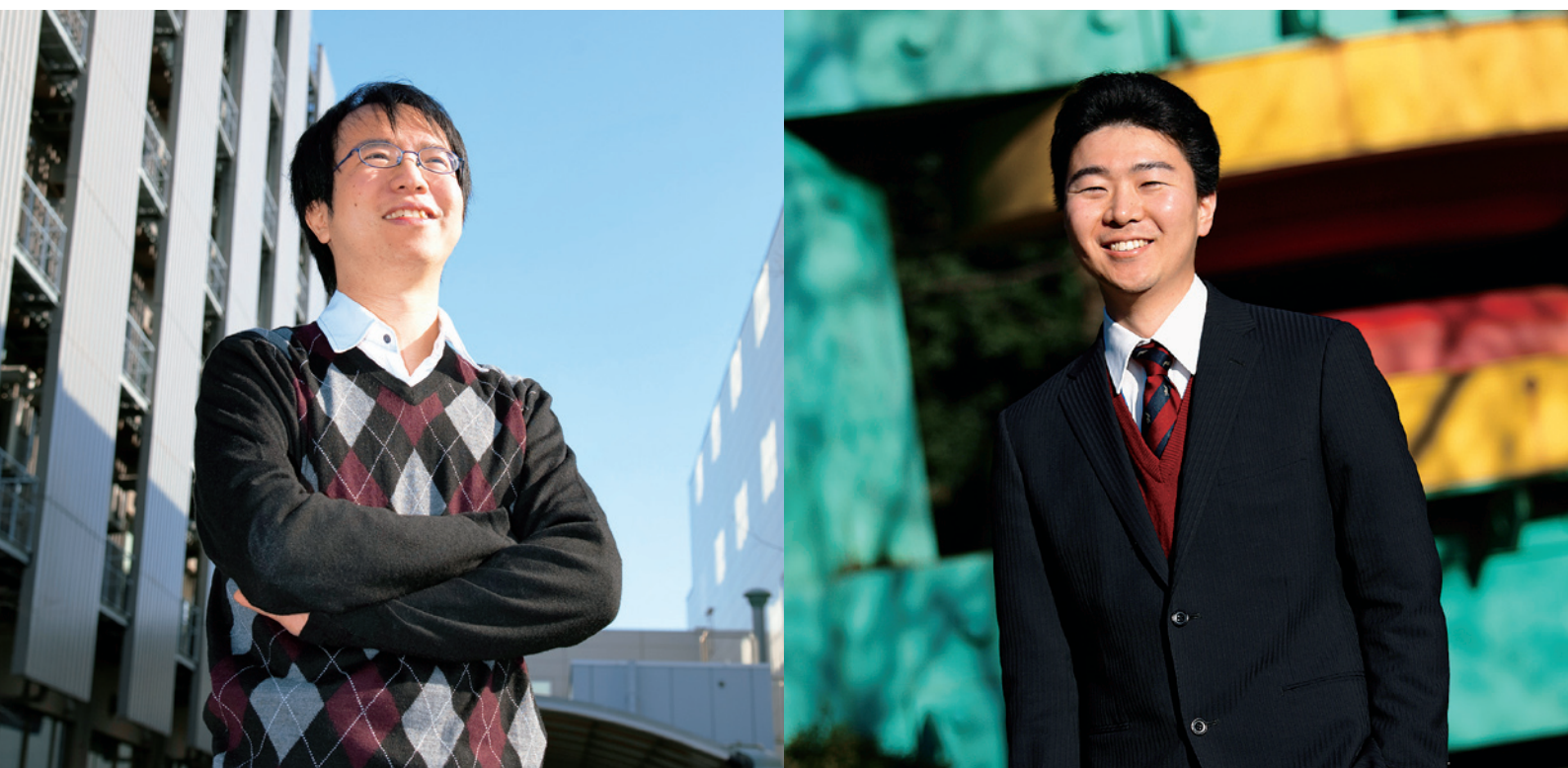
The inspiration for the study actually came from the 18th century Swedish botanist Karl von Linne (more commonly known as Linnaeus), who discovered that one could estimate the time of day simply by watching which types of flowers had opened their blooms. Minami and Kasukawa thought that they might be able to do the same thing by watching the changes in levels of metabolites and other factors instead. The Ueda lab had made a first important step toward that goal by developing a molecular timetable for the mammalian biological clock in 2004, but the method involved analyzing gene expression in the liver, making it unfeasible for simple clinical uses.



The team turned next to a metabolomics approach, which entails sampling a comprehensive set of metabolites in the bloodstream to get a snapshot of the body's state at any given time. However, such an approach had never been applied to substances with oscillating levels in the blood. Minami, Kasukawa and colleagues drew blood at 4-hour intervals from mice for two days and analyzed the samples using a combination of liquid chromatography and mass spectrometry at Keio University. Mathematical analysis of the samples revealed a set of 142 positive ion and 176 negative ion metabolic products that showed clear rises and falls over the course of a 24-hour day. Importantly, mice with known circadian defects showed aberrant metabolic cycles as well.

The team tested this newly developed metabolite timetable by sampling mice at arbitrary times of day and comparing their results with the predictions from the new technique, and found that it provided an admirably accurate picture of the body's circadian state. Additional tests using mice of different genetic backgrounds, genders, ages, and feeding conditions further confirmed the general applicability of the method. They next entrained mice to an altered daily rhythm and looked at whether the timetable would hold up in these "jet-lagged" animals. They found that on the first day of jet-lag, the circadian metabolites remained at their previous levels, but by day 5, they were at an intermediate state between real time of day and the entrained time, and by day 14, the switchover to the new cycle as measured by metabolomics was complete.

"People show much greater diversity than mice in their eating habits and daily schedules, so it would be important to take that into consideration in developing any clinical applications," says Kasukawa. Minami also adds, "The technique will need to be simplified before it can enter widespread use. But I think this metabolite timetable represents a significant step forward in the drive to realizing chronotherapy."



Takeya KASUKAWA

Yoichi MINAMI

Opposing roles for Wnt receptors in the olfactory system



Masao SAKURAI

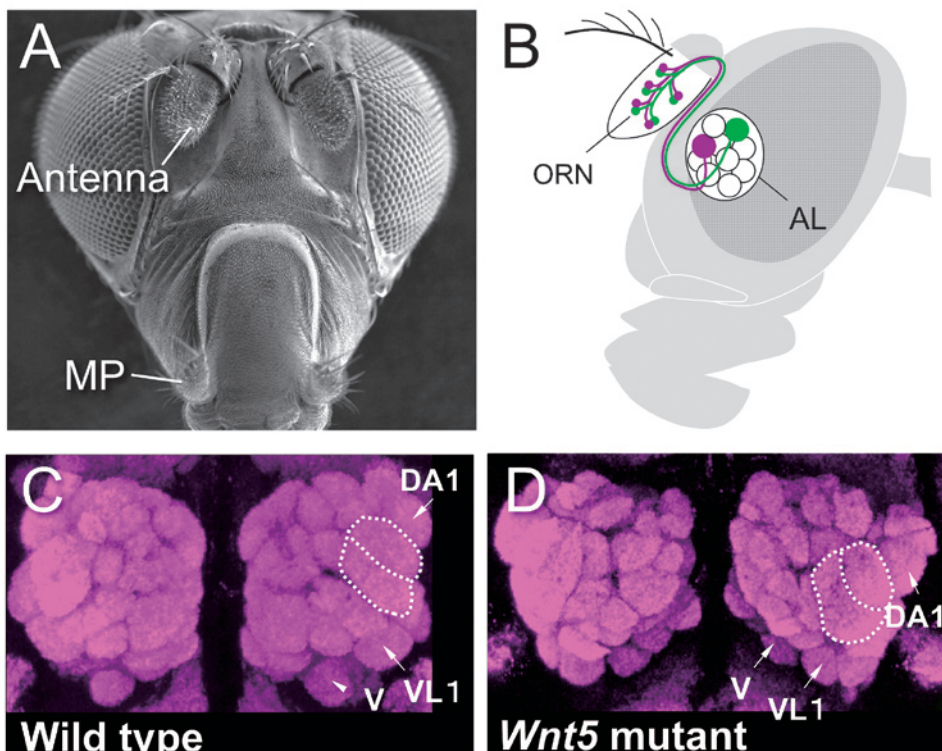
Olfaction is one of the primary sensory modes by which many species, including insects, survey their environments. The olfactory system is therefore highly specialized and exquisitely sensitive to a very broad spectrum of odorant signals. The fruit fly *Drosophila melanogaster*, for example, has around 1300 olfactory receptor neurons each carrying one of around 60 odorant receptors. Adding to the complexity, these neurons bundle together into 50 distinct glomeruli, which can be found arrayed in a consistent pattern in the brain of any given fly. The control mechanisms responsible for ensuring this olfactory patterning are known to involve the signaling factor Wnt5, but otherwise remain largely a mystery.

Work by Masao Sakurai and colleagues in the Laboratory of Neural Network Development (Chihiro Hama; Team Leader) revealed that a pair of receptors – Drl and Drl-2 –are expressed in different olfactory cell types and play opposing roles in Wnt signaling. These findings, published in *The Journal of Neuroscience*, shed new light on the cooperative mechanisms at work in establishing the olfactory circuitry.

The study began by looking at *Wnt5* mutants, which Sakurai found resulted in a phenotype in which peripheral glomeruli in the antennal lobes rotated clockwise of their wildtype positions. Knowing the role of Drl as a Wnt5 receptor, he next checked the *drl* mutant phenotype, but found it to be substantially different from that of Wnt5,



suggesting that other receptors might also be involved. To test this possibility, the team engineered flies to lack function of both *drl* and a related gene *drl-2*, and found that the double mutant phenotype much more closely resembled that of the *Wnt5* mutant, which suggests that both Drl and Drl-2 function as Wnt5 receptors.



A. Flies perceive odors through olfactory receptor neurons (ORNs) on the antenna and maxillary palp (MP).

B. ORNs expressing the same odorant receptor (as shown by the same color) project axons to one of 50 glomeruli in the antennal lobe (AL).

C. A stereotyped organization of glomeruli in the wild-type AL. The glomeruli DA1, VL1, V and VA1d.v (dotted circles) are shown in the right AL.

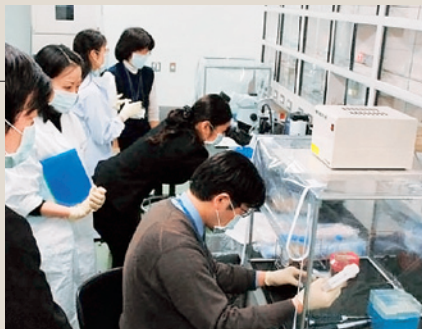
D. The glomeruli in the right AL shift in a clockwise manner in the *Wnt5* mutant compared to those in the wild type.

One reason for the differences in the *Wnt5* and *drl* phenotypes is traceable to Drl's antagonistic effect on Wnt5 signaling. The Drl protein is expressed in only a subset of second-order olfactory neurons and glial cells, and it is in the latter that the repressive function manifests. Drl-2 in contrast is normally expressed in the axons of olfactory receptor neurons. But when the Hama team misexpressed Drl-2 in the glial cells of *drl* mutants, they found it was capable of compensating for the loss of *drl* function, which antagonizes Wnt5 signaling. This led to the somewhat enigmatic conclusion that these two structurally related molecules play contrasting roles with respect to Wnt5, and that further, the choice of role is determined by the cells in which they are expressed.

"The antagonistic function of Drl and Drl-2 is likely to arise from titration of Wnt5 ligand by receptor binding," notes Hama. "This simple physical mechanism invokes the general idea that many other receptors potentially have an antagonistic role in their respective signaling."

2009 Courses

As part of its commitment to promoting awareness and understanding of its field of research, and maintaining close ties with the academic and research communities, the RIKEN CDB dedicates itself to conducting training and public educational programs for a wide range of audiences. Although not a teaching institution, the Center works with students, educators, professional scientists and other organizations to develop, implement and promote a variety of instructional courses throughout the year.



Pluripotent stem cell courses

The CDB organizes a series of beginners' workshops on the use of human stem cells in collaboration with the Kyoto University Center for iPS Research and Application and the RIKEN Bioresource Center. In 2009, these two-day learning programs were held in February, April, and November, and provided instruction for dozens of participants from academia and industry in the basics of human pluripotent stem cell research.

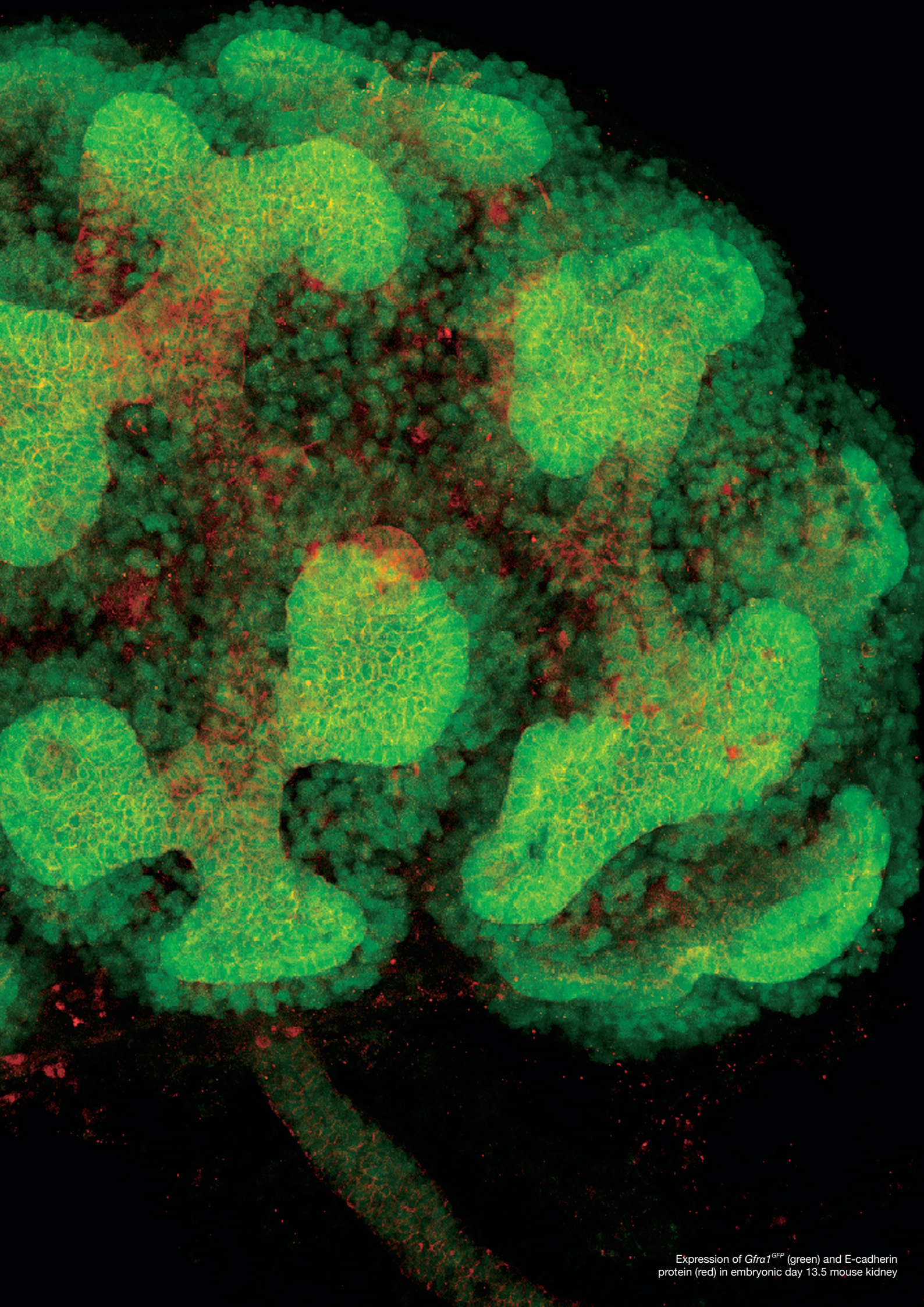
Summer school for high school students

The RIKEN CDB held two summer school days for Kansai area high school students on August 4th and 6th. Now in its third year, this year's program was organized with cooperation from the Laboratory for Morphogenetic Signaling. Both days featured a talk in the morning by Shigeo Hayashi (Group Director, Laboratory for Morphogenetic Signaling), followed by a visit to his lab, and hands-on lab work in the afternoon using *Drosophila* (fruit fly) larvae.



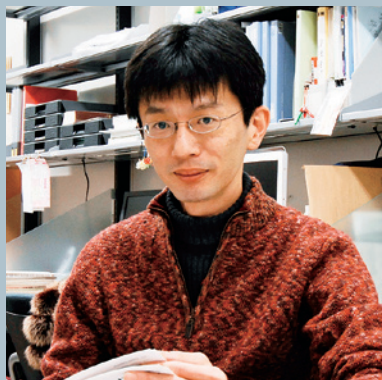
Science lesson for teachers

The RIKEN CDB held its recurring course for high school science teachers in conjunction with the Japanese Society of Developmental Biologists on October 3 and 4. This is the second time the course, which is aimed at making a contribution to high school education in the biological sciences, has been held since its launch last year. This year's main themes were the use of chicken embryos as a classroom material, and organizer transplant experiments, which were introduced through lectures and lab work.



Expression of *Gfra1*^{GFP} (green) and E-cadherin protein (red) in embryonic day 13.5 mouse kidney

Germline Development



Akira NAKAMURA Ph.D.

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

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

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Publications

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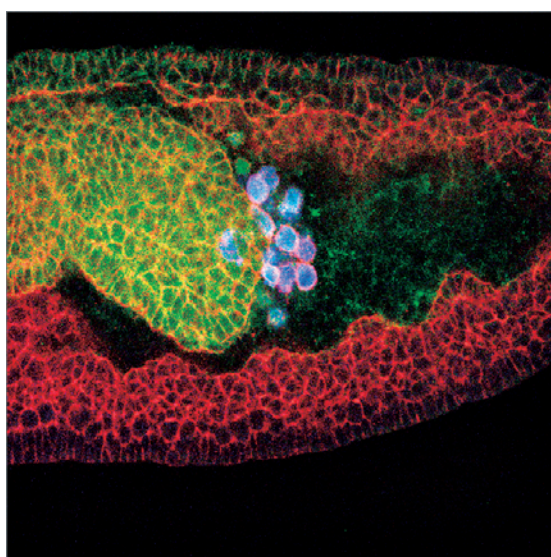
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Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the *Drosophila* fruit fly, the formation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



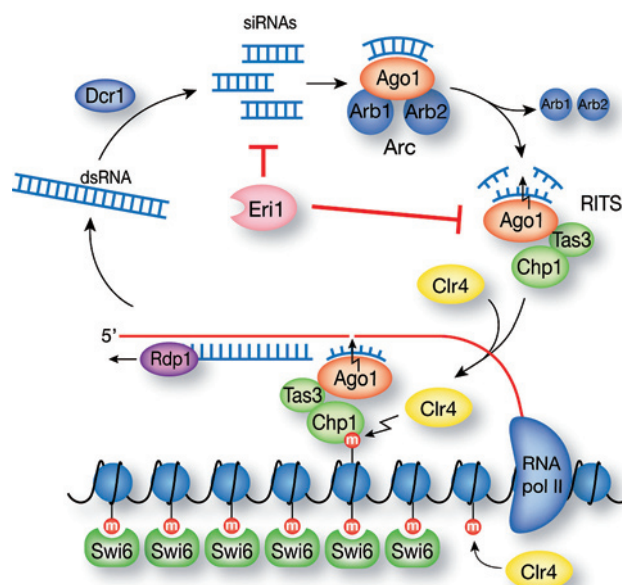
Migrating germ cells (blue) in stage 10 *Drosophila* embryo.

Chromatin Dynamics

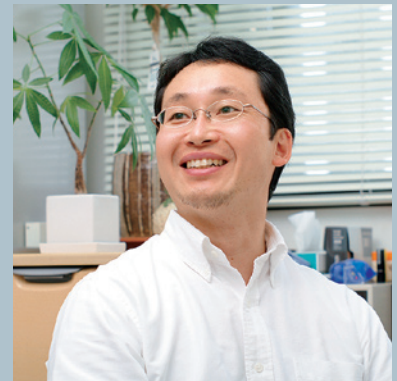
Multicellular organisms are made up of diverse populations of many different types of cells, each of which contains an identical set of genetic information coded in its DNA. Cell differentiation and the process of development itself depend on the ability of individual cells to maintain the expression 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 chromatin. In our current studies, we are particularly interested in determining the specific histone modifications and the molecular recognition processes that enable modified histones to work together to establish and maintain higher-order chromatin structures. We also seek to clarify the picture of how dynamic rearrangements of chromatin structure are triggered by examining the structure and function of protein complexes that bind to and modify histones. Through these approaches we aim to understand the molecular mechanisms that underlie complex epigenetic phenomena in developmental processes.



Model for RNAi-mediated heterochromatin assembly in fission yeast



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 to take a professorship in the Department of Immunopathology, and returned to Kyoto in 1993, as professor in the Department of Molecular Genetics at the Kyoto University Faculty of Medicine. He was appointed CDB group director in 2000.

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Publications

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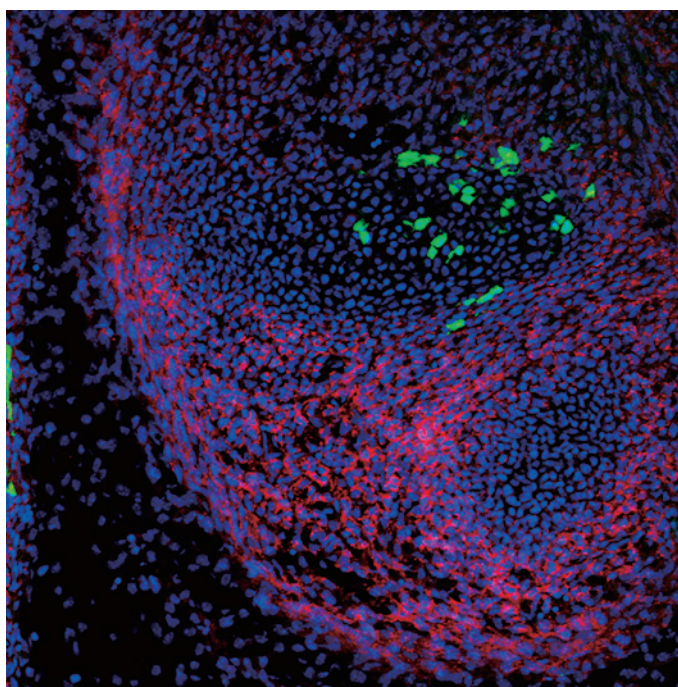
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The stem cell system can be thought of as a means by which organisms maintain cell renewal. All forms of life require the endless recycling of both materials and energy, making it impossible for either organisms or individual cells to live independent 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 milieu. The second is the requirement for preparing new cells in replacement. To investigate the first of these mechanisms, our group uses a system for labeling cells with special dyes, which allows us to monitor their location and behavior. We have also developed a system for differentiating embryonic stem (ES) cells in culture to study the second question of new cell production. Currently, we are focusing on developing different stem cell systems, particularly hematopoietic stem cells. This ongoing research project in our lab will allow us to explore how the self-renewing stem cell system is formed and will lead to the development of methods to generate *bona fide* hematopoietic stem cells from ES cells.



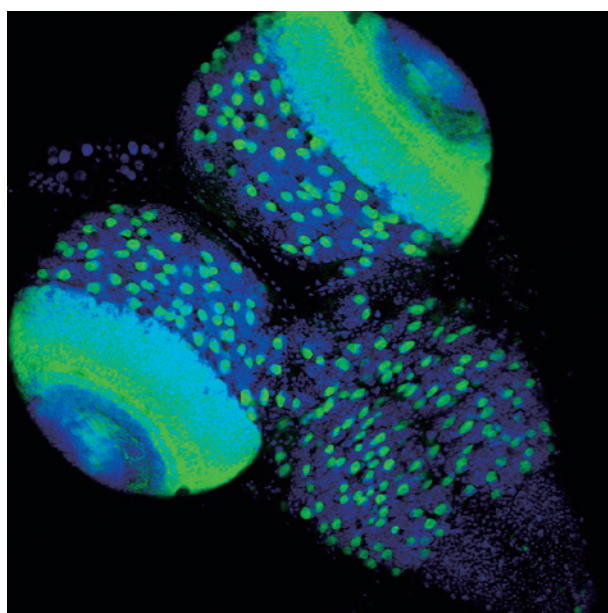
PDGFR α ⁺ mesenchymal stem cells derived from neuroepithelial cells in 14.5 embryo.

Growth Control Signaling

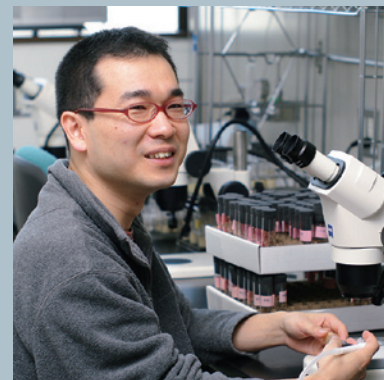
The processes of animal development, including organ size and body size, are genetically predetermined, but these processes are also influenced by environmental factors such as nutrition and temperature. The close link between cell and tissue growth control and environmental cues ensures that developmental 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 late larval brain showing proliferating cells (green)



Takashi NISHIMURA Ph.D.

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

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Publications

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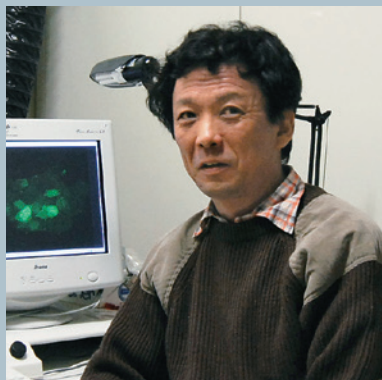
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Pluripotent Cell Studies



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

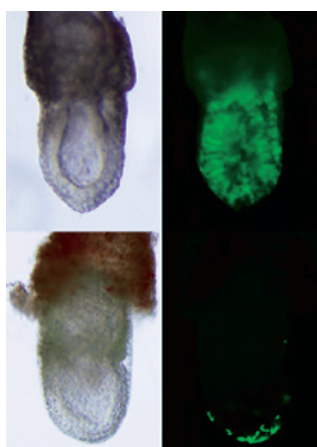


Figure 1

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).

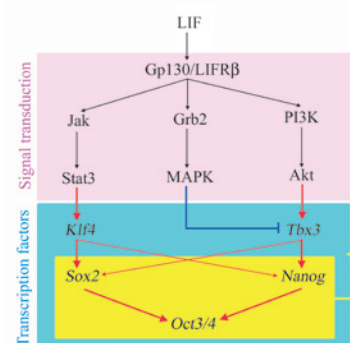


Figure 2

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



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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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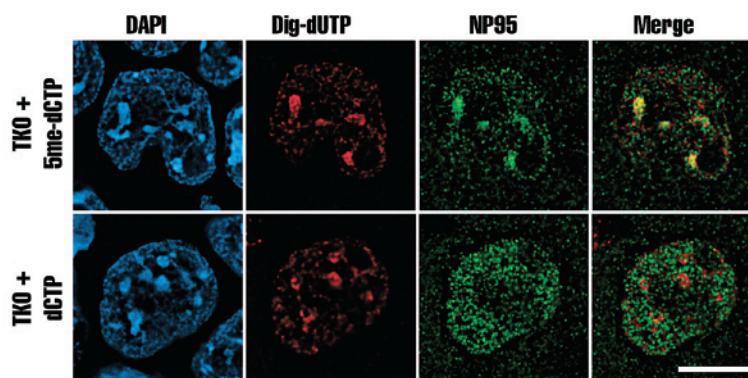
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Immunofluorescence showing Np95 localization to methylated DNA during DNA replication in ES cells.

Mammalian Molecular Embryology



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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.

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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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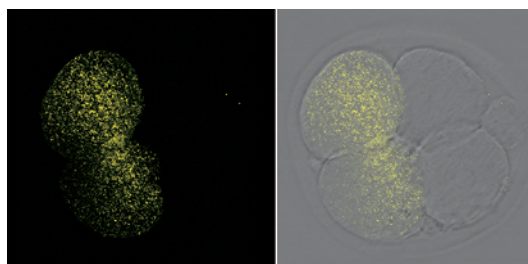
Amanai M, et al. Injection of mammalian metaphase II oocytes with short interfering RNAs to dissect meiotic and early mitotic events. *Biol Reprod* 75. 891-8 (2006)

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. For a fuller explanation, see Shoji et al., *EMBO J.* 25, 834-845 (2006).

Mammalian Germ Cell Biology

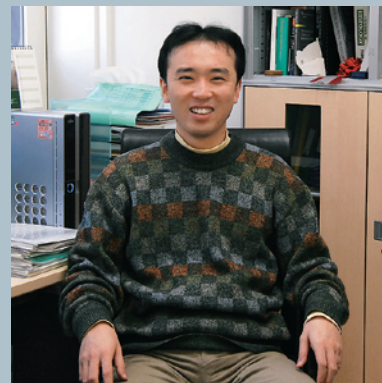
All of the diverse cell types in the body can be broadly classed as either somatic or germline cells. In contrast to somatic cells, which work to maintain the constant, stable form and function of an individual organism's body, germ cells provide the faithfully-replicated information needed to establish 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)



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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 assumed a professorship at the Kyoto University Graduate School of Medicine in 2009.

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Publications

Yamaji M, et al. Functional reconstruction of NANOS3 expression in the germ cell lineage by a novel transgenic reporter reveals distinct subcellular localizations of NANOS3. *Reproduction* 139: 381-93(2010)

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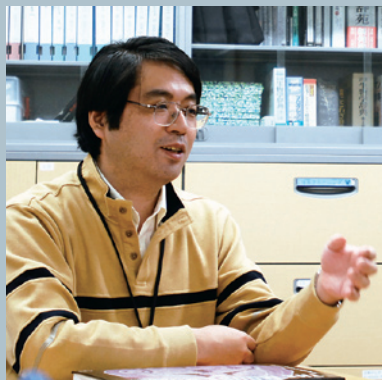
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Organogenesis and Neurogenesis



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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

Sasai N, et al. Ectodermal factor restricts mesoderm differentiation by inhibiting p53. *Cell* 133. 878-90 (2008)

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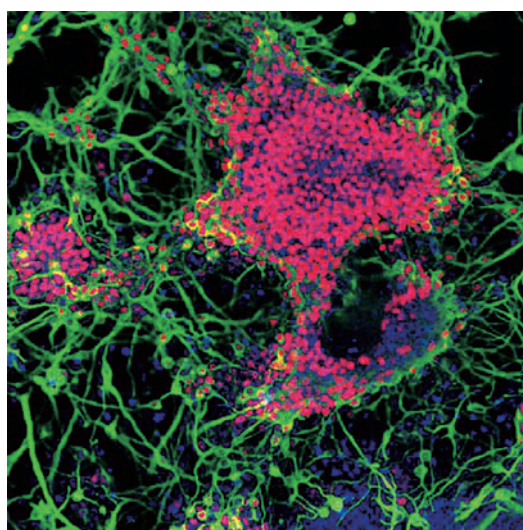
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The complexity of the fully formed brain defies description, yet this organ arises from a nondescript clump of cells in the embryo. The specification of the dorsal-ventral axis is significant in neural development in that the central nervous system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

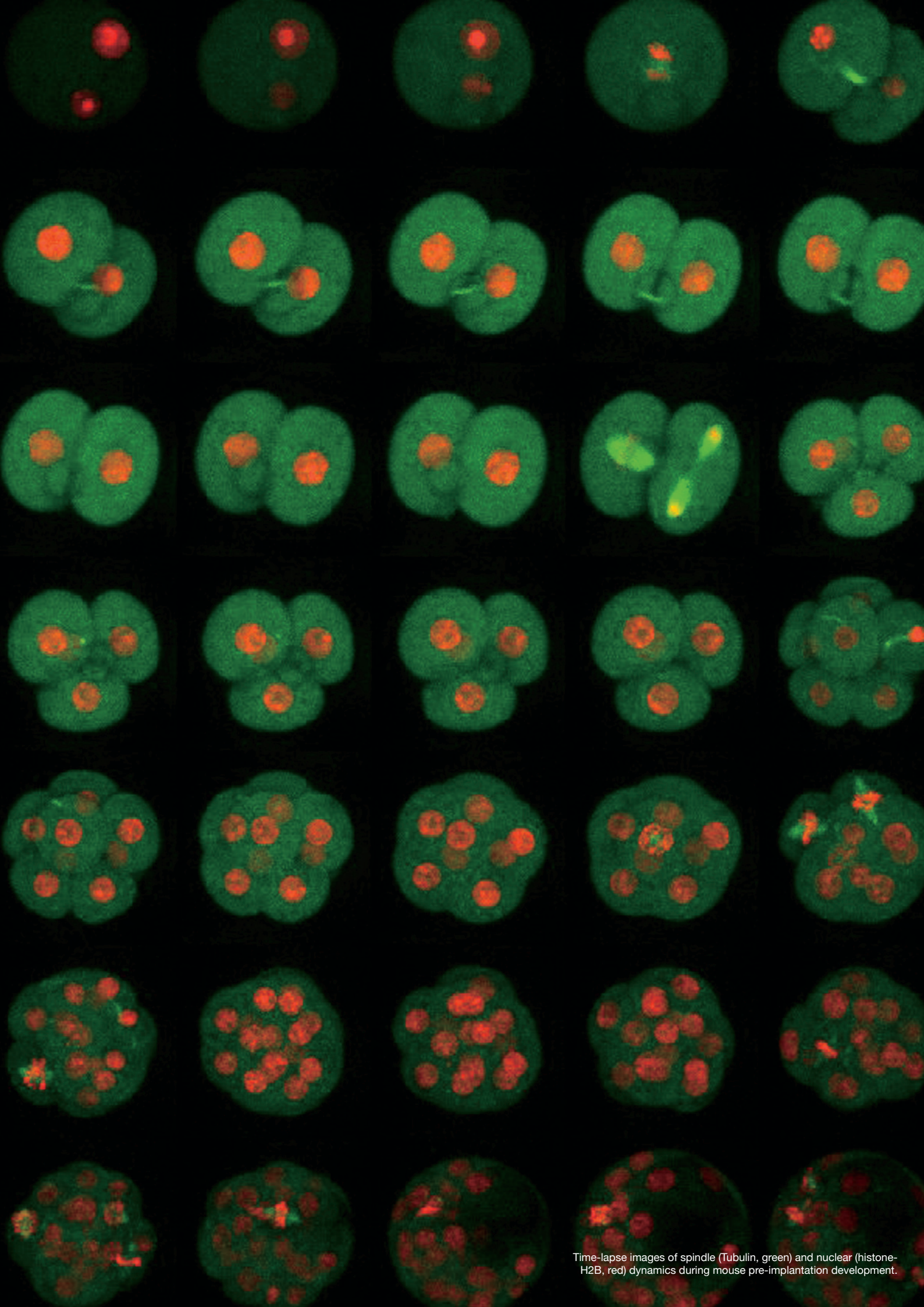
Using the African clawed frog, *Xenopus laevis*, as a model in molecular embryological studies, our group is engaged in clarifying the structure and extent of the signaling networks involved in setting up the dorsal-ventral axis and determining neural fate in the ectoderm. These studies focus on molecules 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 neurogenesis 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.



Telencephalic precursors cells induced by SFEB culture (Bf1 shown in red)



Time-lapse images of spindle (Tubulin, green) and nuclear (histone-H2B, red) dynamics during mouse pre-implantation development.

LIF follows two paths to pluripotency

Mouse embryonic stem (ES) cells, distinct from their human counterparts, rely on a cytokine named leukemia inhibitory factor (LIF) to keep them in their pluripotent, self-renewing state; deprived of LIF, they begin to differentiate. A number of transcription factors, namely Oct3/4, Sox2 and Nanog, form the core circuitry of pluripotency and serve as the molecular hallmarks of undifferentiated ES cells, but how LIF integrates with this triad has remained something of a mystery to ES cell biologists. A better understanding of this mechanism could yield important insights into how embryonic cells maintain their pluripotency in the face of internal dynamics and environmental change.

Findings from the Laboratory of Pluripotent Cell Studies (Hitoshi Niwa; Team Leader) show that LIF sits at the head of a pair of pathways that interact with separate elements in the pluripotency genetic core. These parallel circuits may help to stabilize the transcription factor network that sustains stem cells in culture. This work was published in the journal *Nature*.

It has been known for some time that a complex, interlocking network of transcriptional pathways is involved in ES cell maintenance, but not all of the pathway components have been identified. LIF is essential for keeping mouse ES cells in an undifferentiated state, but previous work has shown that this requirement can be bypassed



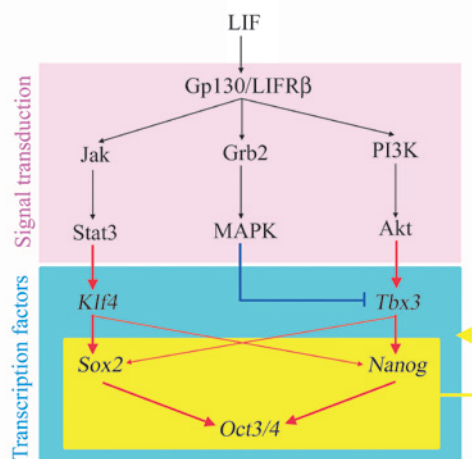
Hitoshi NIWA



by the overexpression of Nanog or the activation of Stat3, a critical factor in a signaling cascade known as the Jak-Stat3 pathway. Other studies involving the analysis of possible targets of the ES cell core circuitry revealed that the transcription factor-encoding genes *Klf4* and *Tbx3* are also able to compensate for the absence of LIF.

Niwa began by trying to piece together the relationships between these multiple factors in maintaining pluripotency. He first looked at the expression levels of pluripotency circuitry elements in mouse ES cells in which the absence of LIF was compensated for by transgenic *Nanog*, *Klf4*, or *Tbx3*. In all three lines, Oct3/4 remained stable, but interestingly in the *Nanog* and *Tbx3* lines, *Klf4* expression dropped to levels similar to that of wildtype ES cells cultured without LIF, which was coupled with reduced expression of Sox2. This jibes well with a previous suggestion that Sox2 and Nanog work independently to activate Oct3/4.

Staining of ES cells with antibodies to Tbx3, Klf4 and Nanog revealed that even as Oct3/4 levels remained homogeneous, the levels of these three factors vary from cell to cell in an apparently un-coordinated fashion. Somehow these fluctuating expression levels must work together to produce a single, uniform output. Returning to the LIF linkage, Niwa next tried reintroducing LIF to the transgenic ES cells, and found that while neither Tbx3 nor Nanog responded, Klf4 levels changed after LIF was added to the medium.



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

Previous studies of LIF have shown it to work via three separate signaling routes, known as the Jak-Stat3, PI(3)K-Akt, and MAP kinase pathways; in mouse ES cells, LIF is the only factor in control of the Jak-Stat3 pathway, while the other two are multiply regulated. Wanting to identify the roles of each pathway in ES cell maintenance, Niwa used specific inhibitors and activators of each of the downstream pathways before stimulating the cells with LIF. The team found that while Klf4 is downregulated by the Jak-Stat3 blockade, Tbx3 and Nanog were not. In contrast, when the PI(3)K-Akt was activated, Tbx3 and Nanog remained switched on, while Klf4 was unaffected. MAP kinase appeared to work as a negative control on Tbx3; when the MAPK pathway was inhibited, Tbx3 levels rose. The larger picture that arises from this work is one of parallel LIF-activated pathways, which engage in crosstalk to ensure that Oct3/4 remains unperturbed in ES cells.

"In this study we showed that the pluripotency-associated transcription factors have a hierarchical network structure defined by pathways that integrate external signals," says Niwa. "In this scheme, Klf4 and Tbx3 work as signal transmitters while Oct3/4 sits at the core, and Sox2 and Nanog are intermediates. This new model may help to improve our understanding of how pluripotency is robustly maintained under various environmental conditions."

Something fold, something new: Evolutionary morphology of the turtle shell



Shigeru KURATANI, Hiroshi NAGASHIMA, Fumiaki SUGAHARA, Masaki TAKECHI

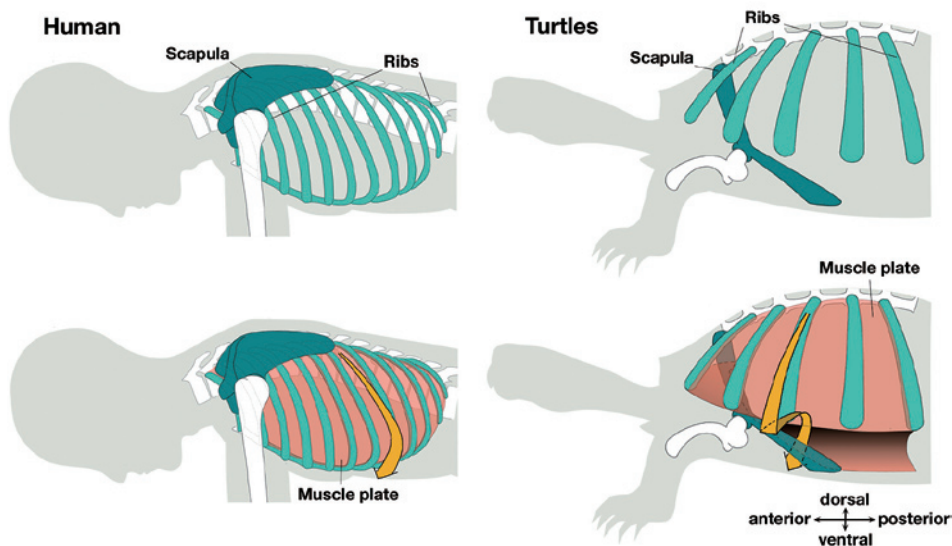
The elephant's trunk, the giraffe's neck, the bat's wing all provide examples of how evolution tinkers with pre-existing body parts, giving rise to adaptations that are simultaneously new and familiar. But there are also cases in which a novelty enters the body plan without evident precursors, defying explanation by such *bricolage*. The turtle's shell, or carapace, is one such invention; it appears to have burst into the fossil record with almost no prior inkling of its emergence.

Work published in *Science* by Hiroshi Nagashima and coworkers in the Laboratory for Evolutionary Morphology (Shigeru Kuratani; Group Director) showed that the musculoskeletal changes that led to abrupt appearance of the turtle shell involved a combination of altered tissue folding and new connectivity. And, by analyzing the structure of a proto-turtle fossil recently unearthed in China, the group revealed an intermediate body plan between that of ancient uncarapaced reptiles in the same lineage and the turtles, tortoises, and terrapins of today.

Nagashima began by making anatomic comparisons of mouse, chicken and turtle embryos at the stages in development at which structural changes that underlie the formation of the carapace first become apparent. Specifically, the group focused on how connections between muscles associated with the ribs and scapulae might differ in turtle and other vertebrate embryos, for although it is known that the shell itself forms through the fusion of ribs, the developmental steps that lead up to this novel structure have been unclear.



What Nagashima found was that, despite the radically different modified appearance of the adult turtle, the changes to the embryo are in fact quite modest. In contrast to the bird and mammal, the embryonic turtle's scapula forms anterior to the ribs, and the muscles that attach to these bones maintain their connectivities. The ribs themselves are shorter, confined to the axial region, meaning that they can only grow to the sides and anterior of the embryo, eventually covering the scapula. The cause of this "axial arrest" of the ribs remains uncertain, but it is this change in rib growth coupled with altered folding of tissue at the boundary between the axis and body wall that fundamentally distinguishes the turtle from other vertebrates.



Comparison of skeletal and muscular structure in human and turtle.

A second innovation the group identified was a number of new attachment points among what are known as "in-and-out muscles," which primarily connect limbs to the trunk. They found that muscles in the turtle's upper thoracic region, such as the pectorals and the latissimus dorsi, arose more anteriorly and established different connectivities to bones than those seen in chicken and mouse.

The insights into turtle musculoskeletal development gained from comparisons with extant animals mesh well with the perspective provided by fossils of extinct species from the lineage that led to the turtles. The skeleton of the most ancient known turtle, *Odontochelys*, which was first described in 2008, shows that axial arrest of the ribs had already begun 220 million years ago. The ribs, however, did not show the splayed arrangement seen in the ribs of modern turtles, nor did they fuse to form a full carapace.

"In this study, we used fossil evidence to develop comparisons in the skeletal architecture of modern turtles and their ancestors, but the question of muscle homologies remains unanswered, due to the lack of soft tissue," says Kuratani. "But we feel that a general knowledge of the vertebrate developmental plan will still allow us to draw reasonable conclusions about how at least some of the muscles might have connected to the skeleton in the most ancient turtles, via an evolutionary developmental biology approach that we feel may find broader application among paleontologists."

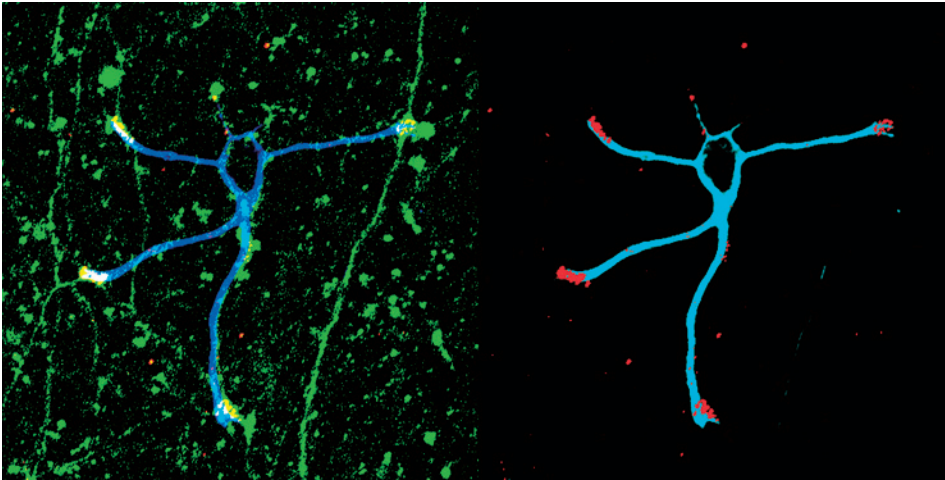
Synapses stay true: Selective neural connectivity in vitro

The brain is a bramble, with thorny branches of neuronal projections intertwining into a dense network. But a closer look reveals an order to its seemingly thicket-like structure, in which axons from one neuron connect with dendrites from specific partners. In cultured neurons, however, these relationships become blurred, and synapses have been shown to form between mismatched neurons, raising questions about the mechanisms that control neuronal recognition in vitro.

Shoko Ito of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) reexamined this notion of in vitro neuronal promiscuity and found that, in contrast to previous allegations, at least some dendrites remain true to their partners. In studies of synapse formation using cerebellar granule cell dendrites, Ito showed that while these may come into contact with axons of all types in cell culture, they synapse only with mossy fiber axons, just as they do in vivo. This work was published in the *Proceedings of the National Academy of Sciences*.



Shoko ITO

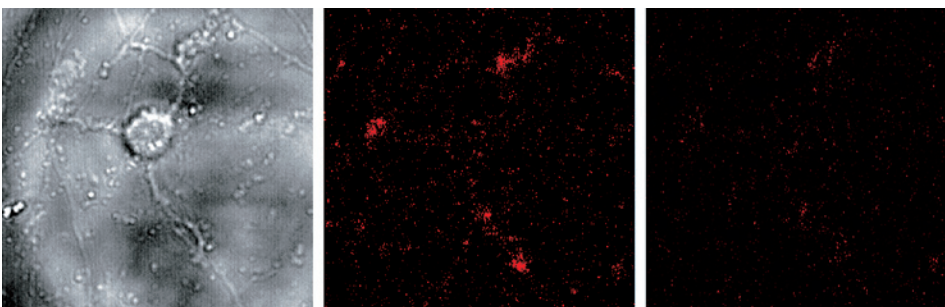
**Figure 1**

Synapse formation between granule cell dendrites (blue or light blue) and mossy fiber axons (green). Synapses are detected by staining for PSD95 (red), a postsynaptic marker.

The cerebellum features two characteristic neuronal cell types: the huge and hugely intricate Purkinje cells, which form synapses with climbing fibers from a medullary region called the inferior olivary nucleus, and granule cells which synapse with mossy fiber axons from a different region known as the pontine nuclei. Ito began with a neuronal co-culture system in which granule cells were grown in the presence of climbing fibers and hippocampal neurons, neither of which would they ordinarily form synapses within the brain. These explants projected axons as normal, and the granule cells extended dendrites, but although they in some cases the neurites came into close contact with each other, synapses of the type seen in vivo failed to form.

Only when granule cells were cultured with their typical binding partners, mossy fiber axons from the pontine, did they form synapses which are functionally normal and morphologically identical to those seen in vivo, as indicated by the expression of synaptic markers. Axons from the inferior olivary and hippocampal cultures also apparently formed synapses, but on closer examination these showed their arrangement was abnormal, and they showed impaired release of synaptic vesicles, which are responsible for the cycling of neurotransmitters.

This new work clearly shows that the selective synapse formation known to occur in vivo is replicable in cultured neurons as well. The mechanisms by which such selection takes place, however, remain unknown and must await further studies now that this fundamental principle has been shown to be conserved in vitro.

**Figure 2**

FM staining of co-cultured granule cells and mossy fibers shows recycling of synaptic vesicles. Differential interference microscopy on left; center and right panels show normal uptake and release of synaptic vesicles in these cultured cells.

2009 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 creativity, impact and quality in its scientific output. The individual achievements of many of its scientists, laboratory heads, junior scientists and student trainees alike, have been recognized for numerous awards from government agencies, private foundations, and industry.

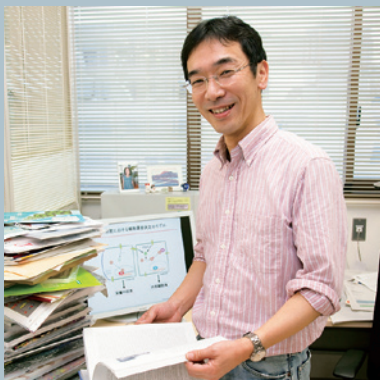
The Center takes great pride in the recognition garnered by its scientists. A list of all prizes awarded in 2009 to CDB scientists follows:

Date	Name	Position	Laboratory	Award	Organization
3/9/2009	Teruhiko Wakayama	Team Leader	Genomic Reprogramming	Japanese Academy Medal	The Japan Academy
3/9/2009	Teruhiko Wakayama	Team Leader	Genomic Reprogramming	5th annual JSPS Prize	Japan Society for the Promotion of Science
3/16/2009	Sayaka Wakayama	Research Scientist	Genomic Reprogramming	2nd Annual Shiseido Research Grant for Female Scientists	Shiseido Co.,Ltd.
4/2/2009	Hiroshi Nagashima	Research Scientist	Evolutionary Morphology	147th Meeting of the Japanese Society of Veterinary Science Outstanding Poster Award	The Japanese Society of Veterinary Science
4/14/2009	Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Minister of Education, Culture, Sports, Science, and Technology Prize for Science and Technology	Japanese Ministry of Education, Culture, Sports, Science, and Technology (MEXT)
4/14/2009	Mitinori Saitou	Team Leader	Mammalian Germ Cell Biology	Minister of Education, Culture, Sports, Science, and Technology Prize for Young Investigators	Japanese Ministry of Education, Culture, Sports, Science, and Technology (MEXT)
4/17/2009	Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Tokyo Techno Forum 21 Gold Medal Prize	Tokyo Techno Forum 21
5/26/2009	Teruhiko Wakayama	Team Leader	Genomic Reprogramming	NISTEP's Nice Step Researcher Prize	National Institute of Science and Technology Policy
10/27/2009	Yoichi Minami	Research Scientist	Systems Biology	16th Annual Meeting for the Japanese Society for Chronobiology Outstanding Poster Award	Japanese Society for Chronobiology
11/10/2009	Hiroko Ueda	Project Leader	Systems Biology	IBM Japan Science Prize (Computer science category)	Japan IBM
11/18/2009	Chong Li	Junior Research Associate	Genomic Reprogramming	Certificate of Award for Excellent Poster Presentation	The Asia Reproductive Biotechnology Society
12/5/2009	Motoki Tada	Research Scientist	Evolutionary Morphology	Leica Microscope Photo Contest 2009 Outstanding Photo Award	Leica Microsystems
12/9/2009	Shigeo Hayashi	Group Director	Morphogenetic Signaling	Leica Microscope Photo Contest 2009 Grand Prize	Leica Microsystems



Parvalbumin7 and Vglut1 are expressed in Purkinje cells (magenta) and parallel fibers of granule cells (green) in zebrafish larval cerebellum.

Embryonic Induction



Hiroshi SASAKI Ph.D.

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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 CDB.

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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)

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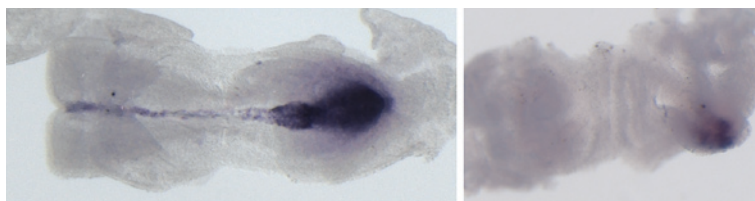
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During vertebrate embryogenesis, simply structured regions of undifferentiated cells organize and cooperate to give rise to the anterior-posterior and dorsal-ventral body axes, the neural tube and other embryonic structures deriving from the three 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.



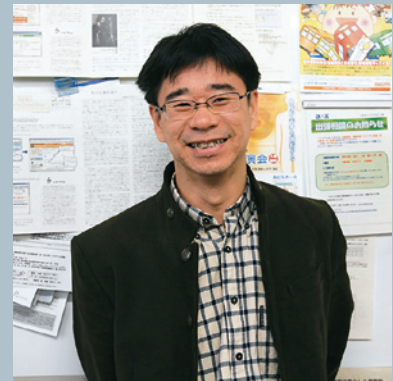
Day 8.5 mouse embryos showing that the notochord and node (signaling centers regulating trunk/tail development; stained purple on the left) is absent in the *Tead1;Tead2* double mutant (right; remaining staining is in the primitive streak). *Tead1;Tead2* double mutants also have defects in cell proliferation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



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.

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Publications

Zinovyeva A Y, et al. Complex network of Wnt signaling regulates neuronal migrations during *Caenorhabditis elegans* development. *Genetics* 179. 1357-71 (2008)

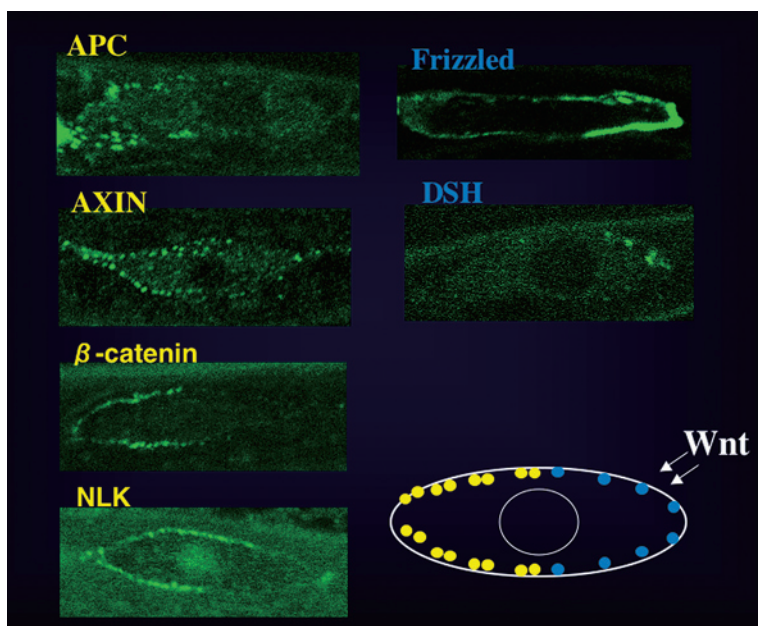
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Asymmetric cortical localization of the Wnt pathway components.

Early Embryogenesis



Guojun SHENG Ph.D.

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

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

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YuPing WU

Publications

Alev C, et al. Dynamic expression of Endoglin, a TGF-beta co-receptor, during pre-circulation vascular development in chick *Int J Dev Biol* 54. 737-42 (2010)

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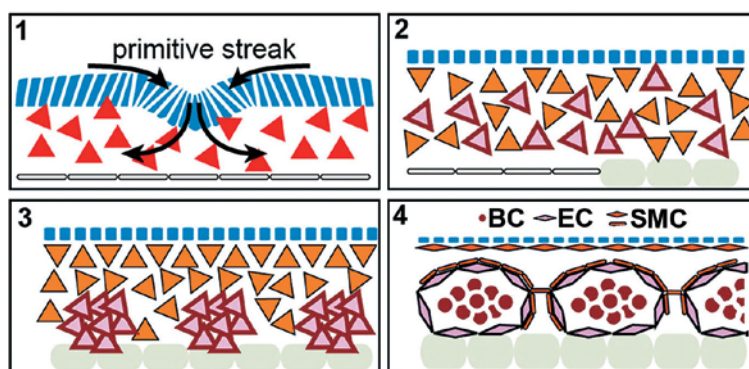
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The lab studies how mesoderm cells form and differentiate during early vertebrate development, using the chick as the main model organism. Our research currently focuses on two aspects: 1) the formation and early regionalization of mesoderm cells during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



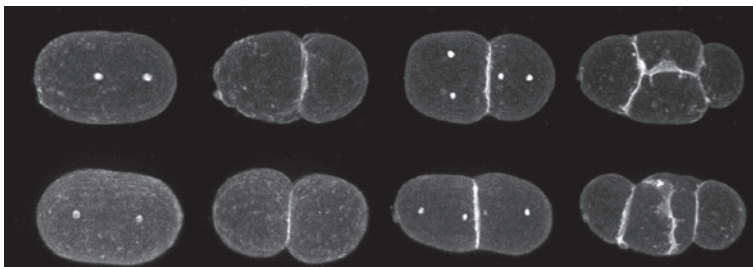
The circulatory system of early chicken embryos (E5 shown here) is complex and contains embryonic, allantoic, and yolk sac sub-systems. The diversity of blood cells during this period is revealed by the transcriptomic analysis of non-red blood cells in circulation.

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.



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.

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Publications

Konishi T, et al. The *Caenorhabditis elegans* DDX-23, a homolog of yeast splicing factor PRP28, is required for the sperm-oocyte switch and differentiation of various cell types. *Dev Dyn* 237. 2367-77 (2008)

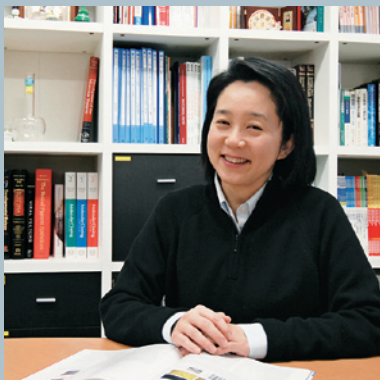
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Retinal Regeneration



Masayo TAKAHASHI M.D., Ph.D.

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Masayo Takahashi received her M.D. from Kyoto University in 1986, and her Ph.D. from the same institution in 1992. After serving as assistant professor in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1996, where she discovered the potential of stem cells as a tool for retinal therapy. She 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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Publications

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

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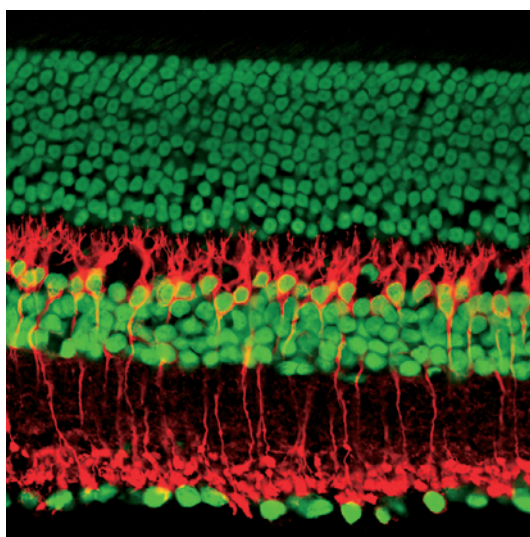
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The retina has been called the “most approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous 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 to differentiate into retinal neurons and pigmented 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.



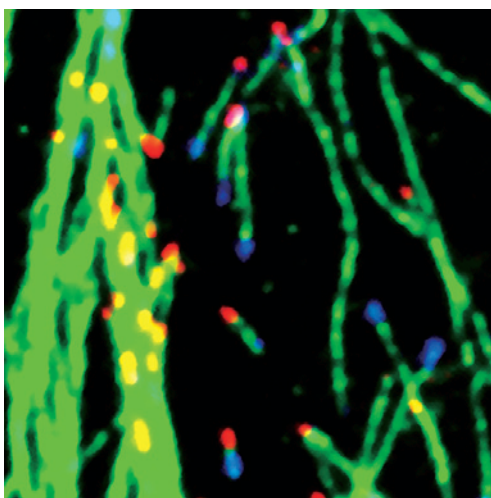
Immunostaining of bipolar cells in the adult mouse retina

Cell Adhesion and Tissue Patterning

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



Identification of a novel protein, termed Nezha, which binds non-centrosomal microtubules at the minus ends. Triple immunofluorescence staining for microtubules (green), Nezha (blue), and EB1 (red), a plus-end binding protein.



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)

Ishiuchi T, et al. Mammalian Fat and Dachsous cadherins regulate apical membrane organization in the embryonic cerebral cortex. *J Cell Biol* 185, 959-67 (2009)

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Systems Biology



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

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

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Publications

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

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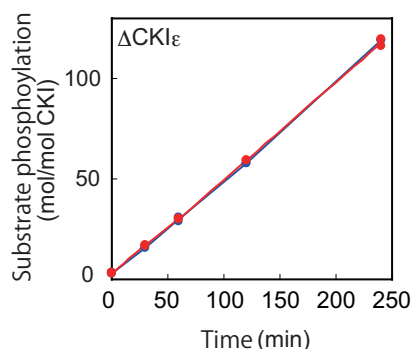
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Recent large-scale efforts in genome-sequencing, expression profiling and functional screening have produced an embarrassment of riches for life science researchers and biological data can now be accessed in quantities that are orders of magnitude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

The Laboratory for Systems Biology (LSB) has specific aims to investigate development of systems-biological approaches and their application to system-level questions of complex and dynamic biological systems, such as mammalian circadian clocks.

In pursuing the research aims outlined above, we have been mainly focusing on the development of a systems-biological approach. We have successfully developed new strategies and technologies for genome-wide profiling, bioinformatics, quantitative measurement, perturbation of cellular state, and implementation of artificial circuits in cells. We have also applied these systems-biological approaches to specific system-level questions, which has led to a number of new discoveries and inventions. Over the next five years, we plan on fully integrating these approaches in an attempt to realize a system-level understanding of the mammalian circadian clock. In order to facilitate these processes, we will also commit to the development of key technologies such as functional genomics used for complete identification of the mammalian circadian clock and Micro Electro Mechanical Systems (MEMS, also known as microfluidics) for quantitative perturbation of the mammalian circadian clock. These key technologies also have the potential to be applied to the study of developmental problems.

In October 2009, the Laboratory for Systems Biology was re-designated as a Project Lab in the Center Director's Strategic Program for Systems Biology research.



Temperature-insensitive activity of CKIε

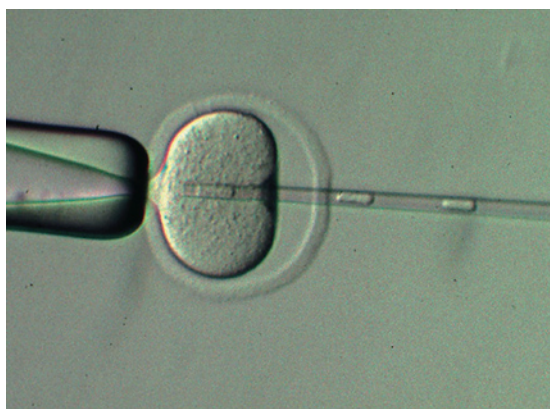
Temperature dependency of the CKIε phosphorylation activity for the PER2-peptide substrate was measured at 25°C (blue) and 35°C (red). CKIε phosphorylated the peptide substrate at similar rates whether at 25°C or 35°C, indicating a strong temperature-insensitivity

Genomic Reprogramming

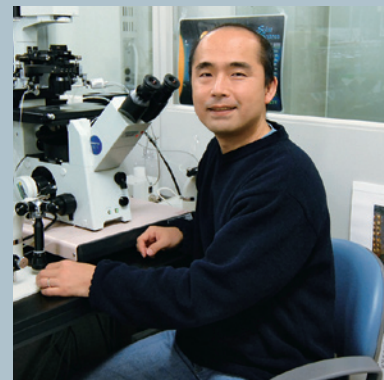
A limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual cells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



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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Publications

Li C, et al. An efficient method for generating transgenic mice using NaOH-treated spermatozoa. *Biol Reprod* 82, 331-40 (2010)

Wakayama S, et al. Detrimental effects of microgravity on mouse preimplantation development in vitro. *PLoS One* 4, e6753 (2009)

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Tabar V, et al. Therapeutic cloning in individual parkinsonian mice. *Nat Med* 14, 379-81 (2008)

2009 Meetings

Scientific gatherings are at the core of international exchanges among researchers, and the RIKEN CDB has devoted a tremendous amount of effort into ensuring that every aspect of the meetings it hosts or sponsors meet the highest standards of quality in their programs, venues, and logistics. In addition to its annual symposium and many CDB Seminars, the CDB hosts or supports numerous meetings every year.



Stem Cell Research in the Asia-Pacific Region

February 2 – 4 at Kobe Chamber of Commerce and RIKEN CDB

In this three-day event, co-organized by the JSPS and the Stem Cell Network: Asia-Pacific, around 40 scientists and experts from other fields gathered to discuss the ethical, legal, commercial, and social aspects of stem cell research and applications in the Asia-Pacific region, and to present their latest findings in the study of a range of stem cells including ES, iPS, mesenchymal, and hematopoietic.

Common Themes and New Concepts in Sensory Formation

April 13 – 15 at RIKEN CDB

Hosted by the Laboratory for Sensory Development and colleagues from the RIKEN BSI and the U.K., this meeting drew leading scientists from around the world to address common themes in sensory development and novel approaches to this research, with particular focus on the specification of sensory cells, mechanisms of sensory cell organization, and the use of non-model systems.



Building the Body Plan: How Cell Adhesion, Signaling and Cytoskeletal Regulation Shape Morphogenesis

September 21 – 23 at Kyoto International Conference Center

The CDB joined with the American and Japanese Societies for Cell Biology to sponsor this meeting, designed to allow grad students and postdocs to interact with leaders in the field from around the world in an intimate setting. Three days of talks focused on topics such as cell junction formation and dynamics, signaling via cell contacts, epithelial remodeling, cell migration for morphogenesis, and neural morphogenesis and axon recognition.

Frontiers in Stem Cell Biology and Embryonic Manipulation

November 9 at RIKEN CDB

In this one-day meeting, leaders in the fields of stem cells and contemporary embryology gathered to discuss their recent work and to participate in a lively discussion of outstanding problems and future prospects in their fields.





Section view of paratympanic organ, stained to show nuclei and cytoplasm; the organ appears as an oval structure, lying next to the future middle ear.

Fat and Daschous cadherins work at the apical membrane in cerebral cortical cells



Takashi ISHUCHI

A study by Takashi Ishiuchi, Takuji Tanoue and others in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) shed new light on an unexpected role for a pair of cadherin molecules in the embryonic brain. The group revealed that, in mammals, the little-studied Fat and Daschous cadherins help to organize the apical membrane structure in neural progenitor cells in the cerebral cortex.

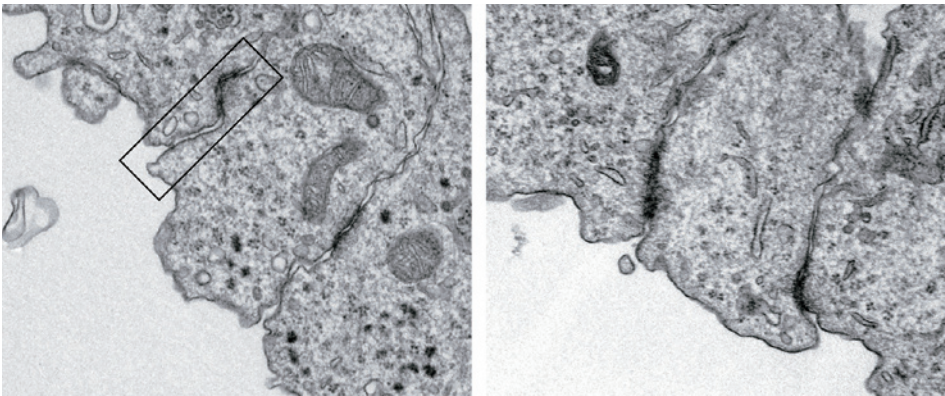
The cadherins are a superfamily of transmembrane molecules best known for the roles of the classic cadherins in cell-cell junctions, where they help cells recognize other cells of similar type and stick together by a like-binds-like mechanism. But there are other cadherin types as well, some of which are of indeterminate function and much greater size than their classic counterparts. The Fat cadherins, for example, have nearly 7 times the number of extracellular "cadherin repeat" segments than does the typical classic cadherin, E-cadherin; the atypical cadherin, Daschous, is similarly oversized. A number of gross phenotypes have recently been reported in mice lacking Fat4 cadherin (one of the four mammalian Fat cadherins), but the molecular and cellular level mechanisms behind these have yet to be worked out.

Ishiuchi began by making antibodies against Fat4 and Daschous1 and using these to map distribution of these proteins by immunostaining, which revealed high concentrations in the cerebral cortex. The two molecules were co-localized in the vicinity of the adherens junction, but unlike the junction proteins beta-catenin and ZO-1, their expression was discontinuous and apically shifted, features that had previously been reported for the distribution of these proteins in *Drosophila*.

Given the known properties of Fat and Daschous in flies, the group next considered they might bind heterophilically (binding between unlike pairs), in contrast to the homophilic binding characteristic of classic cadherins. They transfected Fat4 and Daschous1 into fibroblasts lacking classic cadherins, and found that the molecules became concentrated only at the boundaries between the Fat and Daschous transfectants, where threadlike filopodia made contact with neighboring cells, suggesting a heterophilic mechanism. This balance between Fat and Daschous was dynamically regulated as well – when Fat4 was depleted, Daschous1 levels dropped. When Daschous1 was removed, however, Fat4 was up-regulated.



Looking to the inner side of the junction, Ishiuchi sought after cytoplasmic binding partners of Fat4 and Dachsous1, and using immunoprecipitation found that it associated with members of the Pals protein complex. Fat4 co-localized with Pals1 and a second member of the complex, MUPP1, suggesting a functional interaction apical of the cell-cell junction. Interestingly, Pals1 and MUPP1 localized remained even in the absence of Fat4 and Dachsous1, indicating that their recruitment to the apical region relies on a distinct mechanism.



The apical gap between cells is lost in Fat4 loss-of-function cells (right). Wildtype cell-cell junction (with gap indicated in the boxed area) shown to left.

To investigate the cellular roles of the complex comprising Fat4, Dachsous1, Pals1 and MUPP1, Ishiuchi and co-workers used RNAi to block the functions of each molecule and observed the effects. A minute gap apical to the adherens junction is normally seen between plasma membranes of adjacent cells in the intercellular regional, but when the function of either Fat or Pals was interfered with, this gap disappeared. As this form of intercellular gaps appear to be prominent only in certain cell types, including neural progenitor cells, Fat and Dachsous may exert cell type-specific effects on the organization of the apical membrane.

The current work, published in *The Journal of Cell Biology*, showed both the conserved nature of the molecular properties of the Fat and Dachsous cadherins and a new function for these proteins and their binding partners in the mammalian cortex. "We still don't know how Fat4 and Dachsous1 regulate junction structure in the apical region," says Ishiuchi, "so we are going to need to look at possible functional relationships with other factors that are known to work in that part of the cell, such as Crumbs."

Embryos feel gravity's pull



Sayaka (and Ryuta) WAKAYAMA

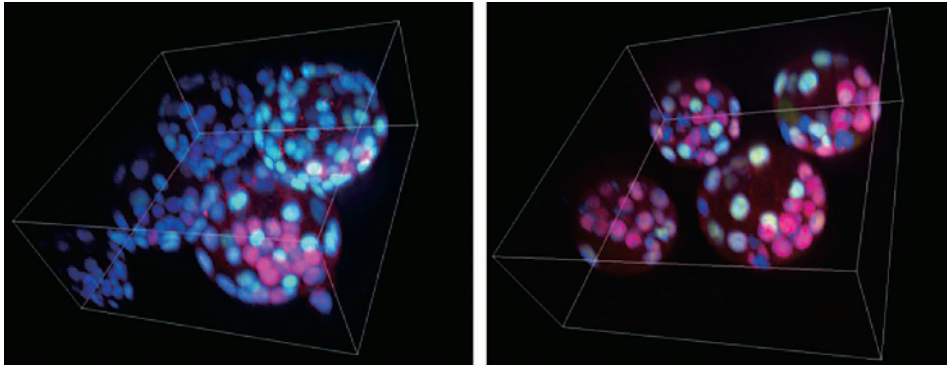
Were lengthy space flights to become a reality, the question of whether humans and other animals could reproduce normally when living in low gravity would need to be addressed. Previous studies have focused on species of fish, amphibians and birds, but little work has been done to date on mammalian reproduction in microgravity due to the limitations imposed by technology and the stress responses in rats and mice.

Sayaka Wakayama and colleagues in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama; Team Leader) studied reproduction and early development in mice in simulated microgravity conditions, and made some unexpected findings. In an article published in *PLoS One*, they showed that while fertilization is possible in low gravity, subsequent development is perturbed.

To achieve the most realistic simulation of microgravity, the team used a newly developed device known as a three-dimensional clinostat, which uses rotation on two axes to evenly distribute gravitational pull in all directions over time (rather than the unidirectional downward pull we normally experience). Previous studies on mammalian development had been performed using a one-dimensional clinostat, but the results were equivocal.

Wakayama first tested whether in vitro fertilization would be possible in microgravity, and found that the success rate for fertilization was no different from that at 1G, which suggests that mouse gametes are unaffected by low gravity.

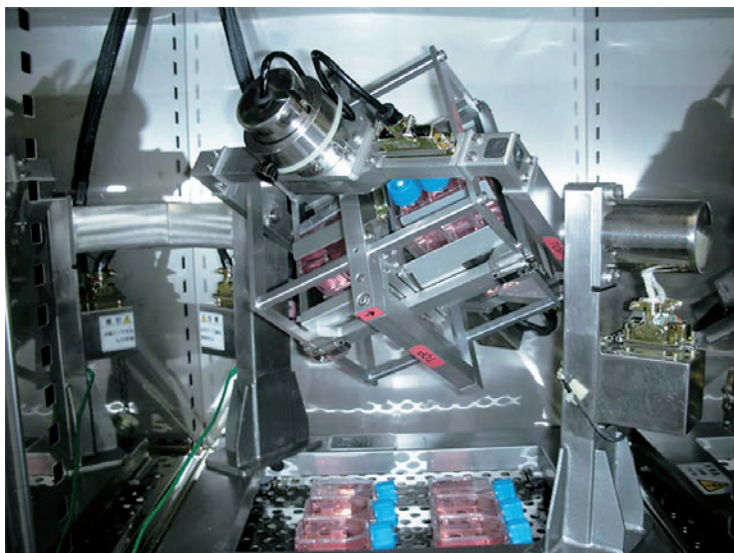
As a next step, they allowed zygotes created by IVF continue to develop in the clinostat before implanting them into recipient females. But while the embryos could develop to the 2-cell and even the blastocyst stage, they did much less well when transferred into a surrogate mother – the rate of generation of offspring from the microgravity embryos was little more than half that when using embryos derived in normal gravity. Fertility of the mice born from microgravity embryos, however, was normal, as shown by their ability to mate and have offspring of their own.

**Figure 1**

Preimplantation embryos developed in simulated microgravity (right) and earth-surface gravity (left) conditions.

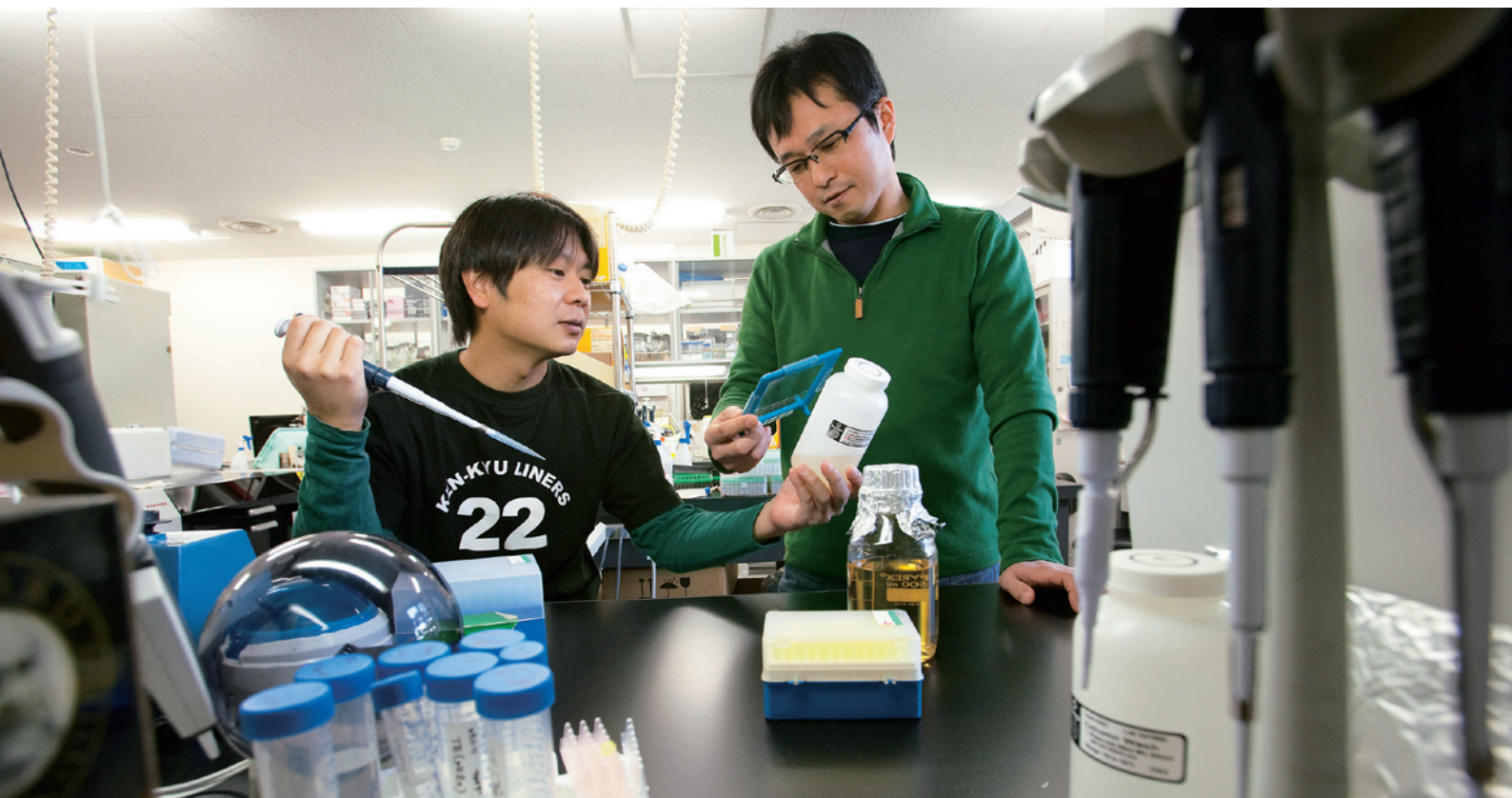
To determine the reason behind the developmental problems, Wakayama took a closer look at the microgravity embryos' cell differentiation and polarization. In the early embryo, there is normally a clear demarcation between regions expressing *Cdx2* (which contribute to the extraembryonic trophoblast) and those expressing *Oct4* (which give rise to the embryo proper). But in the microgravity embryos, an unusual number of cells expressed both. Surprisingly, the blastocysts remained polarized, with the inner cell mass located in the expected position.

These results have important implications for the prospects of reproduction in space, but remain preliminary in their scope. "What we have shown using this new technology will still need to be replicated in actual microgravity conditions," cautions Wakayama. "So we are looking forward to being able to perform those experiments once the Japanese experiment module *Kibō* in the International Space Station is complete."

**Figure 2**

3-D clinostat used in experiments

New basis for the temperature-insensitive period regulation of the biological clock



Hideki UKAI, Masato NAKAJIMA

Although the circadian clock is exquisitely tuned to changes in light intensity and many chemical compounds, it is robust in the face of temperature variations. Although this temperature-insensitivity is seen in a wide range of taxa from bacteria to mammals, its fundamental basis has eluded description. One possibility is that the clock mechanism includes at least one biochemical component that is unaffected by temperature fluctuations, but at first blush this seems counterintuitive, given the highly temperature-sensitive nature of most such reactions.

A collaboration of researchers including Yasushi Isojima from the Advanced Computational Sciences Department, RIKEN, Masato Nakajima, Hideki Ukai and others from the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader), and Joseph S. Takahashi's group from Northwestern University, which was formed to elucidate underlying mechanisms of the circadian clocks by the chemical-biological approach, has now identified an essential regulatory component that shows the selective environmental sensitivity that would be required to explain the flexible-yet-robust workings of the biological clock.

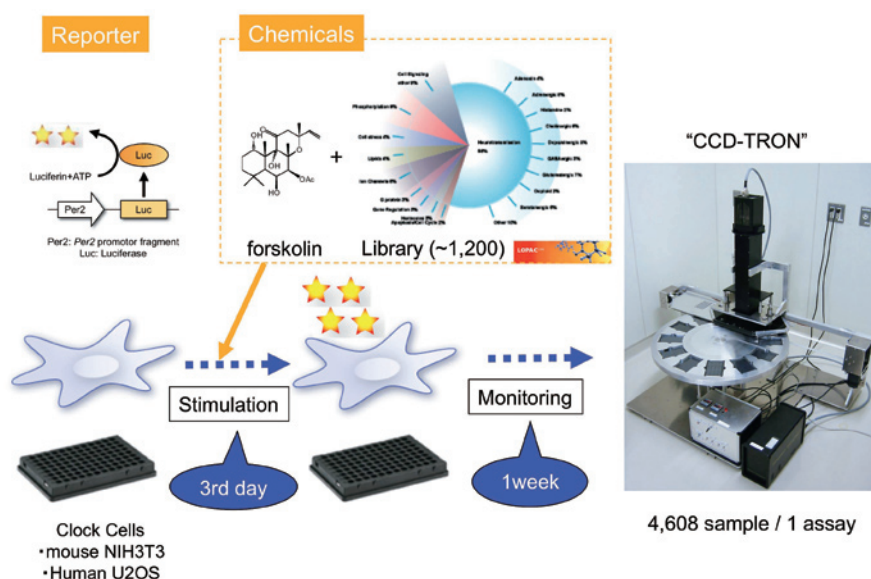
The study began with a screen of more than 1,200 pharmacological compounds to test their effect on clock periodicity, which yielded 10 that had the strongest ability to lengthen clock period in mouse and human cells. The majority of these had never been identified as affecting the length of the circadian period, but two were known to inhibit a family of protein kinases known as CKI (casein kinase I). Taking this lead, the team knocked down various proposed target genes of the 10 compounds to check for possible roles in clock regulation, and found that two – CKI ϵ/δ and CKI δ – had the greatest period-lengthening effects. Going back to their compounds of interest, they found that 9 of the 10 inhibited the catalytic domain of CKI.



CKI ϵ is known to regulate the degradation of an important clock factor known as PER2 (which CKI δ is thought to regulate as well), so Isojima, Nakajima and Ukai next checked whether the two test compounds would inhibit this as well. They found that compounds that putatively inhibit CKI had a significant positive effect on PER2 stability and slowed its degradation, suggesting that these compounds indeed operate via their effects on CKI ϵ/δ .

Using the same two compounds, they next tested the extent to which they could lengthen the normal circadian period, and found that both increased the duration of a single cycle by more than double. Using cells expressing a construct of the circadian gene *Per2* fused with the gene encoding luciferase to assist in the visualization of its expression, they found that its cyclical expression reflected the changes of PER2 stability seen on administration of the potent compounds and, importantly, that the rate of degradation of the PER2 protein was temperature-insensitive.

To confirm whether the underlying CKI ϵ/δ kinase activity is also robust in the face of temperature changes, the collaborators created a peptide substrate derived from the CKI ϵ/δ binding domain thought to be phosphorylated by CKIs, and tested it against the catalytic domain of CKI ϵ at temperatures between 25° and 35° C. Kinase activity remained stable against changes across this range. Interestingly, this temperature-insensitivity was reduced in an autophosphorylating version of CKI ϵ , and when they tested the activity of the kinase with commercially available and general substrates.



Model of the screening system used to test chemical compounds with effects on the circadian clock

As a final confirmatory step, they used the Per2-luciferase construct to compare degradation rates in clock cells to those of luciferase alone (which shows temperature-sensitive degradation). As expected, the Per2 constructs significantly outlasted luciferase alone against a range of temperatures, and this effect was intensified by enhancing the reaction by Per2 and CKI by forced expression of the CKI ϵ catalytic domain.

These results clearly point to casein kinase I ϵ/δ factors as the enzymatic drivers behind temperature compensation in the circadian clock. This new knowledge of the basis for period-determination and temperature-insensitivity may additionally prove to be of value in the development of circadian regulatory drugs.



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

Go SHIOI

Hirofuka TAO

Visiting Scientist

Fardin FATHI

Toshihiko FUJIMORI

Technical Staff

Takaya ABE

Naoko HATAMOTO

Michiko

HIGASHIKAWA

Mari KANEKO

Hiroshi KIYONARI

Makiko OGAWA

Naoko OHSHIMA

Yoshihiro TAKAMIYA

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. We also develop transgenic mice for the CDB and Kansai biological research communities. 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

Masahiro OHNO

Assistant

Mayumi

MIZUSHIRO

Yuki TSUJI

Animal Resource Unit

Kazuki NAKAO

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

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Ima T, Yamazaki T, Kobayakawa R, Abe T, Suzuki M and Sakano H. Pre-target axon sorting establishes the neural map topography. *Science* 325 585-590 (2009)

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.

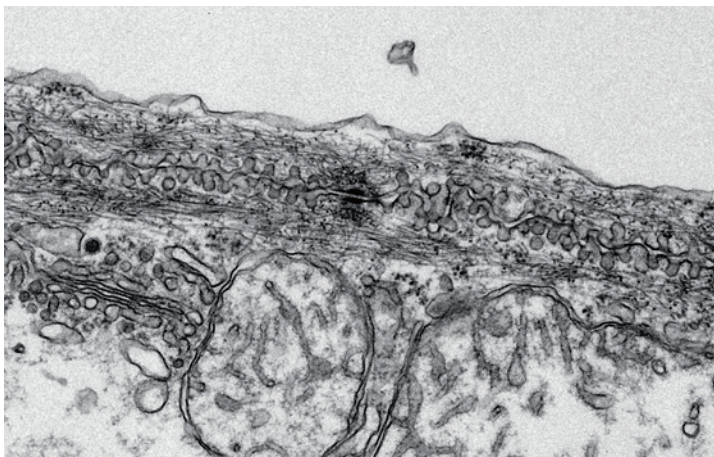


Figure 1
Cells organizing the notochord of zebrafish embryo.

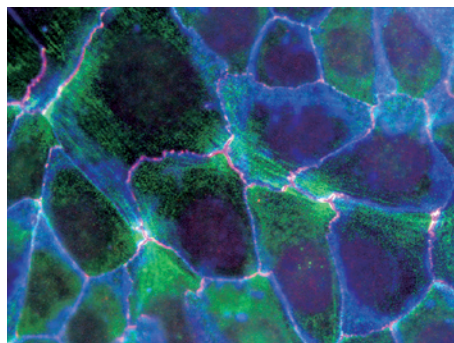


Figure 2
 α -catenin molecules stretched by forces produced by Myosin II (green) are selectively labeled with α 18 antibody (red).



Staff

Team Leader Shigenobu YONEMURA	Sachiko ONISHI Makiko F. UWU
Special Postdoctoral Researcher Masatsune TSUJIOKA	Student Trainee Tomoki FUJITA
Technical Staff Kazuyo MISAki	Research Fellow Ayuko SAKANE
	Assistant Mai SHIBATA

Publications

Takata N, Itoh B, Misaki K, Hirose T, Yonemura S and Okada M. Non-receptor tyrosine kinase CSK-1 controls pharyngeal muscle organization in *Caenorhabditis elegans*. *Genes Cells* 14: 381-393 (2009)

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Shigeo HAYASHI Ph.D.

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.



Staff

Unit Leader

Yuko MIMORI-KIYOSUE

Technical Staff

Tomoko HAMAJI

Agency Staff

Tatsunori HAGIHARA

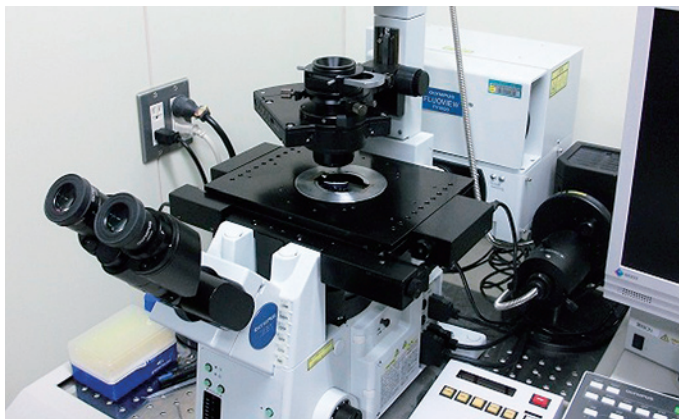
Kayoko NAGAHAMA

Optical Image Analysis Unit

Yuko MIMORI-KIYOSUE Ph.D.

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	Visiting Scientist Osamu NISHIMURA
Technical Staff Tetsutaro HAYASHI Kazu ITOMI Yu LIN Asuka MOMIYAMA Kaori TATSUMI	Part-Time Staff Minako MOTOISHI Assistant Chiharu TANEGASHIMA

Publications

Maki N, Suetsugu-Maki R, Tarui H, Agata K, Del Rio-Tsonis K and Tsonis PA. Expression of stem cell pluripotency factors during regeneration in newts. *Dev Dyn* 238(6), 1613-6 (2009)

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Functional Genomics Unit

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

The Functional Genomics Unit (FGU) provides functional genomics services to CDB labs and introduces and develops cutting-edge technologies to accelerate research. The FGU has been working to develop GeneChip and cell-based screening technologies. For the past 3 years, FGU has provided GeneChip service and informatics for more than 1,250 experiments in a range of research projects conducted using diverse model organisms and protocols. The FGU has constructed protocols including several quality checks of samples to ensure reliable GeneChip service, launched an intranet website, held technical seminars and project meetings for first-time users, and held a service meeting every week for sharing important information and discussing problems our GeneChip service. We have also established several informatics methods to support this service in the future.

The FGU has additionally developed cell-based screening system using a full-length cDNA library, and conducted several genome-wide assays in a pilot study to identify regulators for transcription processes.



Staff

Unit Leader Hiroki R. UEDA	Technical Staff Junko NISHIO Kenichiro UNO
Research Scientist Takeya KASUKAWA Itoshi NIKAIDO	Agency Staff Chikako IMAI
Visiting Scientist Hiroki DANNO	Assistant Ikuko TADA

Publications

Nakazawa F et al.: PBRL, a putative peripheral benzodiazepine receptor, in primitive erythropoiesis, *Gene Expr Patterns*. 9, 114-21 (2009)

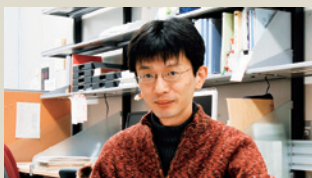
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Shigeo HAYASHI Ph.D.

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.



Staff

Lab Head

Shigeo HAYASHI

Unit Leader

Akira NAKAMURA

Technical Staff

Kaori SHINMYOZU

Publications

Kakihara K, Shinmyozu K, Kato K, Wada H and Hayashi S. Conversion of plasma membrane topology during epithelial tube connection requires Arf-like 3 small GTPase in *Drosophila*. *Mech Dev* 125, 325-336 (2008)

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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.



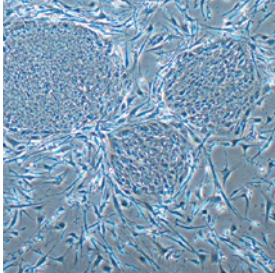
LC-MASS spectrometry system used in the Mass Spectrometry Analysis Unit.

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.

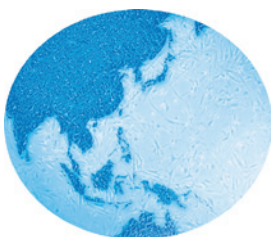


Human embryonic stem cells cultured on a fibroblast feeder layer.

Human Stem Cell Technology Unit

Yoshiki SASAI M.D., Ph.D.

Despite their great promise and fundamental interest, stem cells – both embryonic and somatic – can be challenging to study and manipulate in vitro. The 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 technological expertise, training and support in hESC culture, maintenance, distribution and management, meeting the needs of both experienced and first-time users in the life sciences research community. Working with labs in academia and industry, 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. In parallel to hESCs, technical development for the use of human iPS cells is being planned.



Studying global phenomena in stem cell research.

Science Policy and Ethics Studies Unit

Douglas SIPP

The field of stem cell research has received a great deal of attention due to the combination of fundamental scientific interest, therapeutic promise, and commercial potential it entails. But it has also been surrounded by 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 will 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 social and ethical perspectives on the translation of human stem cell research, with an emphasis on the Asia-Pacific region.



Staff

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Michiru MATSUMURA-IMOTO

Company-Sponsored Research Trainee
Maki MINAGUCHI

Company-Sponsored Research Trainee
Tokushige NAKANO

Publications

Nagase, T., Ueno, M., Matsumura, M., Muguruma, K., Ohgushi, M., Kondo, N., Kanematsu, D., Kanemura, Y., Sasai, Y. 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)

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Staff

Unit Leader
Douglas SIPP

Publications

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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.

RIKEN Kobe Institute

Budget and Staff

CDB Symposium

CDB Seminars

About RIKEN

RIKEN Campuses



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

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

RIKEN Kobe Institute

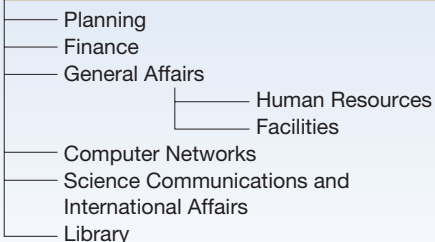
The RIKEN Kobe Institute was established in April 2000 as an organizational framework for the newly launched Center for Developmental Biology (CDB), which conducts a wide range of research, from fundamental studies of development and stem cells, to cutting-edge work with the potential to make a contribution 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



Kobe Safety Center



Center for Molecular Imaging Science

In 2005, the Molecular Imaging Research Program was selected by Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT) as an important part of its efforts to promote research and development in life sciences that respond to societal needs. Since that time RIKEN strove to establish a core center for drug development molecular imaging. In the fall of 2006, the Kobe MI R&D Center Building was completed with the support of the Kobe municipal government, and served as home to the RIKEN facility for the Molecular Imaging Research Program (MIRP). In October 2008, MIRP was reorganized and inaugurated as the Center for Molecular Imaging Science (CMIS) to further advance this field of research, and in April 2009 the Center expanded to 5 teams and 2 units. The CMIS has developed many novel probes for molecular imaging, contributing to drug discovery and the development of diagnostic and therapeutic indicators.

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, science communications and international affairs, and the CDB library.

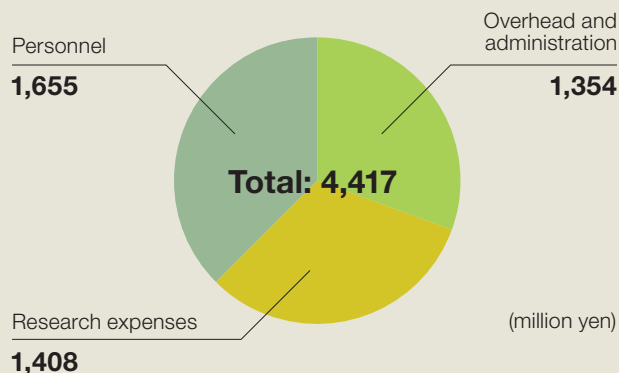
Safety Center

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.

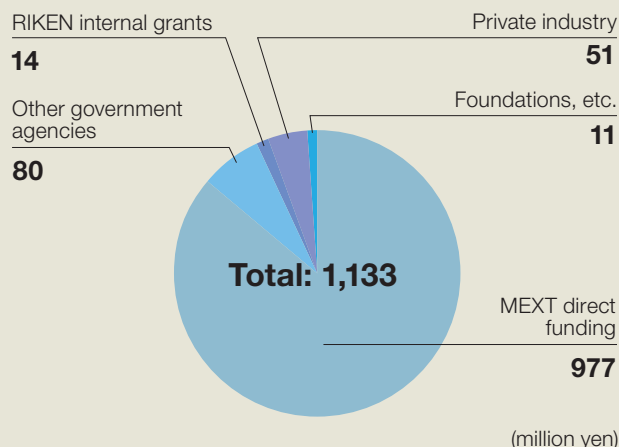


Center for Molecular Imaging Science

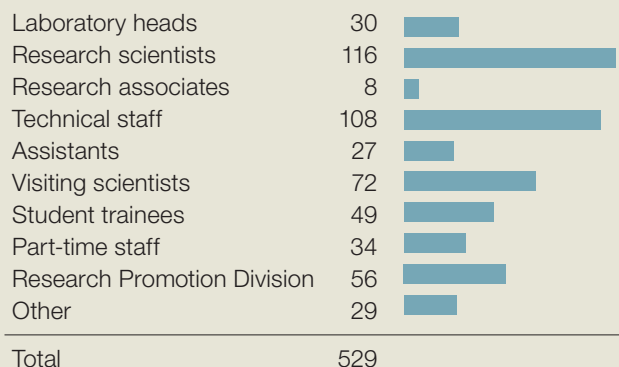
2009 CDB Budget



In addition to these intramural funds, 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), other government agencies, private foundations, and industry. These external funds form an important component of the CDB's overall funding mix, representing more than 1 billion yen in external funding for CDB research programs in the fiscal year ending in March 2009.



2009 CDB Staff



2009 CDB Symposium

Shape and Polarity

March 23–25, 2009

The CDB held its seventh annual symposium on the theme, “Shape and Polarity” on March 23 – 25. The three-day program featured a wide range of talks on aspects of how cells become polarized and the roles of cell polarity, and how cell populations organize into complex tissues and organs through rearrangement of polarized cells. In addition to more than 20 invited talks, each day included a poster session, at which more than 80 presenters introduced and discussed their work. The annual symposium series was launched in 2002 to establish a forum for addressing diverse aspects of developmental biology and related fields, and continues to promote the free, timely, and global exchange of research achievements.

Session 1

Shigeo Ohno (Yokohama City University, Japan)
Julie Ahringer (University of Cambridge, UK)

Session 2

Anne Ephrussi (EMBL, Germany)
Pankaj Dhonukshe (Utrecht University, The Netherlands)

Session 3

Jürgen A. Knoblich (IMBA, Austria)
Sarah M. Russell (Peter MacCallum Cancer Centre, Australia)
Hitoshi Sawa (RIKEN CDB, Japan)

Session 4

Fumio Matsuzaki (RIKEN CDB, Japan)
Xavier Morin (IBDML, France)
Jon Clarke (King's College London, UK)

Session 5

Tadashi Uemura (Kyoto University, Japan)
Matias Simons (University Hospital Freiburg, Germany)

Session 6

Ping Chen (Emory University, USA)
Christopher Kintner (The Salk Institute, USA)
Rudolf Winklbauer (University of Toronto, Canada)

Session 7

Carl-Philipp Heisenberg (MPI-CBG, Germany)
Thomas Lecuit (IBDML, France)
Mirjam Mayer (MPI-CBG, Germany)

Session 8

Christophe Marcelle (IBDML, France)
Shigeo Hayashi (RIKEN CDB, Japan)
Bob Goldstein (University of North Carolina at Chapel Hill, USA)





Session 9

Robert Insall (Beatson Institute for Cancer Research, UK)
Hiroko Sano (Ochanomizu University, Japan)
Roberto Mayor (University College London, UK)

Session 10

Takuya F. Sakaguchi (UCSF, USA)
Patrick O. Humbert (Peter MacCallum Cancer Centre, Australia)
Zhenbiao Yang (University of California-Riverside, USA)
Kenji Matsuno (Tokyo University of Science, Japan)

Session 11

Jerome Gros (Harvard Medical School, USA)
Hiroki Nishida (Osaka University, Japan)
Hiroshi Hamada (Osaka University, Japan)



2009 CDB Symposium Frontiers in Organogenesis March 23–25, 2010

The eighth CDB Symposium "Frontiers in Organogenesis" will be held on March 23 – 25, 2010 in the CDB Auditorium. The focus will be on questions such as spatiotemporal patterning, inductive signals and cellular dynamics, mechanical interactions in histogenesis, growth control and shaping and evolutionary modulation of organogenesis. By facilitating communication between the leaders and promising younger scientists in these fields at a collegial forum, this meeting seeks to create a unique atmosphere that provides a comprehensive perspective into the current knowledge of the dynamics of organogenesis.

Invited speakers

Arkhat Abzhanov
Harvard University, USA

Benoit G. Bruneau
Gladstone Institute of Cardiovascular Disease, USA

Hideki Enomoto
RIKEN Center for Developmental Biology, Japan

Scott E. Fraser
California Institute of Technology, USA

Yukiko Gotoh
The University of Tokyo, Japan

Brigit Hogan
Duke University Medical Center, USA

Jukka Jernvall
University of Helsinki, Finland

Ryoichiro Kageyama
Kyoto University, Japan

Mark A. Krasnow
Stanford University School of Medicine and
Howard Hughes Medical Institute, USA

Shigeru Kuratani
RIKEN Center for Developmental Biology, Japan

Gail R. Martin
University of California, San Francisco, USA

Andy McMahon
Harvard University, USA

Atsushi Miyawaki
RIKEN Brain Science Institute, Japan

Toshihiko Ogura
Tohoku University, Japan

Stefano Piccolo
University of Padova, Italy

Olivier Pourquie
Howard Hughes Medical Institute,
Stowers Institute for Medical Research, USA

Yoshiki Sasai
RIKEN Center for Developmental Biology, Japan

Neil Shubin
University of Chicago, USA

Didier Stainier
University of California, San Francisco, USA

Cliff Tabin
Harvard Medical School, USA

Hiroyuki Takeda
The University Tokyo, Japan

Masatoshi Takeichi
RIKEN Center for Developmental Biology, Japan

Naoto Ueno
National Institute for Basic Biology, Japan

Joachim Wittbrodt
University of Heidelberg, Germany

Christopher V. E. Wright
Vanderbilt University, USA

Elazar Zelzer
Weizmann Institute of Science, Israel

CDB Seminars

The CDB has made special efforts to develop a full and diverse program of invited seminars by scientists from around the world. To date, the Center has hosted around 450 such talks, in addition to numerous meetings, internal forums and colloquia. The following speakers presented CDB Seminars in the period from January to December 2009.

date	title	speaker
01-06	Genetic regulation of renal progenitor cells	Akio KOBAYASHI
01-06	<i>C. elegans</i> : A simple approach to understanding stem cells	Myon-Hee LEE
01-19	Atypical cadherin Fat4 governs planar cell polarity (PCP) in vertebrates	Sakura SABURI
01-19	A molecular mechanism for balancing self-renewal with differentiation in <i>Drosophila</i> neural stem cells	Takashi NISHIMURA
02-17	Transcriptional silencing and lineage commitment in mice	Brian D. HENDRICH
03-03	Signaling in the neural crest lineages during cardiovascular and peripheral nervous system development	Yuka MORIKAWA
03-09	Stem Cells: Niche, competition, aging and applications	Ting XIE
03-12	Chemokine-mediated migration control of osteoclast precursors visualized by in vivo bone marrow imaging: A novel point of regulation for osteoimmunology	Masaru ISHII
03-12	Regulation of Xist function in X chromosome inactivation	Hiroyuki KISHIMOTO
03-18	Regulation of planar cell polarity by Smurf ubiquitin ligases	Masahiro NARIMATSU
04-03	Wnt signaling in mammalian embryonic development	Yingzi YANG
04-09	Developmental regulation of avian stem cells and the application for organ regenerations	Hiroshi KAGAMI
04-16	Novel functions of tumor suppressor RB protein and p53 in nucleus and near plasma membrane	Yoichi TAYA
04-20	Embryonic stem cells and pluripotency	Austin SMITH
04-24	Notch signaling is essential for coordinating cell fate, morphogenesis and migration in the lateral line primordium	Miho MATSUDA
05-27	Roles of Dpr2 in TGF- β signaling and in development	Anming MENG
05-27	Regulation of zebrafish embryonic cell migration and patterning by steroids	Bon-chu CHUNG
06-01	Extrinsic and intrinsic regulators of synapse formation in <i>C. elegans</i>	Kang SHEN
06-02	Epiblast stem cells	Paul TESAR
06-02	Towards generation of visceral endoderm from XEN cells	Tilo KUNATH
06-03	From precursor to product: nephron induction, patterning and repair	Andrew Paul McMAHON
06-04	Regulation of TCF3 phosphorylation and function by Wnt signaling	Hiroki HIKASA
06-05	Post-translational regulation in the <i>Drosophila</i> germ line	Paul LASKO
06-08	Induction and trans-generational inheritance of responses to a novel challenge presented to developing flies	Yoav SOEN
06-08	Prospective isolation of blastocyst- and epiblast-stage precursors from human embryonic stem cells	Micha DRUKKER
07-29	Signalling at tight junctions and regulation of gene expression in epithelial cells	Karl MATTER
07-29	Tight junction remodeling participates in cytoskeletally-mediated barrier regulation: The unique role of zonula occludens-1	Jerrold R. TURNER

date	title	speaker
09-07	Developmental evolution of digit identity in birds	Günter P. WAGNER
09-10	Identification and characterization of molecules directing axonal growth and target field innervations	Kenji MANDAI
09-11	Stemming vision loss with stem cells	Martin FRIEDLANDER
09-14	Novel family of neurotrophic factors – structure, biology and therapeutic potential	Mart SAARMA
09-19	Tribal networks in cerebral cortex	Henry KENNEDY
09-19	Role of G1 phase regulation in mouse and primate corticogenesis	Colette DEHAY
09-19	Managing stem cells and modulating mitosis: Key roles for centrosomes and their regulation	Mark PEIFER
09-25	Genetic mechanisms of axon regeneration after injury in <i>C. elegans</i> : roles of second messengers and MAP kinase pathways	Andrew CHISHOLM
09-30	<i>C. elegans</i> as a model system to study autophagy	Hong ZHANG
10-06	Role of membrane-deforming proteins and endocytosis in neuronal migration and morphogenesis.	Franck POLLEUX
10-14	Using zebrafish genomics to elucidate human disease	Thomas BECKER
10-14	Genome architecture and new strategies to analyze gene regulation and activity	Silke RINKWITZ
11-02	Nuclear dynamics in fission yeast <i>Schizosaccharomyces pombe</i>	Pernilla BJERLING
11-11	Characterization of microtubule-end structure and γ -tubulin in plants	Yoshinobu MINEYUKI
11-13	Role of cell polarity genes for photoreceptor morphogenesis in <i>Drosophila</i>	Kwang-Wook CHOI
11-13	Effects of neuropeptide in early postnatal brain development	Ichiko NISHIJIMA
11-19	Niche-associated polarization of a stem cell in <i>Drosophila</i>	Chip FERGUSON
12-04	Control of frontal cortical size and neuronal number during development	Setsuko SAHARA
12-08	Delineating the molecular mechanisms of chromatin protein dynamics in embryonic stem cells	Eran MESHORER
12-11	Elucidating the mechanism underlying embryonic stem cell self-renewal	Qi-Long YING
12-14	"Hitchhiking" to the microtubule plus end: identification of a molecular mechanism	Anna AKHMANOVA
12-15	RNAi-dependent DNA methylation of repeat elements and gene expression homeostasis in <i>Arabidopsis</i>	Vincent COLOT
12-17	The Tetrahymena Argonaute-binding protein Giw1p directs a mature Argonaute-siRNA complex to the nucleus	Kazufumi MOCHIZUKI
12-21	Understanding the epigenetical functions of histone H3-lysine 56 modification in DNA repair, transcription, and chromatin structural regulation.	Toshiaki TSUBOTA
12-24	Odorant receptors and neural map formation	Takeshi IMAI
12-24	Statistical analysis and mathematical modeling of spontaneous activities of chemotactic cells	Tatsuo SHIBATA
12-25	How can a protein that protects the brain during injury be harmful during brain development?	Seong-Seng TAN

About RIKEN

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

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

RIKEN Website



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

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



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On The Cover

Overexpression of alpha-catenin-ezrin fusion protein (green) in human R2/7 cells induces filopodia-like structures. Actin filaments (magenta) are also shown.

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