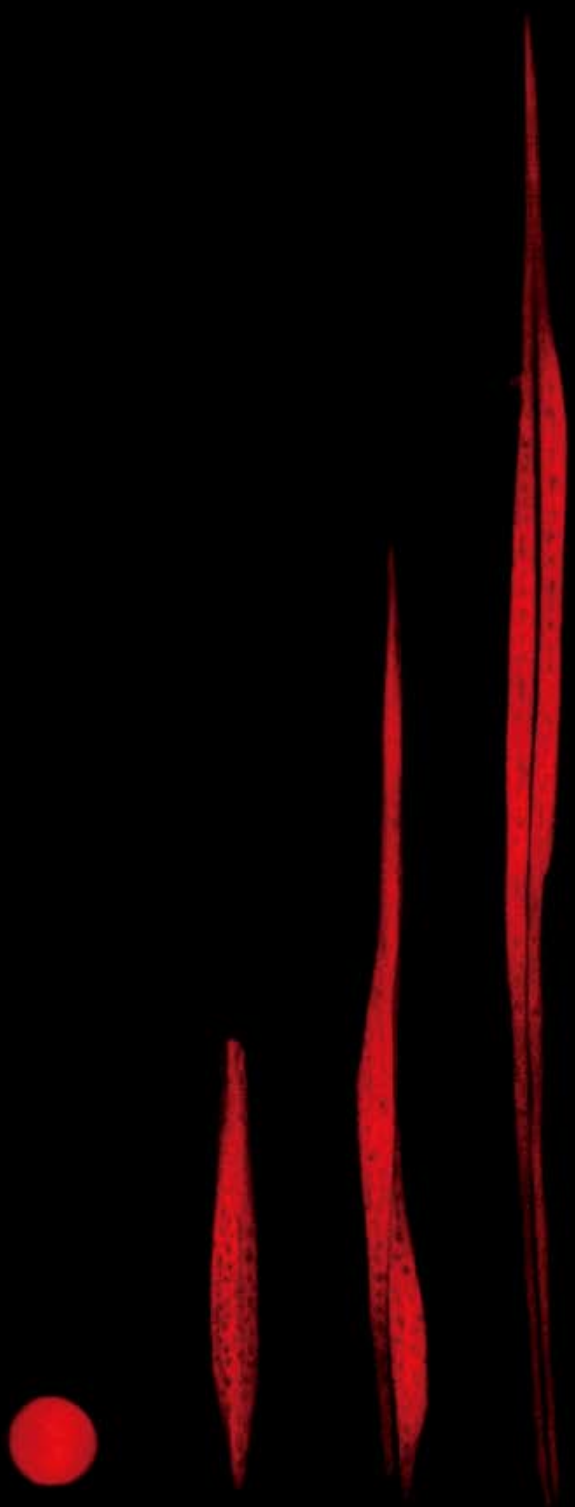
RIKEN Center for Developmental Biology

2011 Annual Report



RIKEN Center for
Developmental Biology

2011
Annual Report

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

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

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

Message from the Director

History will remember 2011 as a year in which Japan was confronted with an unprecedented series of disasters, natural and manmade. Our research center was fortunately spared any physical damage, but like all of Japan, we are still coming to terms with the impact of these events. I would like to being this year's end reflection by expressing our deepest gratitude to all of the many people who reached out to Japan in its moment of need, and particularly to the many scientists and research organizations that made such generous offers of help and support in the days following the Great East Japan Earthquake.

Our own achievements and challenges over the past year here at the RIKEN Center for Developmental Biology pale in significance against this backdrop, but it seems clear that the most effective way for us to contribute to the nation's rebuilding efforts is to continue in our scientific endeavors and support the greater community. Our research programs have certainly continued to open new insights into a range of developmental phenomena, with novel findings into cell signaling, migration, asymmetry, and morphological change that have honed our appreciation for the complexity of the embryo. Stem cell research was a particular highlight in 2011, with CDB labs revealing cell culture techniques capable of inducing the self-organization of embryonic stem cells into tissue-like structures closely resembling the optic cup and the anterior pituitary, as well an assay using patient-specific induced pluripotent stem cells as a platform for drug validation and disease modeling in retinitis pigmentosa.

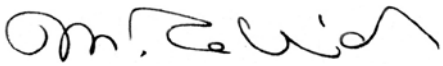
We continue to recruit capable young PIs into the center as part of our commitment to supporting the careers of talented scientists in a research-intensive environment; in 2011, we welcomed a new lab led by Erina Kuranaga that will focus on histogenetic dynamics to the CDB. We also saw a number of our earliest team leaders move on to new positions in academia and government research institutes, a sign of the continuing success of our emphasis on fixed-term commitments to promising young scientists in the field. With two new labs scheduled to open in early 2012, we remain on a course of continuous self-renewal, chasing the horizons in these rapidly evolving fields of science.

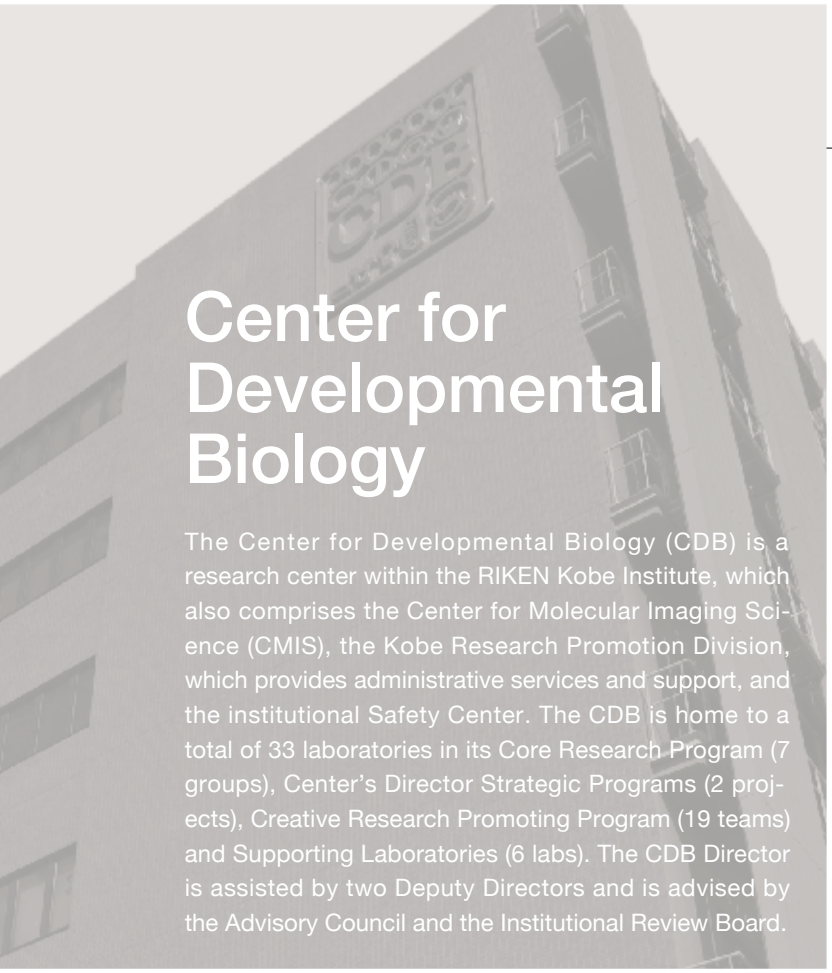
We have also maintained our commitment to outreach and interaction, both with the international scientific community and the lay public. This fall, we hosted our largest Open House event, in conjunction with the other RIKEN Kobe research centers, giving over 1,000 visitors the opportunity to speak with our scientists and learn more about the CDB's work. We were delighted to host a delegation led by the Japanese Minister of Education, Culture, Sports, Science and Technology in December, during which he visited labs and spoke with a number of our research leaders. As in previous years, we also organized a number of popular hands-on programs for local high school students and teachers, designed to stimulate and reward the curiosity of learners and educators alike.

Our location in the city of Kobe remains a key factor in our ability to contribute to and conduct translational and cross-disciplinary studies. Not only has RIKEN's presence in the city expanded to include four life sciences research institutions and the world's fastest supercomputer, but in July Kobe's new municipal medical center opened just a short walk from the CDB, joining the more than 200 public and private biomedical organizations that make their home on Port Island.

And so, we look back proudly on 2011 as a year of successes for the CDB, but with the recognition that these were overshadowed by events of greater historical magnitude. We could not have sustained this record of achievement without the dedicated work of our many investigators, student trainees, and administrative staff, or without the encouragement and well wishes of our colleagues around the world. And so I close, as I opened, with a message of gratitude to all who have supported us in our efforts over the past year, and wish you all the best for the one to come.

Masatoshi Takeichi
Director, RIKEN Center for Developmental Biology





Center for Developmental Biology

The Center for Developmental Biology (CDB) is a research center within the RIKEN Kobe Institute, which also comprises the Center for Molecular Imaging Science (CMIS), the Kobe Research Promotion Division, which provides administrative services and support, and the institutional Safety Center. The CDB is home to a total of 33 laboratories in its Core Research Program (7 groups), Center's Director Strategic Programs (2 projects), Creative Research Promoting Program (19 teams) and Supporting Laboratories (6 labs). The CDB Director is assisted by two Deputy Directors and is advised by the Advisory Council and the Institutional Review Board.

Center for Molecular Imaging Science

Research Promotion Division

Safety Center

Institutional Review Board

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

Core Program

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

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

Center Director's Strategic Program

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

- Pluripotent Stem Cell Studies
  Hitoshi NIWA M.D., Ph.D.
- Systems Biology
  Hiroki R. UEDA M.D., Ph.D.

  Physical Biology
  Tatsuo SHIBATA Ph.D.

Advisory Council

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

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

- Austin Smith
  University of Cambridge, UK
- Christopher Wylie
  Cincinnati Children's Hospital Medical Center, USA
- Margaret Buckingham
  Institut Pasteur, France
- Patrick Tam
  University of Sydney, Australia
- Stephen Cohen
  Temasek Life Sciences Laboratory, Singapore
- Haifan Lin
  Yale university, USA
- Toshio Suda
  Keio University, Japan
- Yoshimi Takai
  Kobe University, Japan
- Ryoichiro Kageyama
  Kyoto University, Japan
- Hiroshi Hamada
  Osaka University, Japan

Director

Deputy Directors

Creative Research Promoting Program

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

- Neuronal Differentiation and Regeneration
  Hideki ENOMOTO M.D., Ph.D.
- Neocortical Development
  Carina HANASHIMA Ph.D.
- Sensory Circuit Formation
  Takeshi IMAI Ph.D.
- Histogenetic Dynamics
  Erina KURANAGA Ph.D.
- Sensory Development
  Raj LADHER Ph.D.
- Germline Development
  Akira NAKAMURA Ph.D.
- Chromatin Dynamics
  Jun-ichi NAKAYAMA Ph.D.
- Growth Control Signaling
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- Mammalian Epigenetic Studies
  Masaki OKANO Ph.D.
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  Guojun SHENG Ph.D.
- Developmental Genomics
  Asako SUGIMOTO Ph.D.
- Retinal Regeneration
  Masayo TAKAHASHI M.D., Ph.D
- Genomic Reprogramming
  Teruhiko WAKAYAMA Ph.D.

Supporting Laboratories

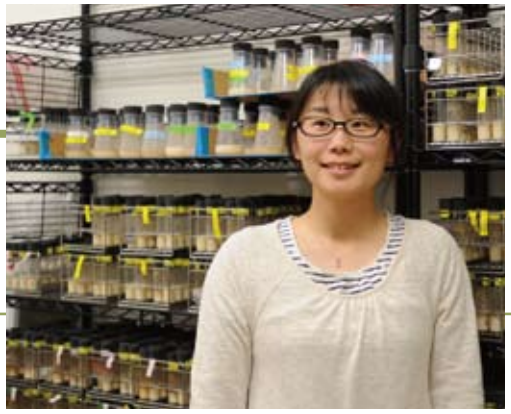
The Supporting Labs provide a range of technical support functions available to all researchers working at the CDB. They also conduct research and development of new lab equipment and analytic software, and provide training on the use of research technologies.

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

2011 Highlights

New Team Leader Erina Kuranaga joins the CDB

Erina Kuranaga, the new head of the Laboratory for Histogenetic Dynamics, studies the roles played signaling pathways that underlie programmed cell death in tissue development. The Kuranaga lab joined the Center in January.



Examining emu embryology

Scientists from the Labs of Early Embryology and Sensory Development created a stage series of emu embryogenesis, showing parallels to that of the more widely studied chick in that while these two very different bird species proceed through similar embryonic stages, a number of differences in timing and pace set them on the widely diverging courses manifested in their adult forms.

Project leader Hiroki R. Ueda wins JSPS Prize

Hiroki R. Ueda, leader of the CDB Center Director's Project on Systems Biology, was awarded the prestigious JSPS Prize by the Japan Society for the Promotion of Science for his studies of the design principles underlying the mammalian circadian clock system. Ueda takes a systems biology and quantitative approach to developing a better understanding of the mechanisms at work in regulating the approximately 24-hour circadian cycle operating in diverse forms of life.

Course in multicolor fluorescent in situ hybridization

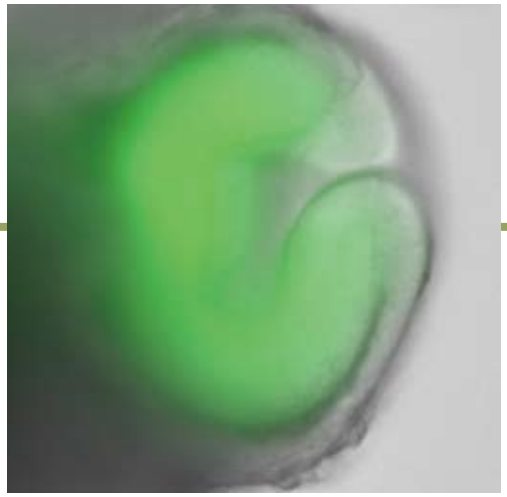
In February, the CDB organized a course on multicolor fluorescence in situ hybridization (FISH) for the nuclear analysis of human stem cells. This program was the latest in a series of training workshops coordinated by the Division of Human Stem Cell Technology.

CDB Symposium: Epigenetic Landscape in Development and Disease

The RIKEN CDB hosted its ninth annual symposium, “Epigenetic landscapes in development and disease” on March 14–15. More than 180 people gathered to share and discuss the latest developments in the study of epigenetics as it relates to ontogeny and pathology.

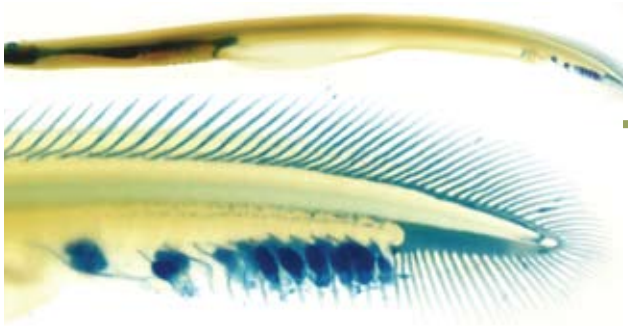
Self-organized optic cup from ES cells

Researchers from the Four-dimensional Tissue Analysis Unit and the Laboratory for Neurogenesis and Organogenesis described how mouse embryonic stem cells (ESCs) are able to differentiate and self-assemble assemble into an optic cup-like structure.



CDB scientists awarded MEXT Prizes

Group Director Shigeru Kuratani and Yasuhide Ohinata, a research scientist in the Pluripotent Stem Cell Studies lab, were honored with awards from the MEXT Minister for their accomplishments in scientific research. Kuratani was noted for his career of achievements in comparative vertebrate evolutionary developmental biology, while Ohinata was recognized for his contributions to the understanding of germline formation in the early embryo and its recapitulation in vitro.



Hagfish vertebra-like structures found

The Laboratory for Evolutionary Morphogenesis demonstrated the presence of cartilaginous vertebra-like elements and analyzed their anatomical features as genuine vertebrae in a hagfish species, solidifying the phylogenetic position of hagfish as true cyclostomes.

Summer school for highschool students

The CDB held its annual summer school for local high school students in early August. The pair of one-day courses featured lectures, lab visits, and hands-on experiments on fluorescent labeling. After staining the cells, the students watched their creations under a fluorescence microscope, enabling them to observe phenomena, such as cell division.

Biology at the Interface

The CDB was delighted to co-organize and host the meeting “Cellular Development: Biology at the Interface” from September 29 to October 1 to commemorate the tenth anniversary of the publication of Developmental Cell, in cooperation with Cell Press, and Fondation Ipsen, with support from the Days of Molecular Medicine Global Foundation.

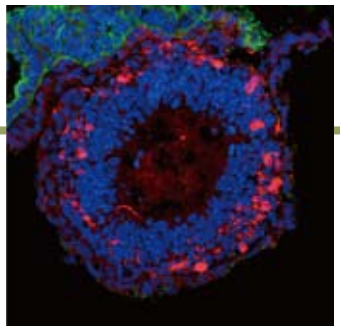
Hands-on course for high school teachers

A course in development for teachers of high school biology, organized by the Office for Research Communications (ORC), was held at the CDB in October. This course is held annually in conjunction with the Japanese Society of Developmental Biologists, and gives groups of teachers an opportunity to learn hands-on techniques in embryology, using methods and materials which they can adapt to their own classrooms.

Open House

The Center held its annual visitors' day activities in November as part of the RIKEN Kobe Institute Open House event, which featured talks, science-themed crafts and games, demonstrations and open laboratories highlighting the research being done at the Institute's research centers. More than 1,200 visitors from around the Kobe area participated in the event.

ES cells give rise to organized pituitary tissue in vitro

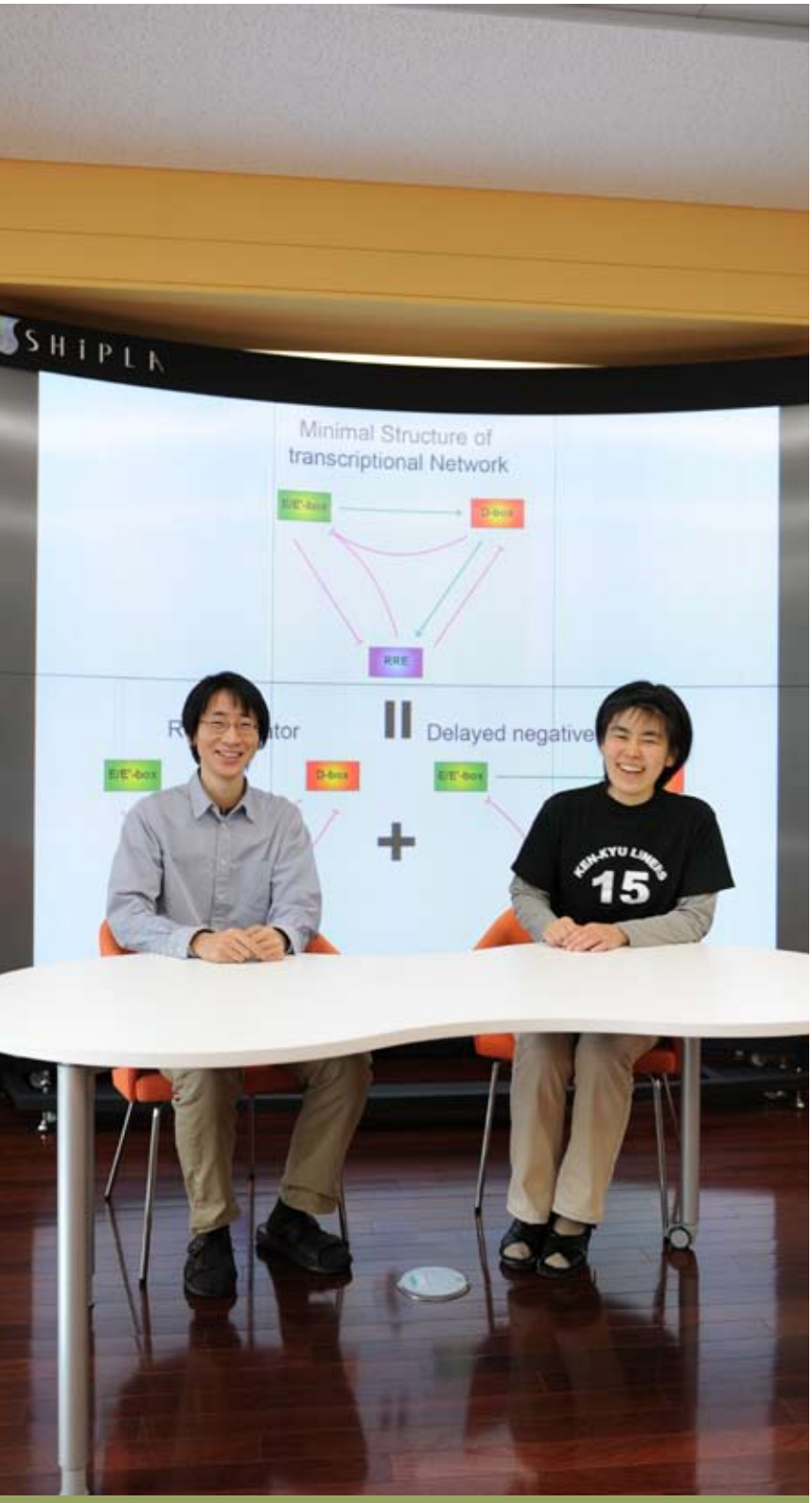


A collaboration between the Division of Human Stem Cell Technology and the Laboratory for Organogenesis and Neurogenesis made a new breakthrough in self-organized tissue differentiation, steering mouse ESCs to give rise to tissue closely resembling the hormone-secreting component of the pituitary, known as the adenohypophysis, in vitro.

MEXT Minister visits Center

Masaharu Nakagawa, Japan's Minister of Education, Sports, Culture, Science and Technology (MEXT) paid a visit to the RIKEN Center for Developmental Biology in December to hear about research at a number of CDB labs. He showed especial interest in the leading work being done by stem cell researchers at the Center, and their potential for applications in regenerative medicine.

Key factor combines day and night to hold back morning



Rikuhiro G. YAMADA, Maki UKAI-TADENUMA

As in manmade timepieces, the movements of the genetic clockworks that lie behind circadian cycles involve a remarkable amount of complexity. The mammalian circadian clock, for example, is thought to arise from the interactions of around 20 transcription factors with specific DNA sequences associated with morning, day, and night expression. Existing models of this genetic network can readily explain the basis for the day and night activities, but the mechanism underlying morning expression remains incompletely understood. It is thought that delayed negative feedback exerted by the morning (E/E' box) inhibitor Cryptochrome 1 (*Cry1*), which is itself expressed in evening, plays an important role in keeping the biological clock on time. But just how it achieves this effect is unknown.

Maki Ukai-Tadenuma and Rikuhiro G. Yamada of the Laboratory for Systems Biology (Hiroki R. Ueda, Project Leader), along with colleagues in the Universities of Memphis (USA) and Fribourg (Switzerland), now report how delayed feedback repression is a key factor in mammalian clock function. Published in *Cell*, this work shows the role of *Cry1* as mediator of delayed negative feedback repression and fleshes out the current understanding of the circadian circuitry.

The team began by looking into the basis for the evening expression of *Cry1* using reporter genes coding for the luciferase protein to detect transcriptional activity, and found that the *Cry1* promoter region induces the expression of genes carrying the daytime expression motif. A closer look at *Cry1*'s DNA revealed that its intronic region contains a separate sequence that induces nighttime clock genes. They next stitched together a construct including these promoter and intron regions, ran another reporter assay to observe its behavior, and found that its expression switched on in circadian evening, suggesting that this in-between expression time is a result of the combination of day and night regulatory elements. To test this model, the team tried to rescue clock function in cells with homozygous deletions of both *Cry1* and *Cry2* by inducing the evening expression of exogenous *Cry1*. They found that while

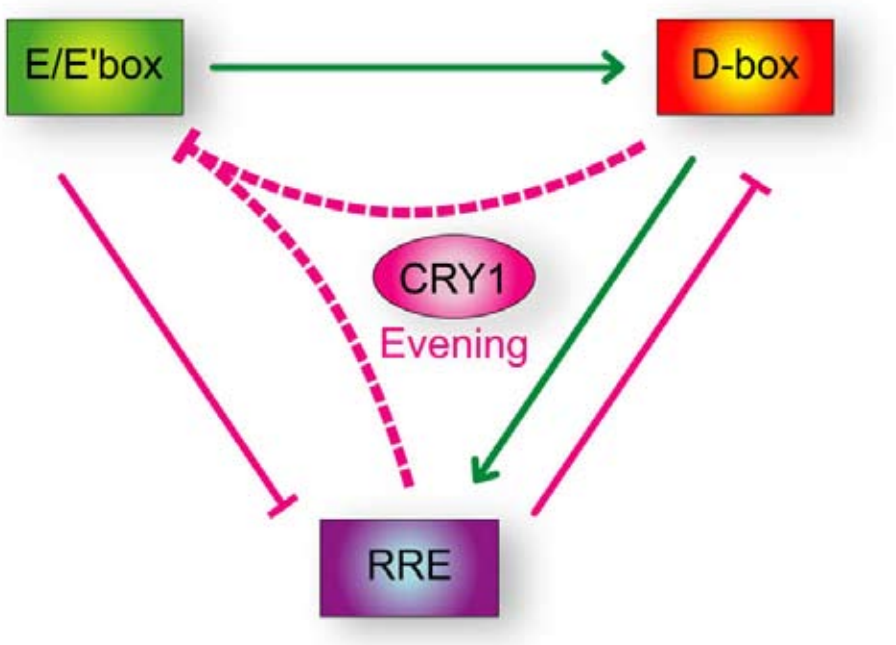
Ukai-Tadenuma M, et al. Delay in feedback repression by cryptochrome 1 is required for circadian clock function. *Cell* 144.268-81 (2011)

the *Cry1* promoter region alone was ineffective, when the promoter and intron regions were used in conjunction, the gene's circadian rhythmicity was restored.

Using this same set-up, Ukai-Tadenuma and Yamada next tried changing the onset time of *Cry1* expression, and found that as expression neared midday, meaning that the normal phase delay was reduced, the amplitude of circadian oscillations grew smaller, in line with predictions. Similarly, prolonging the delay of exogenous *Cry1* expression caused an increase in the length of the restored cycle.

The team's findings were recapitulated by a relatively simple phase vector model, which not only successfully reproduces the findings from the current study, but numerous other aspects of the circadian clock network as well.

"In 1990, Paul Hardin at Texas A&M pointed out the importance of delayed feedback repression in biological clocks, but it has taken 21 years to work out the mechanism behind it," says Ueda. "We will continue exploring whether the current minimal transcriptional network model is complete, or whether new regulatory systems remain to be discovered."



A minimal circuit model for the mammalian circadian transcriptional network. Morning (E/E'box), day (D-box) and night (RRE) DNA elements lie at the heart of the circadian clock. Solid lines indicate activating (green) and inhibitory (pink) interactions between clock gene types. The dotted lines indicate how *Cry1* represses morning expression through the combined action of day and night sequences.

Emus emulate chick developmental stages

Hiroki NAGAI, Siu Shan MAK, Guojun SHENG, Raj LADHER

Birds are useful models in developmental biology, given their large, external eggs and an array of classic embryology techniques, but the study of avian development has been dominated by a single species, the chicken *Gallus gallus*. While a few other bird varieties have been studied for purposes of comparison, these have all been from the "modern" species (neognaths), such as quail, duck and pheasant. A number of more basal bird species (palaeognaths), including ostrich, rhea, kiwi, and emu survive, but their development has remained largely unstudied.

In a report published in *Developmental Dynamics*, Hiroki Nagai of the Laboratory of Early Embryogenesis (Guojun Sheng, Team Leader) and colleagues from the same lab as well as the Laboratory for Sensory Development (Raj Ladher, Team Leader) provided a comparative description of the development of the emu, *Dromaius novaehollandiae*, with that of the better-known chick. The team found that while the two birds proceed through similar embryonic stages, a number of differences in timing and pace set them on the widely diverging courses manifested in their adult forms.

Nagai's approach centered on identifying emu equivalents for Hamilton-Hamburger stages in the chick. The Hamburger-Hamilton (HH) system uses definitive morphological characteristics to determine the state of development, and is the gold standard in staging chick embryos. As a general rule, emus take 2–3 times longer than chicks to reach the same HH stage.

The early development of the emu resembles that of the chick until HH7. During subsequent stages in which somites form, however, the emu forms a greater number of these structures than the chick. The emu somitogenic period was calculated to be 100–110 min, slightly longer than the 90 minutes in chick embryos.

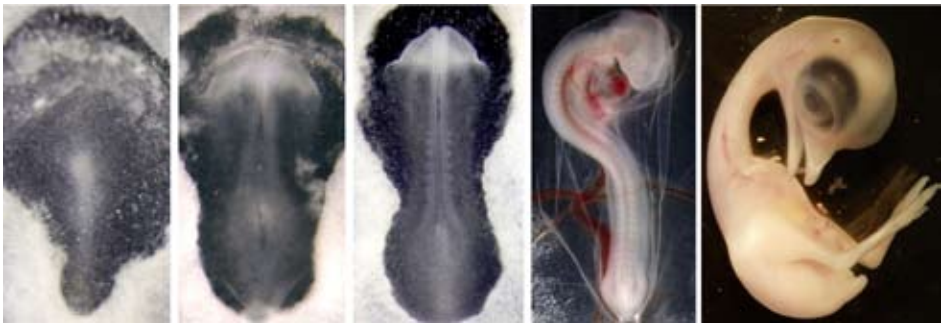
Nagai H, et al. Embryonic development of the emu, Dromaius novaehollandiae.
Dev Dyn 240.162-75 (2011)

The forelimbs in adult emus are diminutive, which is reflected in development as well. The forelimb buds form and undergo initial patterning, but these appendages fail to grow apace with the rest of the embryo body, including the hindlimbs. Interestingly, adult emus also have fewer forelimb digits than do other birds, a difference that is also observable at the stage of the limb's patterning. Once the limb buds have formed, the emu embryo begins a growth spurt that results in the enormous size differential with the chick.

The emu, *Dromaius novaehollandiae* (left), and a comparison of emu and chicken eggs (right)

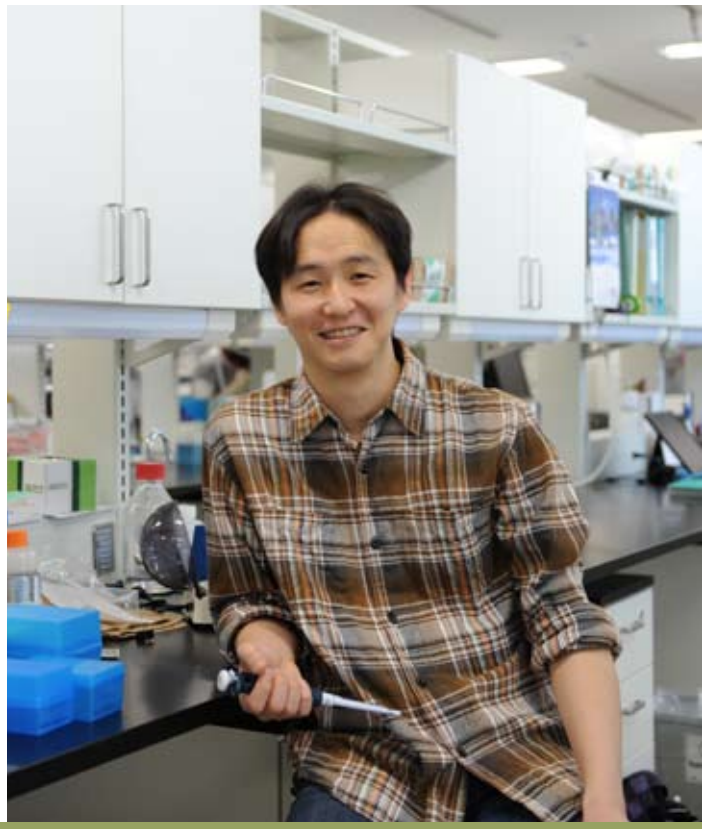
In addition to their comprehensive morphological observations, the team looked at the expression of a number of genes, such as *Sonic hedgehog (Shh)*, *Brachyury*, and *Chordin*, known to be important in early development. Expression patterns were similar to those in chick up to stage 7, when the first somite appears, with the single exception that *Brachyury* expression begins slightly later in emu.

"Since a brief description by Haswell in 1887, there have been no published studies of emu development," says Sheng. "In staging these embryos, we learned of the high level of conservation of developmental routines across bird orders, which suggests that findings from the chick may well apply generally to birds. That said, we also saw heterochrony in the development of specific tissues and structures between chick and emu, so we look forward to studying these embryos in more detail through cell labeling, transplants, and imaging techniques."



Emu embryos at HH3^{+}, HH7, 6-somite, HH18 and HH36 stages of development

Modeling retinitis pigmentosa with iPS cells



Zi-Bing JIN, Satoshi OKAMOTO

Retinitis pigmentosa (RP) is a cluster of genetically determined eye disorders that cause visual defects, such as night blindness and narrowing of the field of vision, due to progressive loss of rod photoreceptors. As many as 45 different genes have been linked to the inheritance of this disease, which suggests a diverse etiology and makes development of a standardized animal model problematic. Thus, despite a range of clinical trials of nutritional and drug-based interventions for RP, the disease remains untreatable. Better platforms for modeling the disease and testing drug candidates in vitro are urgently needed.

New work by Zi-Bing Jin and colleagues in the Laboratory for Retinal Regeneration (Masayo Takahashi, Team Leader) looks to add a set of powerful new tools for those searching for treatments for RP. In an article published in *PLoS One*, the team reports the generation of induced pluripotent stem cells (iPSCs) from patients carrying mutations in several RP-associated genes, and the subsequent differentiation and characterization of rod photoreceptors from these genetically distinct, patient-derived pluripotent cells.

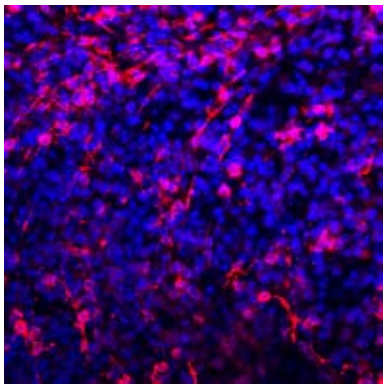
After obtaining informed consent from five RP patients with distinct mutations in the *RP1*, *RP9*, *PRPH2*, or *RHO* gene, the team took samples of skin cells and used the fibroblasts as a starting point for generating iPSCs. Using the classic reprogramming cocktail of *Oct4*, *Sox2, Klf4*, and *c-Myc* delivered via a retroviral vector, Jin and colleagues generated cell lines from each patient and verified their conversion by tests for appropriate morphology, genetic and karyotypic integrity, and teratoma formation.

Using these iPSCs, the team next generated photoreceptors carrying the genetic signatures of each of the five patient donors using a previously established stepwise protocol that steered the cells over four

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

months in culture from an undifferentiated ES cell-like state through retinal progenitor, and photoreceptor precursor stages to the desired rod photoreceptor phenotype. The differentiated cells were shown to express the rod photoreceptor marker rhodopsin at high levels, and to have similar electrophysiological function.

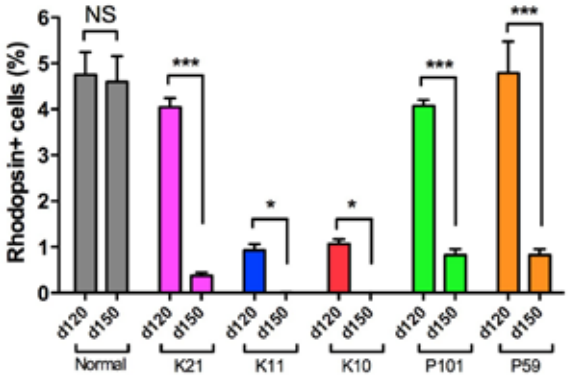
Interestingly, rod photoreceptor cells generated from iPSC colonies carrying RP-linked mutations showed a tendency to degenerate, while cone photoreceptors and bipolar cells derived from the same iPSCs were stable. The mechanisms underlying this instability turned out to be dependent on the affected gene. Rod photoreceptors generated from iPSCs with a mutation in *RP9* showed evidence of DNA oxidation, while those from iPSCs with a mutation in the gene encoding rhodopsin showed signs of stress on the endoplasmic reticulum, the site of protein synthesis.



Rod cells induced from patient-derived iPS cells.
The cells express rod-specific transmembrane protein, rhodopsin (red).

As an initial proof-of-concept test of their suite of RP-specific rod photoreceptors in drug validation, Jin and colleagues examined the effects of antioxidant vitamins in preventing degeneration of these cells in vitro. Ascorbic acid, α-tocopherol, and β–carotene have all been tested in clinical trials as anti-oxidant therapies for RP, but none had proved very effective. When the team tested these on individual cells lines by treating them with one of the three antioxidants for seven days at around the stage at which rod photoreceptor degeneration occurs, they found that α-tocopherol increased cell survival in the lines generated from two patients both carrying mutations in *RP9*. The same treatment was ineffective in cells from other patients, and ascorbic acid and β–carotene had no effect in any of the lines. These results, which show the efficacy of α-tocopherol in promoting survival in *RP9* rod photoreceptors, highlights the potential of patient-derived induced pluripotent stem cells in the study of disease mechanisms and in vitro testing of treatment approaches.

"Using iPSCs from cells donated by RP patients with different underlying genetic mutations, we were able to show that rod photoreceptors generated from these cells underwent apoptosis in vitro, and showed differing responses in a genetically determined manner to drug treatment," says Takahashi. "This is one of the first reports to demonstrate that patient-derived iPSCs may be useful in personalized medicine, as differential responses within a genetically diverse study group will tend to be lost in the crowd. Future improvements in differentiation protocols, screening techniques, cost and efficiency and the establishment of methods for isolating photoreceptors may open up new possibilities for the use of these cells in drug screening."



Patient-derived rod photoreceptors undergo degeneration in vitro. The bars show differentiation efficiencies of the iPS cells derived from five retinitis pigmentosa patients.

New role for phosphorylation in heterochromatin

Kyoko HIRAGAMI-HAMADA

A great many cellular processes are switched on or off by the modification of a given enzyme or other protein by addition of a phosphate molecule, known as phosphorylation. This regulatory activity occurs widely in the cytoplasm, but can take place in the nucleus as well. Recent work has shown the HP1α, a protein that guides the formation of heterochromatin, a form of the DNA-protein structure know as chromatin, is also subject to this post-translational modification, but the biological meaning of this event has remained unresolved.

A new study by Kyoko Hiragami-Hamada and colleagues in the Laboratory for Chromatin Dynamics (Jun-ichi Nakayama, Team Leader), working in collaboration with labs in Kobe University, Kwansei Gakuin University, and AIST, have shown that phosphorylation of HP1α boosts its ability to bind to heterochromatin, resulting in stabilization of chromosomes. Published in *Molecular and Cellular Biology*, this work opens up new insights into the interplay between protein modification and chromatin dynamics.

HP1 was first identified in *Drosophila*, and is now recognized as a highly conserved regulator of transcriptional repression in heterochromatin in eukaryotes. The protein has two similar binding domains: a chromodomain (CD) region in its N-terminal region, and a chromo shadow domain (CSD) at the carboxyl end. The CD binds to a methylated site on histone H3 (H3K9me3), and importantly the CSD promotes binding to other HP1 proteins, which is essential to the formation and maintenance of heterochromatin. It has been suspected that HP1 may also be regulated by phosphorylation as well, but this has never clearly been shown.

Hiragami-Hamada began by testing for binding activity between mammalian HP1 variants HP1α, HP1β and HP1γ and H3K9me3, and found that while phosphorylation had no evident effect on the

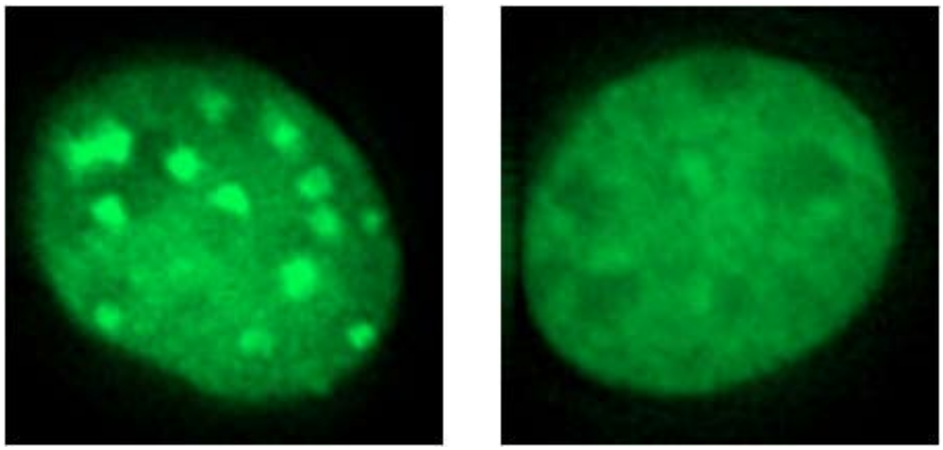
Hiragami-Hamada K, et al. N-terminal phosphorylation of HP1α promotes its chromatin binding. *Mol Cell Biol* (2011)

strength of this association in HP1β or HP1γ, binding of HP1α to methylated histone H3 appeared to be markedly enhanced by the modification. She next used an electrophoresis technique known as Phos-tag-PAGE to examine HP1α in its phosphorylated state, and found not only that multiple sites on the protein were thus modified, but that phosphorylation increased during cell division as well.

To resolve the specific phosphorylation sites, she next used Phos-tag-PAGE in combination with targeted amino acid substitutions and identified serine 14 in the N-terminal and serine 93 in the protein's hinge region as major phospho-targets. Mass spectrometry revealed additional sites in serine 11–13 in the N-terminal as well. Tests of binding affinity showed that it was the N-terminal sites, but not S93 in the hinge, that are responsible for binding to H3K9me3 in a phosphorylation-dependent manner. Interestingly, while this modification was important for establishing the interaction, it was not required for its stabilization.

With a better understanding of the biochemistry behind this interaction, Hiragami-Hamada next turned to phosphorylated HP1α's role within the cell. She found that, when phosphorylation of S14 was prevented by an amino acid substitution, HP1α accumulation at heterochromatic regions was reduced. Similar experiments on N-terminal serines 11 to 13 showed that their phosphorylation also plays a role in targeting HP1α to heterochromatin. When the team replaced all four serines 11 through 14 with substitute amino acids in cultured mouse cells, they observed an increase in chromosomal abnormalities. Serine 93 in the hinge region in contrast had no such effects.

"This work suggests that the chromodomain alone is not sufficient for binding the methylated histone; the connections needs to be strengthened by phosphorylation of sites on the HP1α N-terminus, which interestingly contributes to chromosomal stabilization as well" says Nakayama. "We're now curious about how this phosphorylation is regulated, and what role, if any, it plays in serine 93."



HP1α accumulates in heterochromatic regions in wildtype cells (left), but not in cells engineered to lack phosphorylation of the protein's N-terminal region (right).

Conserved kinase protects cells from endosomal traffic jam

Tetsuhisa OTANI

Some molecules have a single, highly specific function in physiology, while others have a much broader remit. Kinases in the IKK (inhibitor of nuclear factor κB (NF-κB) kinase) family are of the latter sort, playing a variety of roles in the immune system, cancer, and differentiation. Previous work with a related kinase, IKKε, in *Drosophila* has shown that this protein regulates the cytoskeleton and cell elongation in a number of contexts. It has yet to be shown, however, how it accomplishes this task.

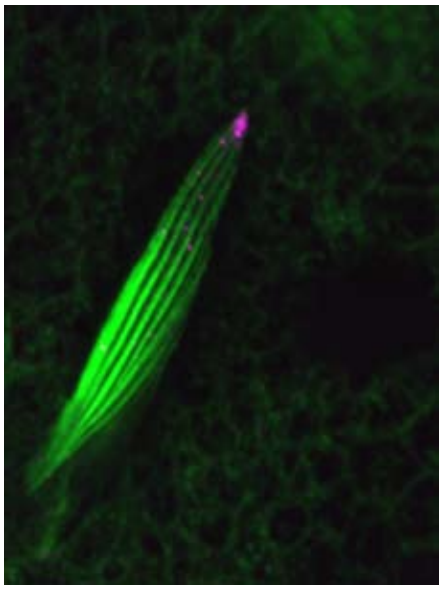
A report by Tetsuhisa Otani in the Laboratory of Morphogenetic Signaling (Shigeo Hayashi, Group Director) and colleagues, working in collaboration with the CDB Electron Microscope and Proteomics labs, has done just that. In an article published in *Developmental Cell*, the groups show that this factor regulates shuttling of endosomes in the tips of growing mechanosensory bristles, converting the direction of their trafficking from incoming to outward bound.

Bristles form from single cells during the fly's pupal stage. A previous report had indicated that IKKε is expressed at the tips of these structures. Otani looked at the active, phosphorylated form of the molecule and found that it indeed accumulated at the tips of growing bristles. He next focused more closely at cytoskeletal organization in these structures in wildtype and IKKε mutants, and found that in the mutant bristles, actin bundles were poorly organized and frequently failed to attach to their anchor points in the cell cortex. Transmission electron microscopy further showed that while microtubules in mutants maintained their usual orientation with the respect to the bristle, an abnormal number of vesicles accumulated in this space, suggesting that vesicle transport was somehow affected.

An examination of vesicle markers turned up a promising candidate in Rab11, a recycling endosome protein known to be essential in bristle morphogenesis. In contrast to its tendency to cluster around the tip in control animals, Rab11 was clumped in the bristle shaft in IKKε mutants. Other endosome markers also showed aberrant localization. The effect was replicated in cultured cells, in which mutant IKKε caused Rab11 to aggregate.

Otani T, et al. IKKε Regulates Cell Elongation through Recycling Endosome Shuttling. *Dev Cell* 20.219-32 (2011)

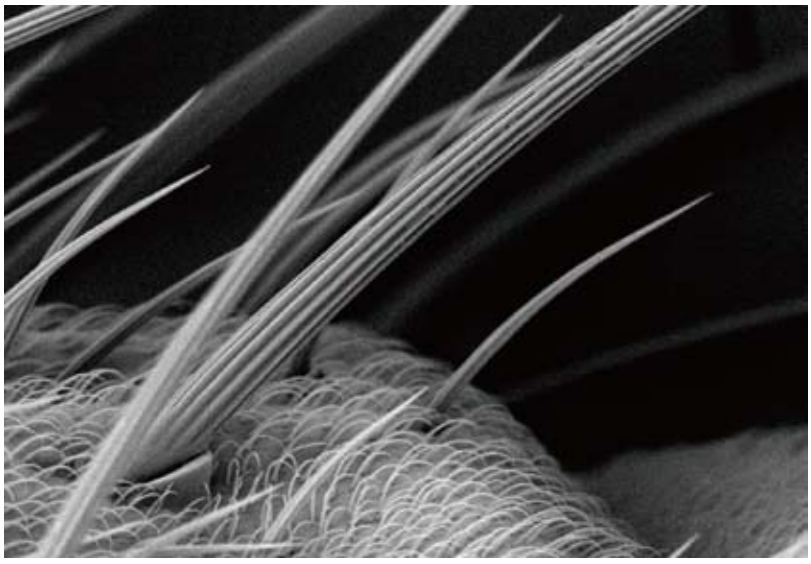
What then was the link between the defects in actin bundles and endosome distribution in IKKε mutants? Looking at the timing of these events, Otani found that Rab11 accumulation precedes the misrouting of actin, and was unaffected by various mutations that disturb the bundling of actin filaments, suggesting that IKKε's roles in Rab11 distribution and actin bundling are independent.



Activated IKKε (magenta) accumulates at the tips of growing bristles, and actin bundles (green) run along the long axis of growing bristles.

He next used photobleaching and time-lapse imaging to track the localization of Rab11 over time. Rab11 normally moves along the bristle, shuttling between shaft and tip in a state of dynamic equilibrium. The rate and overall directionality of this back-and-forth varied, and was at its highest during bristle elongation. Looking for a mechanism, the group examined functional relationships between IKKε, Rab11, and the Rab11 effector, Nuf, which binds to Rab11 and the Dynein light intermediate chain (a component in microtubule trafficking). What they found pointed to opposing roles for IKKε and Nuf in the trafficking of Rab11. This antagonistic effect appears to be due to the phosphorylation of Nuf at a specific amino acid (serine 225) by IKKε. Preliminary experiments using IKKε homologs in mammalian cells suggested that the function of this factor in the trafficking of recycling endosomes is conserved.

"In addition to their role as vehicles for cellular materials, endosomes also serve as a platform for various signaling activities in cell proliferation and immunity," says Hayashi. "The identification of new downstream targets of IKKε sheds light on the roles of endosomal trafficking in morphogenesis, innate immunity, and cancer."



Electron microscopy image of bristles

Novel factor behind ES cells' neural default

Daisuke KAMIYA

Embryonic stem cells (ESCs) are highly regarded for their ability to give rise to the full range of cellular lineages found in the adult body, a capacity known as pluripotency. In most cases, this differentiation needs to be steered by molecular cues that drive the stem cells' progeny to adopt a specific fate, such as blood, muscle or bone. But left to their own devices, ESCs tend to differentiate into neural lineages. Indeed, in the absence of an inhibitory signal, such as BMP4, the preferred destination for ESC differentiation is neural ectoderm. This has been known for years, following demonstrations of the phenomenon in undifferentiated tissue in amphibians, but the mechanism behind this cell-intrinsic propensity has never been explained.

Daisuke Kamiya and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director), working in collaboration with the Laboratories for Stem Cell Biology and Animal Resources and Genetic Engineering, have revealed how the nuclear protein *Zfp521* is key to the default neural fate. In an article published in *Nature*, the group reports that this factor is both necessary and sufficient in driving the intrinsic neural differentiation of mouse ESCs.

The study started from a GeneChip survey of mouse ES cells that had taken their first step down the road to neural differentiation, as revealed by GFP knocked into *Sox1*, a neural progenitor-specific marker. This turned up 104 genes of interest, 29 of which were even more interesting for their lack of expression in mesodermal tissues. Of these, *Zfp521* was particularly attractive as it was found to be a potent promoter of neural differentiation when added to ESCs. Forced expression of *Zfp521* led to increased expression of early neural markers such as *Sox1*, *Sox3*, and *Ncad*. The last of these, which encodes N-cadherin, is normally suppressed by the neural inhibitor BMP4, but Kamiya found that overexpression of *Zfp521* overrode this effect. In situ hybridization showed that *Zfp521* expression

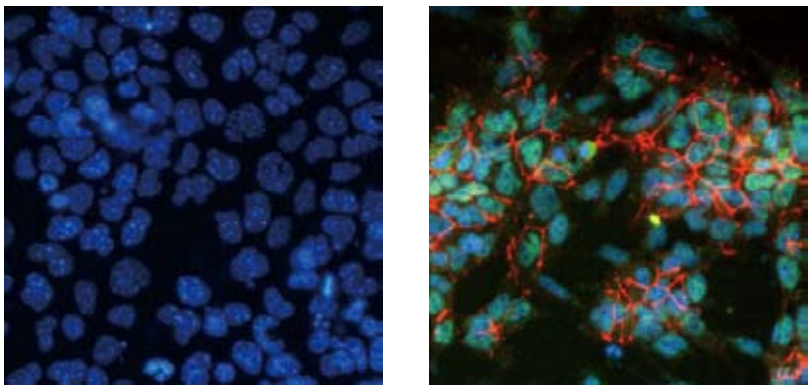
Kamiya D, et al. Intrinsic transition of embryonic stem-cell differentiation into neural progenitors. *Nature* 470.503-09 (2011)

switches by around day 6.5 of development, and grows more pronounced in the neuroectodermal region of the epiblast, while it is absent in non-neural tissues.

To determine whether *Zfp521* is truly necessary for neural differentiation of ESCs, Kamiya next knocked down its function by short hairpin RNA, resulting in a dramatic reduction of cells expressing neural markers. Importantly, this was not rescued by the addition of control cells, indicating that that action was cell autonomous. When the *Zfp521*-knockdown ESCs were injected into blastocysts, they failed to contribute significantly to the rostral neural tube, indicating a failure in primary neurulation.

Wanting to know the specific window of time in which *Zfp521* exerts its function, the group examined its effects on a range of stage- and lineage-specific markers, from the earliest stages of undifferentiated ES cells, which reflects the developmental status of the inner cell mass (ICM), to various primary lineages differentiating from epiblast tissues. They found that while ICM development to epiblast was unaffected by loss of *Zfp521* function, epiblast cells themselves failed to make the transition to neuroectoderm. Chromatin immunoprecipitation (ChIP) assays revealed that *Zfp521* associates with a number of neuroectoderm-specific genes, but not with genes lined to the maintenance of pluripotency or differentiation into other lineages, suggesting that its effect is achieved by activating neural genes rather than inhibiting non-neural ones. This may be attributable to an interaction with a second factor, the histone acetylase p300, with which *Zfp521* also associates. The model of default neural differentiation that emerges from these findings suggests that *Zfp521* functions as a transactivator of neural specification, autonomously steering undifferentiated ESCs to adopt a neuroectodermal fate.

“This set of findings has given us insight into the mechanisms behind how a colony of undifferentiated cells such as ESCs can begin neural differentiation prompted early in development by *Zfp521* in the absence of countering signals, such as BMP4,” says Sasai. “What we would like to work out next is whether this factor has similarly intrinsic effects in the epiblast as well.”



Mouse ESCs overexpressing *Zfp521* in SFEB culture express neural markers Sox1 (green) and N-cad (red) by day 5 of culture (right panel). Wildtype ESCs shown on left (blue shows DAPI staining of nuclei).

2011 Events

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

Transcripts newsletter launched

The Office for Research Communications began publication of a quarterly bulletin, Transcripts, to keep staff informed about recent news and upcoming events. The newsletter contains information on new hires, important calendar events, RIKEN-wide programs, and even interesting spots in and around the city of Kobe.

Junior high school student internships

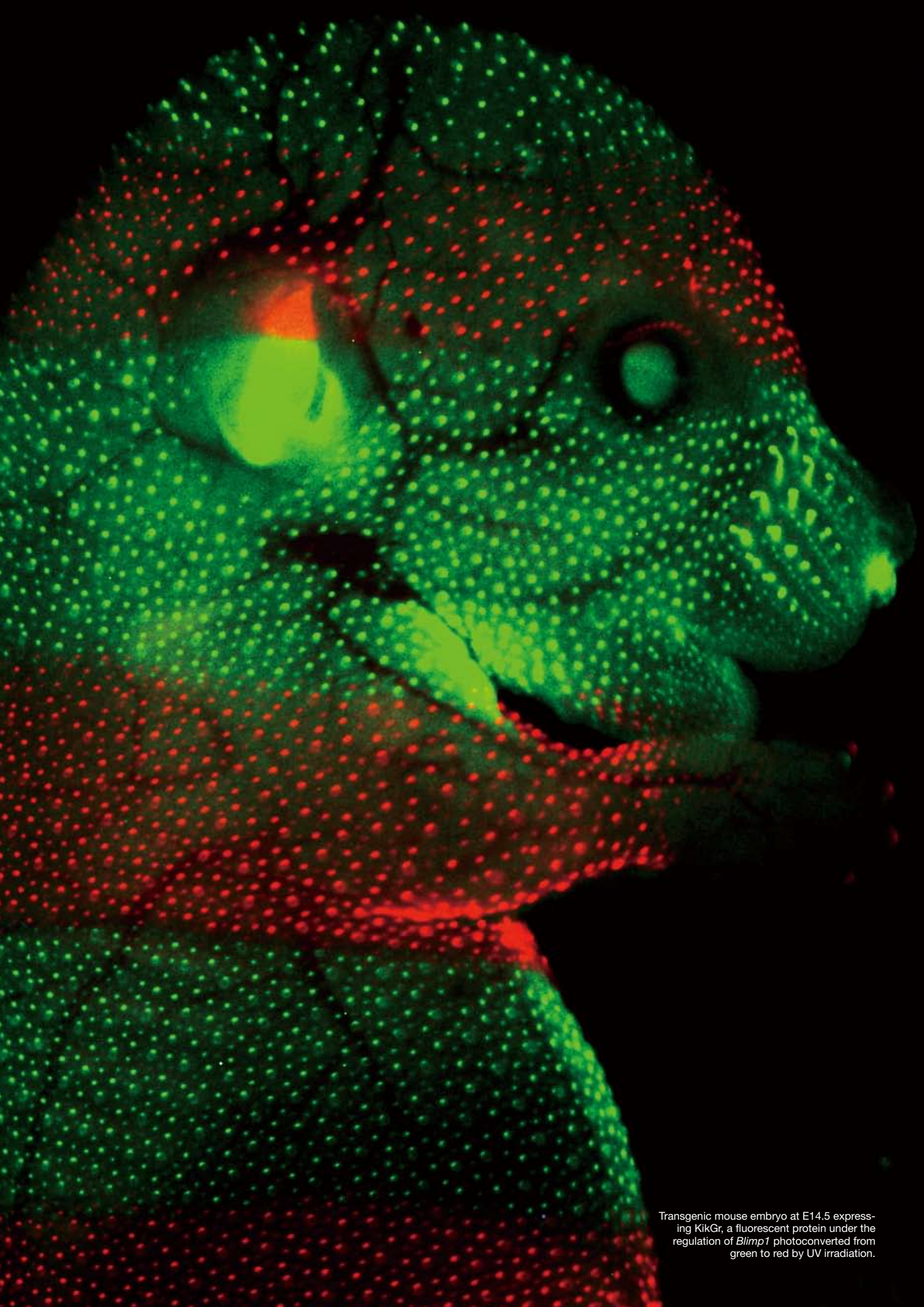
RIKEN Kobe Institute welcomed four students from local junior high schools affiliated with Kobe University as part of a weeklong program to introduce participants to work in various settings. The young trainees spent three days at the CDB, learning basic scientific techniques in the demo lab maintained by the Office for Research Communications, and visiting the Laboratory for Retinal Regeneration.

2011 Open House

The RIKEN Center for Developmental Biology held its annual visitors' day activities on November 5 as part of the RIKEN Kobe Institute Open House event, which featured talks, science-themed crafts and games, demonstrations and open laboratories highlighting the research being done at the Institute's research centers. More than 1,200 visitors from around the Kobe area participated in the event. Group Director Yoshiki Sasai gave a morning talk on the organ-forming potential of pluripotent stem cells in vitro, explaining how ES and iPS cells can be induced to give rise to complex tissue structures in a self-organized fashion. Multiple CDB labs also opened their doors to visitors, including the Laboratories for Cell Asymmetry, Early Embryogenesis, and Neocortical Development, and the Division for Human Stem Cell Technology, encouraging interaction with the Center's student trainees and scientists. Other features included a an exhibition of model organisms used in the CDB's research, a science café event, and a craft-making corner co-organized by the Kobe Science Museum

Awaji Retreat

The CDB held its annual retreat for its research staff on the nearby island of Awaji. CDB lab leaders, research scientists and student trainees gathered in a closed meeting to share and discuss the latest developments from their labs, and to get better acquainted through social activities and casual conversation away from the bench. This year, a number of former research scientists and leaders joined the Retreat, adding a voice of broad international scientific experience and insights into the career paths followed by CDB alumni.



Transgenic mouse embryo at E14.5 expressing KikGr, a fluorescent protein under the regulation of *Blimp1* photoconverted from green to red by UV irradiation.

Vertebrate Body Plan

Shinichi AIZAWA Ph.D.

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

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

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Publications

Kurokawa D, et al. A lineage specific enhancer drives Otx2 expression in teleost organizer tissues. *Mech Dev* (2011)

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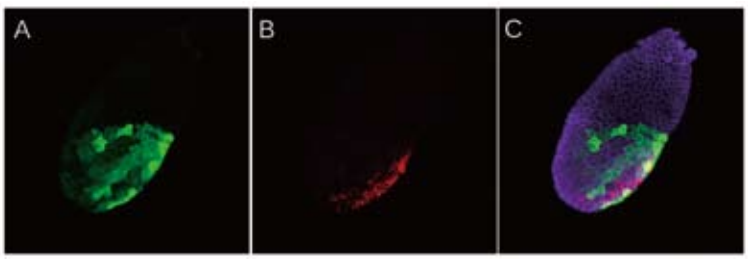
All vertebrate species share certain features in the development of the brain, in that all vertebrate brains comprise four regions—telencephalon, diencephalon, mesencephalon and metencephalon (or cerebellum)—an organizational trait that is thought to have appeared with the advent of the vertebrate lineage.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



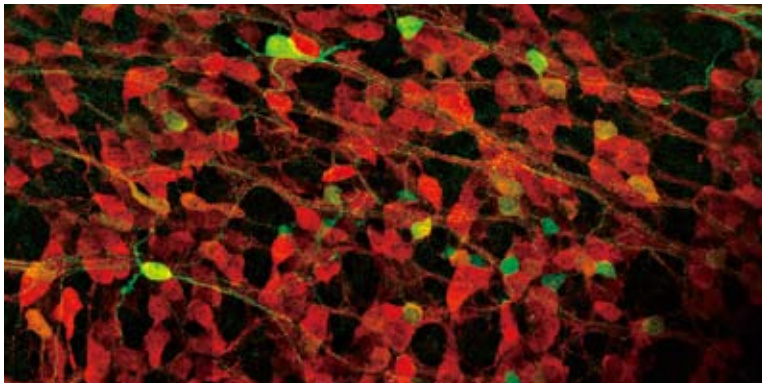
(A) gives descendants of distal visceral endoderm cells that expressed a head organizer gene (*hog*), (B) the descendants keeping the *hog* expression, and (C) their merged view.

Neuronal Differentiation and Regeneration

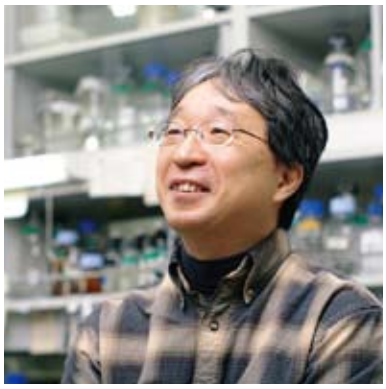
The architecture of the nervous system, which underlies much of the dynamism of animal life, is frequently described as complex, but closer examination reveals great beauty as well. The construction of this exquisite and elaborate structure involves the birth, growth and death of countless individual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

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

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



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



Hideki ENOMOTO M.D., Ph.D.

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

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

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Publications

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

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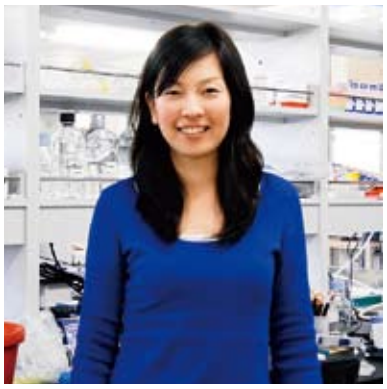
Neocortical Development



Carina HANASHIMA Ph.D.

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

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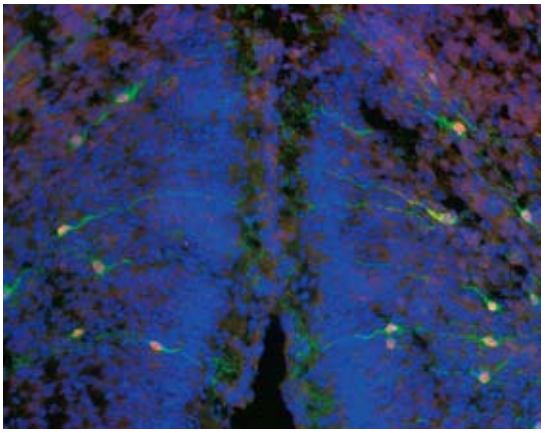
Hanashima C, et al. Foxg1 suppresses early cortical cell fate. *Science* 303. 56-9 (2004)

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

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



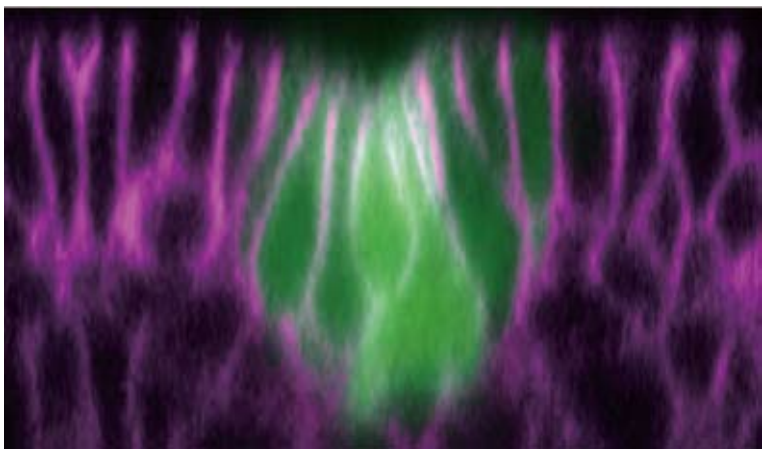
Recruitment of late-born neocortical neurons expressing membrane-targeted GFP (green) and nuclear lacZ (red) to the motor cortex.

Morphogenetic Signaling

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

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

In addition, we study intracellular mechanisms of cell polarization and elongation using model systems of mechanosensory bristles and spermatids, and higher level organization in insect limbs.



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

Shigeo HAYASHI Ph.D.

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

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Publications

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Sensory Circuit Formation

Takeshi IMAI Ph.D.

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

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Publications

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

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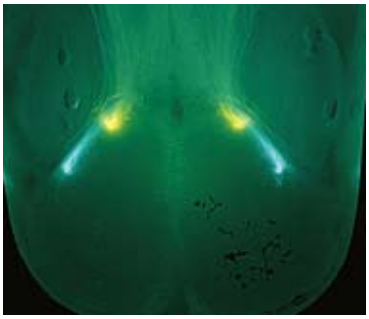
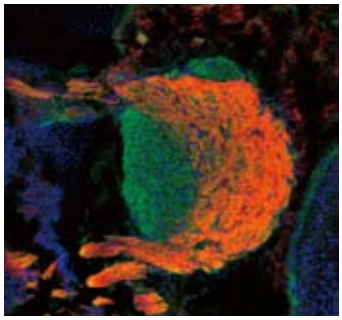
The mammalian nervous system is composed of enormous numbers of neurons, but how do these cells take on diverse fates and organize and array themselves during development? In recent years, it has become clear that the mouse olfactory system provides an excellent platform for addressing these questions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

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

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



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

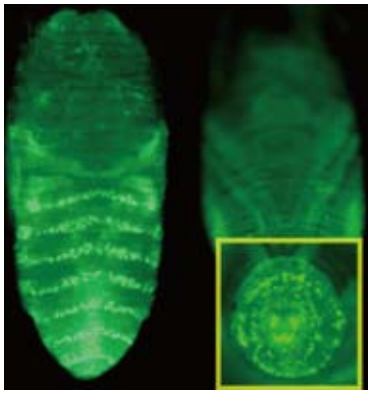


A graded olfactory map forms in the absence of the olfactory bulb. Fibrocellular mass of a bulb-less mutant mouse, stained with anti-gap43 (green) and anti-Neuropilin-1 (red).

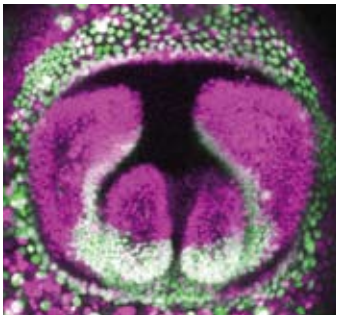
Histogenetic Dynamics

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

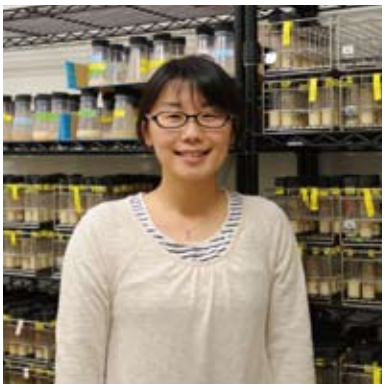
We have chosen the fruit fly *Drosophila melanogaster* as our primary research model, seeking to take advantage of its utility in developmental studies and wealth of genetic data in studying the coordination of histogenesis through live imaging and genetic screens. To elucidate the role of cell death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



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



Caudal view of a *Drosophila* male terminalia showing cells in posterior compartment (green).



Erina KURANAGA Ph.D.

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Erina Kuranaga received her doctorate in medical science from the Osaka University Department of Cell Biology and Neuroscience in 2004, after which she moved to the University of Tokyo Graduate School of Medicine as assistant professor in the Department of Genetics. In 2006, she was promoted to associate professor in the same department, where she remained until she joined the RIKEN Center for Developmental Biology as Team Leader in 2011. She was awarded the Molecular Biology Society of Japan Young Scientist Award in 2010.

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

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Evolutionary Morphology

Shigeru KURATANI Ph.D.

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

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

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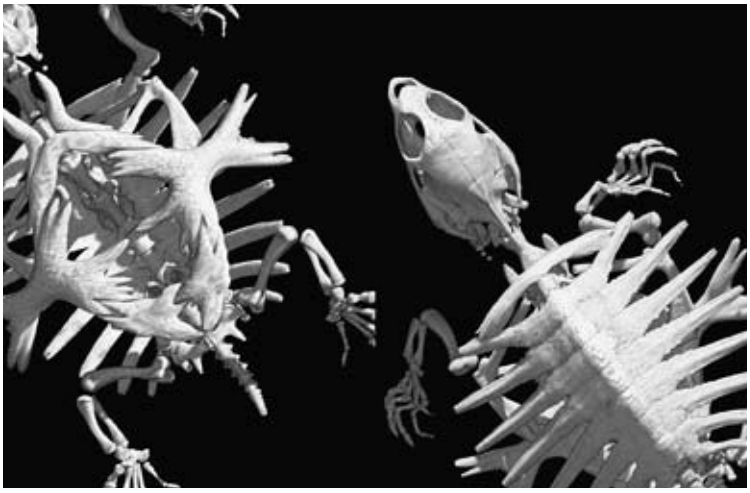
By studying the evolutionary designs of diverse animal species, I hope to gain a deeper insight into the secrets behind the fabrication of morphological designs. Integrating the fields of evolutionary morphology and molecular genetics, our lab seeks to expand the understanding of the relationship between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



CT-scanned skeleton of *Pelodiscus sinensis* juvenile

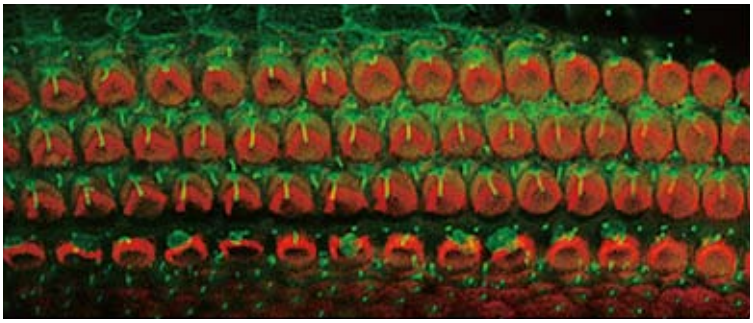
Sensory Development

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

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

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

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



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

Raj LADHER Ph.D.

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

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

Nagai H, et al. Embryonic development of the emu, Dromaius novaehollandiae. *Dev Dyn* 240.162-75 (2011)

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The self-made eye: Formation of optic cup from ES cells

Nozomu TAKATA, Eriko SAKAKURA, Mototsugu EIRAKU, Masako KAWADA

Developmental processes are increasingly well-characterized at the molecular and cell biological levels, but how more complex tissues and organs involving the coordinated action of multiple cell types in three dimensions is achieved remains something of a black box. One question of particular interest and importance is whether signaling interactions between neighboring tissues are essential to guiding organogenesis, or whether these can arise autonomously from developmental routines inherent to a given primordial tissue. Finding answers to these questions will be critical both to a better understanding of embryonic phenomena and to the ability to control the differentiation of cell populations into desired configurations.

A breakthrough new report by Mototsugu Eiraku, deputy leader of the Four-dimensional Tissue Analysis Unit and colleagues in the Laboratory for Neurogenesis and Organogenesis (Group Director, Yoshiki Sasai), as well as the RIKEN VCAD Program, and Kyoto and Osaka Universities, describes how mouse embryonic stem cells (ESCs) are able to differentiate and assemble into an optic cup, capable of giving rise to a tissue exhibiting the stratified structure characteristic of the retina in vivo. Published in *Nature*, the study used a cutting-edge three-dimensional tissue culture system not only to demonstrate this self-organizing capacity of pluripotent stem cells, but the underlying cell dynamics as well.

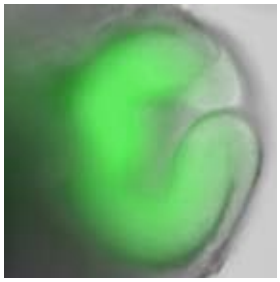
The mechanistic basis for the formation of the optic cup, with its complex two-walled structure, has been a longstanding question in embryology. The retina, with its origins in the lateral midbrain, is part of the central nervous system. Its development begins with the formation of the optic vesicle, a pocket of epithelium that deepens and pinches to form the optic cup, which develops a double layer of cells, with pigment epithelium on the outer, and neural retina on the inner wall. It has generally been thought that this transformation is triggered by chemical and physical influences from other tissues, such as lens or cornea, but some, including the father of experimental embryology, Hans Spemann, have suggested that perhaps external induction or force is not necessary.

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

To resolve this question, Eiraku et al. built on a series of techniques and findings emerging from the use of the SFEBq (serum-free culture of embryoid body–like aggregates) ES cell culture system developed by the Sasai lab, which had previously been used to differentiate these pluripotent stem cells into a wide range of neuronal cell types, including, recently, structurally organized cerebral cortical neurons. By adding extracellular matrix proteins to the SFEBq medium, the group was able to epithelially-organized retinal precursors at high efficiencies by day 7 of culture. One day later, optic vesicle-like structure began to form, followed by bi-layered optic cup-like structures by day 10. The pigmented and neuronal character of the outer and inner layers of cells in these spontaneously formed tissues were confirmed by gene expression, indicating that optic cup development had been recapitulated in vitro, and importantly, in the absence of any external signaling sources, such as lens, demonstrating the capacity for self-organization.

They next used multi-photon microscopy to explore the mechanisms behind this process of self-assembly in 3D. They found that after the ES cell-derived retinal precursors differentiated into pigmented epithelial and neuronal layers, the tissue underwent a four step morphological rearrangement on its way to assuming the optic cup structure. When they examined cytoskeletal behaviors in this process, they noted that myosin activity dropped in the region of the epithelium that bend inward to form the cup, giving the flexibility needed to form a pocket driven by expansion of the epithelium through cell division.

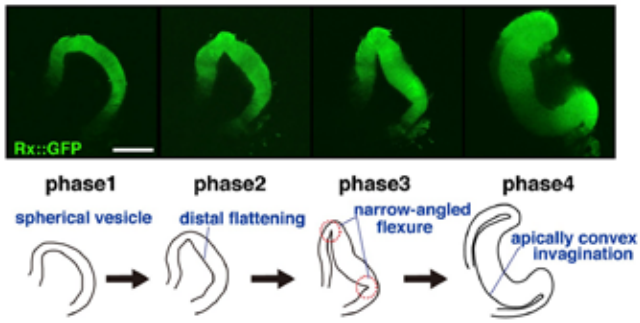
Computer simulation of the mechanics behind this revealed that three principal forces can explain the optic cup-forming event. First, the a region of the epithelium must lose rigidity, allowing it to buckle inward, after which cells at the hinge points (defined by the border between presumptive pigment epithelium and neuronal regions) must undergo apical constriction, giving them a wedge-like shape. Once these conditions are met, expansion of the tissue surface by cell division results further involution of the cup, all of which are very much in line with the experimental findings.



Optic cup derived from mouse ES cells. GFP shows expression of Rx.

As a final test of the in vitro structure's ability to mirror its embryonic counterpart, Eiraku excised the neuronal layer from the ES cell-derived optic cup and allowed it to develop in 3D cell culture under conditions optimized for spurring neuronal maturation. He found that the retinal neurons underwent active mitosis and ultimately organized into a six-layer stratified and synapse-forming neuronal structure closely resembling that of the post-natal retina.

"What we've been able to do in this study is resolve a nearly century-old problem in embryology, by showing that retinal precursors have the inherent ability to give rise to the complex structure of the optic cup," says Sasai. "It's exciting to think that we are now well on the way to becoming able to generate not only differentiated cell types, but organized tissues from ES and iPS cells, which may open new avenues toward applications in regenerative medicine." Potential applications include regenerative medicine approaches to the treatment of retinal degenerative disorders, such as retinitis pigmentosa.



Four-step morphological transformation in formation of optic cup in vitro revealed by two-photon microscopy over three-period.

Transcriptomic insights into the vertebrate phylotypic stage

Shigeru KURATANI, Naoki IRIE

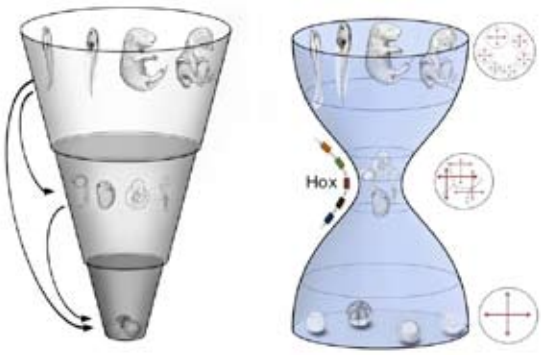
April 10, 2011 –The concept of the phylotypic stage traces its roots back to early comparative observations of embryos from different vertebrate taxa, in which it was noted that embryonic morphologies appeared to converge on a shared body plan before veering off in specialized directions. This gave rise to a profound debate over the evolutionary basis for this phenomenon; specifically, whether it could best be explained by a “funnel” model, in which the commonality of traits is highest at the earliest stages of embryogenesis, and gradually but unilaterally narrows over time, or an “hourglass” model, where homology is highest at a point later in development as the body plan is being established, and differs more widely before and after.

A new comparative transcriptomic analysis of four vertebrate species conducted by Naoki Irie in the Laboratory for Evolutionary Morphology (Shigeru Kuratani, Group Director) has now revealed that genetic expression is most highly conserved across taxa at the pharyngula stage of development. Published in *Nature Communications*, these latest findings strongly suggest that the hourglass model is the more accurate description of how the vertebrate phylotype manifests.

Irie’s decision to study this question using a gene expression approach broke with the long history of morphological comparisons. He sampled tissue from mouse, chicken, and frog embryos across multiple developmental stages to allow for comparisons of changes in gene expression, and further supplemented this data set with information from previously published transcriptomic studies in a fourth taxa, zebrafish, thus providing representative samples from mammal, bird, amphibian and fish species. He took advantage of the supercomputing capabilities at the RIKEN integrated Cluster of Clusters (RICC) for the processing power needed for comparison of this enormous set of data points.

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

As development proceeds at different paces in different species, and organs likewise emerge at different points, making straightforward comparisons can be extremely problematic. To overcome this challenge, Irie compared gene expression similarities from all combinations of developmental stages among four species using various statistical models. For two species comparisons, he found that the highest similarity was seen in intermediate stages of embryogenesis (from neural to late pharyngula stages). More sophisticated computational analysis with four species pairing analysis revealed that pharyngular embryos had the highest transcriptomic similarity of any stage.



Funnel (left) and hourglass (right) models of changes in commonality and diversity in vertebrate ontogeny.

To ascertain the detailed molecular characteristics of this phylotypic stage, the group went on to identify genes showing conserved expression during the pharyngula stage, but that were not constitutively expressed throughout embryogenesis, and identified 109 gene sets, including *Hox* genes, transcription factors, cell-cell signaling genes, and morphogens. Interestingly, within these sets, developmental genes were more highly represented than in sets with different expression profiles. The data generated in this study has been deposited in the ArrayExpress and Gene Expression Omnibus repositories.

"It seems that the notion that genetic programs underlying early development are resistant to change needs to be reconsidered in light of this data," says Irie. "We'll be interested in working out how early genetic flexibility is achieved while maintaining the robustness of gene expression at the phylotypic pharyngula stage."

Embryos at stages of greatest similarity in gene expression. Clockwise, from top right: mouse (E9.5), chicken (HH16), zebrafish (24 hours), and African clawed frog (stages 28 and 31)

PGL proteins work in germ granule assembly

Masafumi YONETANI, Momoyo (and Shiyori) HANAZAWA-MATSUYAMA

The separation of the germ cells responsible for the transmission of genetic information across generations through reproduction from the somatic tissues that form the rest of the body is a critical event in early development. In many organisms, germ cells are characterized by the inclusion of organelles known generally as germ granules, whose function appears to be linked to germ cell specification and differentiation. In the nematode *C. elegans*, these organelles are historically known as P granules, and are made up of a combination of messenger RNAs and protein components, only some of which have been identified.

Momoyo Hanazawa and Masafumi Yonetani in the Laboratory for Developmental Genomics (Team Leader, Asako Sugimoto; now at Tohoku University) have now sorted out a crucial role for a pair of proteins in germ granule formation. The study, published in the *Journal of Cell Biology*, shows how PGL-1 and -3 self-assemble into scaffolds that recruit ribonucleoprotein components to developing granules.

The team began by developing an assay system to test individual components of P granules for the ability to form granules independent of other granule-associated proteins in a mammalian cell-based system. Of the fourteen proteins tested, only two – PGL-1 and PGL-3 – assembled into granular clusters. To test this capacity in *C. elegans* cells, Hanazawa induced the expression of these PGL proteins in embryonic and adult somatic cells and confirmed the formation of granules in these cellular environments as well, suggesting that these factors are able to self-organize into structure that may help to recruit other P granule components.

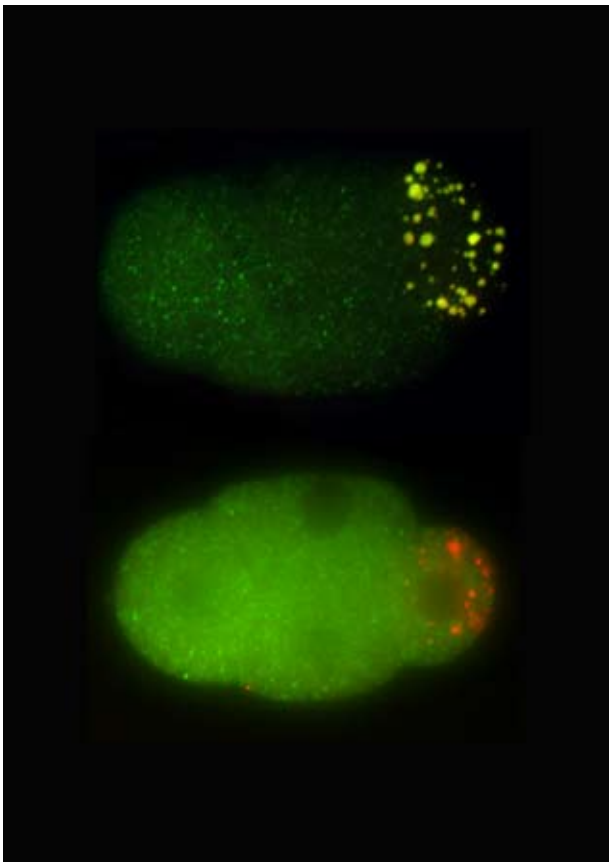
To test this notion, they next checked whether RNAs and proteins co-localized with the PGL complexes. They found that the granules stained positive by a ribonucleic acid-specific dye, and that eight of

Hanazawa M, et al. PGL proteins self associate and bind RNPs to mediate germ granule assembly in C. elegans. *J Cell Biol* 192.929-37 (2011)

12 granule proteins tested also co-expressed with PGL-3. They next used a loss-of-function approach in which they depleted the PGLs from *C. elegans* embryos using mutations or RNAi, and determined that granule formation was greatly reduced in the PGL-deficient animals. This was true for members of the GLH family (homologs of VASA in fruit fly), which typically co-localize with PGL granules in germ cells as well. Analysis of cells in which both GLH-1 and -4 were knocked down below the detection threshold showed that while PGL proteins were still able to self-aggregate, their GLH partners were required for maintaining the multicomponent granular structures.

Yonetani and Hanazawa next performed a structural analysis of PGL-3 to identify possible functional domains. They found two domains are essential to form germ granules: one is an RNA binding domain in the protein's C-terminal, which appeared to be essential for the capture and incorporation of RNAs to granules, and the other a self-association domain in the N-terminal, which is essential for forming globular granules. These findings suggest that the self-association of RNA binding proteins, such as PGL proteins, is crucial for forming germ granules containing diverse RNA and proteins.

Sugimoto notes, "There are many RNA-and-protein containing granules (ribonucleoprotein granules, or RNP granules) in a cell, which play important roles in gene expressions. Our finding may provide a general mechanism for the formation of RNP granules."



4-cell *C. elegans* embryos. Green: PGL-3, Red: GLH-1. Top: normal PGL-3 proteins form granules with GLH-1 in the germ lineage cell (rightmost cell). Bottom: PGL-3 protein that lacks the self-association domain is dispersed in the cytoplasm of both somatic and germ lineage cells.

Apoptosis accelerates morphogenesis

Ayako ISOMURA, Erina KURANAGA, Aimi TSUKIOKA, Emi MAEKAWA

The process of programmed cell death, known as apoptosis, plays a critical role in many developmental events, such as the sculpting of digits in vertebrate limbs, in which the death of the cells that would otherwise form inter-digital webbing enables individual fingers and toes to be formed. The importance of the removal of cells in such contexts is well known, but in recent years, we have begun to learn more about additional roles for apoptosis in the formation of tissues and organs.

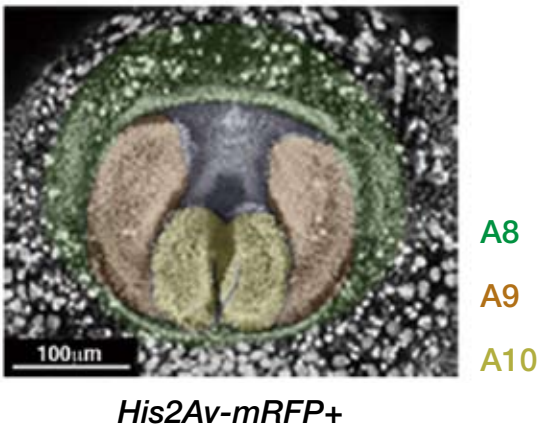
A report by Erina Kuranaga, Team Leader of the Laboratory for Histogenetic Dynamics, and colleagues revealed one such novel function for apoptosis in speeding the rotation of ring-like structures in the developing male reproductive system in the fruit fly *Drosophila*, a process that is necessary for its formation. This work, which was initiated at the University of Tokyo, is published in the journal *Development*.

Kuranaga began by analyzing the part played by apoptosis in morphogenetic processes using the *Drosophila* system with its powerful genetics and decades-old store of research findings. In 1930, it was noted that in this fly the male reproductive structure, known as the terminalia, makes a full 360-degree rotation during development. More than half a century later, researchers observed that defects in apoptosis result in incomplete rotation, and consequently reduced reproductive function and shortening of the structural links with the terminalia. In her recent study, Kuranaga brought live imaging technology to bear on observation of the rotational kinetics of this system.

The terminalia originate in the rostral section of the embryo, and comprise a ring-shaped region known as A8 that encircles the two inner regions A9 and A10. Kuranaga used fluorescent proteins to visualize the nuclei of cells in these domains and observe their movements in living embryos. They found that rotation begins approximately 24 hours after the formation of the puparium, and takes around 12 hours to complete a full turn. But when the team blocked the activity of caspases responsible for

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

inducing apoptosis, they found that while this rotation began at the same developmental time point and continued over the same 12-hour period, it failed to complete a 360° spin. To gain a better understanding of how this happened, they quantitatively analyzed the terminalia rotation in developing flies, and determined that in wildtype the process involves four distinct steps: initiation, acceleration, deceleration, and termination. In apoptosis-defective flies, however, the acceleration stage was not maintained, leading to incomplete rotation.

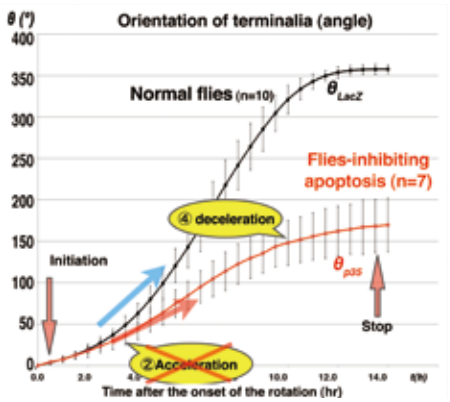


Caudalview of a *Drosophia* male terminalia
(See CDB website for video)

The team next conducted more sophisticated live imaging observations, allowing them to track the behaviors of individual cells. They saw that in the ring-like A8 domain the inner and outer populations of cells behaved differently – the interior cells rotated 360° in synchrony with the cells of the A9 domain, but the outer cells only rotated half that distance, or 180°. Kuranaga also noted that the outer ring cells began their rotation just as the inner cells were entering the acceleration phase, suggesting a link between these events. She also observed for the first time that apoptosis occurs in the A8 domain.

In embryos in which apoptosis was blocked, the inner cells in A8 moved as in wildtype, but the rotation of the outer cells failed to begin. By analyzing the frequency of apoptotic events and speed of rotation of outer cells in wildtype embryos as well, the team was able to show clear correlation between the two. Taken together, the findings suggest that apoptosis is required for the rotation of the outer ring, which in turn drives the acceleration of the inner ring of the A8 domain. And indeed, upregulation of the pro-apoptotic pathway resulted in faster rotation of the terminalia.

"The outer ring seems to function as a kind of moving walkway," says Kuranaga. "When the inner ring steps onto it, its speed increases, allowing it to complete its rotation within the normal period. We are still working out how might apoptosis drive this, but suspect it may be related to the relief of some mechanical stress generated by cellular movements."



Comparison of the speed of genitalia rotation between normal flies (black) and flies-inhibiting apoptosis (red)

Mitochondria shape cell morphology

Michiko KOIZUMI, Tatsuhiko NOGUCHI

Animals employ a surprising array of tricks to ensure their reproductive chances, and successful innovations, such as the feathers in a male peacock's tail, can drive evolutionary change. In another remarkable example, some species of *Drosophila* develop sperm as long as 6 cm, around 30 times the length of the fly itself. Such elongated sperm are more successful in reaching eggs, resulting in the genes of their possessors being evolved through natural selection. But how are such dramatic cellular morphologies achieved?

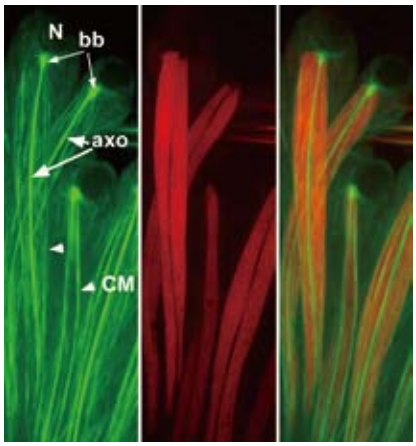
Tatsuhiko Noguchi and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) have now shown in the fruit fly, *Drosophila melanogaster*, how mitochondria play a critical role in sperm elongation in this species. The report, published in *Current Biology*, reveals that interactions between giant mitochondria and microtubules underlie and maintain the sperm's extraordinary stretch.

Drosophila sperm cells undergo changes in shape during their maturation that leave them 200 times longer without any increase in volume. Four primary structures run along the sperm's longitudinal axis: the flagellar axoneme, cytoplasmic microtubules, giant mitochondria, and cables of F-actin. It has previously been shown that the axoneme is not required for sperm elongation, but the role of the other components has remained unknown. In their recent work, Noguchi et al. extracted intact sperm cells and maintained them in culture, allowing them to apply live imaging techniques to get to the bottom of these questions.

The group began by chemically interfering with actin and microtubule function, which revealed that while actin was dispensable, microtubules were necessary for sperm tail elongation. They followed up by inhibiting microtubule growth in a localized fashion, and found indications that the sperm tail end is the focus of elongation activity. They looked next at the role of mitochondria. Giant mitochondria form from the fusion of multiple smaller mitochondria, but Noguchi found that when he inhibited this fusion, sperm elongation was markedly reduced. Even more interestingly, the crucial property of these organelles was shown to be their length and shape, rather than their better-known energy-generating respiratory activity.

Noguchi T, et al. Sustained Elongation of Sperm Tail Promoted by Local Remodeling of Giant Mitochondria in Drosophila. *Curr Biol* 21,805-814 (2011)

The discovery that both microtubules and mitochondria are essential for sperm elongation naturally suggested the next experiment, in which the group asked if they act on each other. When microtubules were ablated in the absence of axoneme, giant mitochondria reverted from their elongated form to a rounded shape. When mitochondrial fusion was inhibited, microtubules assembled preferentially on fragmented mitochondria. Electron microscopic imaging further revealed structures cross-linking both between microtubules and between microtubules and mitochondria.

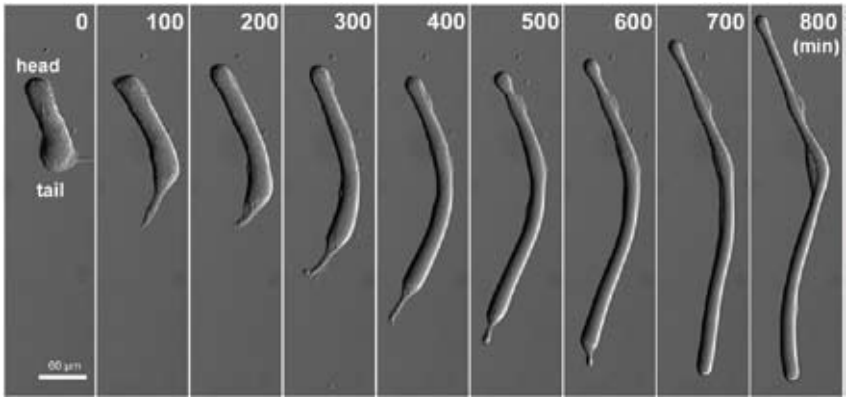


Sperm cells undergoing elongation. Microtubule (green) and mitochondria (red) are labeled. N: nucleus, bb: basal body, axo: axoneme, CM: cytoplasmic microtubule.

In loss-of-function mutants for Milton, a protein cross-linking mitochondria to microtubule motor kinesin, this sliding action, and consequently sperm elongation, were lost, suggesting a role for this motor protein in the process. Further experiments in which microtubules were transiently depolymerized and allowed to regrow indicated that these cytoskeletal structures use the mitochondrial surface as a substrate for growth.

Given these findings, the group proposes that sperm elongation plays out as follows. First, microtubules form at the surface of giant mitochondria in the sperm tail, after which microtubules pair up by cross-linking and slide along the mitochondrial surface, causing them to lengthen and thin, creating a foothold for more microtubules to accumulate and continue the elongation process, as well as to stabilize the structure.

These results represent the first finding that mitochondria can contribute to cell morphogenesis. “The discovery that the mitochondria that occupy our cells can act not only as cellular power plants, but in germ line development as well is intriguing,” says Hayashi. “Mitochondria have a strong bi-layered membrane, and can additionally provide the energy needed for flagellar motion, so the fact that they are used as a scaffold in this context makes perfect sense.”



Snap shot view of spermatid elongation

Willin and Par3 work together in apical constriction

Takashi (and Yuri) ISHIUCHI

Epithelial cells often take the shape of truncated wedges, with an apical surface narrower than the basal face. This form of cellular polarization plays a central role in morphogenetic processes in which sheets of homogeneous tissue bend, fold over, or roll up due to minute changes in the geometry of their component cells. The primary connections between cells in such epithelial sheets are known as apical junctional complexes (AJCs), which both bind cells of like type to their neighbors and engage with the cytoskeleton elements called actomyosin cables to modify and reinforce cell shapes and behaviors. The contractility of such cables is under the control of a family of kinases known as ROCK proteins, which form complexes near AJCs and enhance actomyosin contraction through phosphorylation of myosin light chain. But how ROCK interacts with factors involved in cell polarity remains something of a mystery.

Takashi Ishiuchi in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) has begun to tie the threads together with the discovery that a previously enigmatic protein called Willin works cooperatively with the known polarity protein Par3 to phosphorylate ROCK, a critical trigger of apical constriction. Published in *Nature Cell Biology*, this new work shed light on the complex regulation of this important cellular morphogenetic process.

The study began with an examination of Willin's localization, which revealed that it clusters near the apical junction. A subsequent immunoprecipitation assay turned up an interaction with Par3, catching the group's attention, due to Par proteins' involvement in cell polarization. They tested whether expression of Willin would affect Par3 localization, and found that while this had no effect, it did increase the accumulation of Par3 partner aPKC at the cell membrane. A second round of immunoprecipitation showed that Willin associates with aPKC as well, which deletion mutant studies revealed to be dependent on a region in the Willin protein known as JFR.

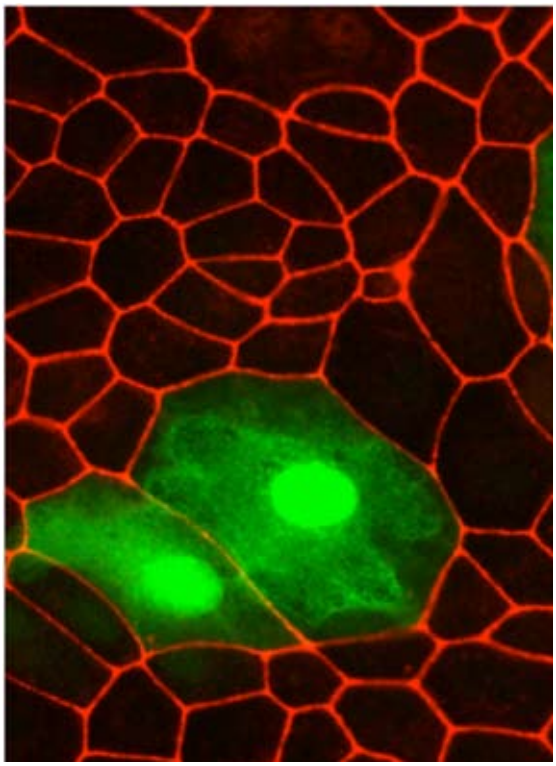
Ishiuchi T. and Takeichi M. Willin and Par3 cooperatively regulate epithelial apical constriction through aPKC-mediated ROCK phosphorylation. *Nat Cell Biol* 13.860-6 (2011)

As Willin expression had no measurable effect on Par3 levels, Ishiuchi tried the converse experiment and showed that Par3 is not required for Willin's upregulation of aPKC. Willin was also not required for the Par3-aPKC association, indicating that while Willin could interact with both of these proteins, it appeared to do so by independent mechanisms.

Curious about Willin's true role, the group knocked down the function of both Willin and Par3 in cells, and found that this resulted in tightening of the apical junctional complex, secondary to a dramatic reduction in the localization of aPKC near the membrane. It appeared that aPKC was the critical downstream factor for limiting apical constriction. This was confirmed by experiments using a membrane-targeted aPKC construct, which was able to rescue excessive actomyosin contractility.

But how does aPKC achieve this effect? Ishiuchi had observed that ROCK inhibition abrogated apical constriction, so he turned his focus to possible interactions between the two factors, with an intuition that this activity might rely on phosphorylating activity by aPKC. On treating ROCK1 with a phosphatase, he found that this caused a change in its molecular mass, suggesting that the protein is indeed phosphorylated in vivo, which the group was then able to verify using a system to detect phosphorylation by band-shift. Analysis of the distribution of wildtype and phosphorylated ROCK proteins revealed that while wildtype ROCKs tended to aggregate at the apical junction, when phosphorylated they remained in the cytoplasm.

"The Par3-aPKC complex is known to regulate the apico-basal polarity of cells, and Willin is thought to be a relative of the *Drosophila* protein Expanded, a component of the Hippo pathway for growth control," says Takeichi. "It will be interesting to work out how the ability of these proteins to regulate actomyosin contraction at AJCs is related to their other physiological functions in future studies."



Apical constriction is lost on knockdown of ROCK in cells lacking Par3 and Willin(red: Z0-1, green: cells expressing ROCK shRNA)

2011 Courses

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

Training in multicolor FISH technqiues for scientists

The Center organized a course on multicolor fluorescence in situ hybridization (FISH) for the nuclear analysis of human stem cells on February 3 and 4, providing instruction in techniques for the detection of chromosomal abnormalities that can appear in ES and iPS cells when cultured for long periods. Twenty participants from academia, industry and other research institutes joined the course, which was organized under the auspices of the MEXT Project for the Realization of Regenerative Medicine and support from Carl Zeiss MicroImaging and Abbot Japan.

Developmental biology over summer break

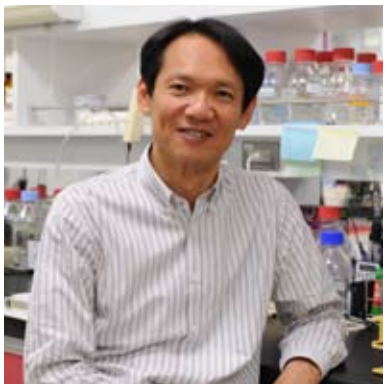
The CDB held its annual summer school for local high school students on August 2 and 4. The one-day courses featured lectures, lab visits, and hands-on experiments on fluorescent labeling. Unit Leader Shigenobu Yonemura (Electron Microscope Laboratory) opened the day with a lecture on how cells respond to tissue damage, and his work on studying the mechanisms of wound healing in vitro. The lab practicum began with a demonstration of how to use a micropipette, followed by the generation of fluorescent staining solutions, which the class used to label tubulin red and DNA blue. Once the cells had been stained, the students watched their creations under a fluorescence microscope, enabling them to observe phenomena such as cell divisions.

Course in development for local HS teachers

The recurrent course in developmental biology for teachers of high school biology, organized by the Office for Research Communications (ORC), was held at the CDB on October 1 and 2, 2011. This course is held annually in the fall in conjunction with the Japanese Society of Developmental Biologists, and gives the teachers an opportunity to learn hands-on techniques in embryology. The program featured a lecture by Prof. Sadao Yasugi of Kyoto Sangyo University on the history of embryology studies in chicken and on the molecular biological mechanism of cell differentiation, followed by a two-day training course in which the teachers learned how to extract, culture, and observe chick embryos under the microscope.

Chick embryo at day E6, showing extensive extra-embryonic vascular network.

Cell Asymmetry



Fumio MATSUZAKI Ph.D.

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

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. 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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Publications

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

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

Wang C, et al. An ana2/ctp/mud complex regulates spindle orientation in Drosophila neuroblasts. *Dev Cell* 21.520-33 (2011)

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

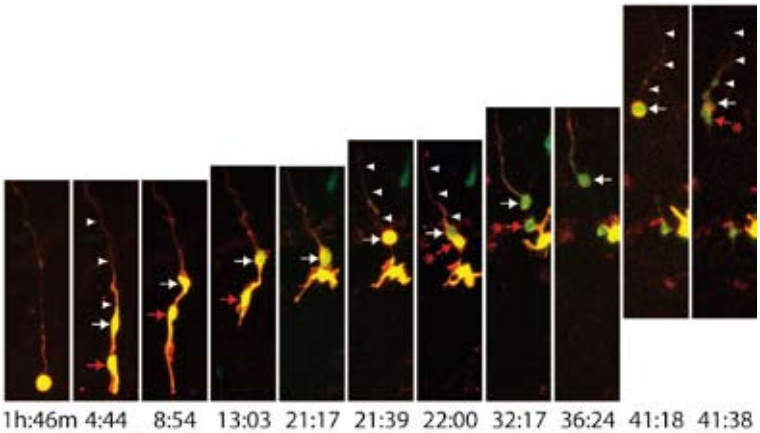
Kitajima A, et al. Progenitor properties of symmetrically dividing Drosophila neuroblasts during embryonic and larval development. *Dev Biol* 347. 9-23 (2010)

Kato T. M, et al. Lunatic fringe potentiates Notch signaling in the developing brain. *Mol Cell Neurosci* 45.12-25 (2010)

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

Drosophila neural stem cells, called neuroblasts, provide an excellent model system for the investigation of fundamental aspects of asymmetric division, a process essential to the generation of cells of divergent type during proliferation. We have been investigating mechanisms controlling asymmetric divisions, including the cell polarity and spindle orientation. We also extend our research scope to understand how neurogenesis is controlled in tissue space depending on the environments that surround the nervous system. We recently identified an extrinsic mechanism that controls the orientation of division (cell polarity) in neuroblasts relative to the overlying ectoderm (Yoshiura et al., 2011), which determines the orientation of neural tissue growth.

The vertebrate brain evolved rapidly resulting in an expansion of the size of the brain, which comprises a larger number of neurons arranged in a vastly more complex functional network than that in invertebrate. Neural stem cells typically adopt three states – proliferative (symmetrically dividing), neurogenic (asymmetrically dividing), and resting – and undergo transitions among the states, on which the size, complexity and basic organization of the brain depend. We investigate mechanisms that determine the individual states of neural stem cells, and control transitions between states in mouse. We recently discovered a novel transition in the developing mouse cortex from radial glia (typical neural stem cells with the epithelial structure) to another type of neural stem cell, basal radial glia (Shitamukai et al., 2011 and see figure), which is known as a major population of neural stem cells in mammals with large brains, such as primates. We are investigating the mechanisms that underlie the formation, maintenance, and expansion of this type of stem cells, by developing model mice that produce large numbers of basal radial glia. We have also revealed a principle underlying the dynamic behavior (interkinetic nuclear migration) of populations of neural stem cells (Kosodo et al., 2011).



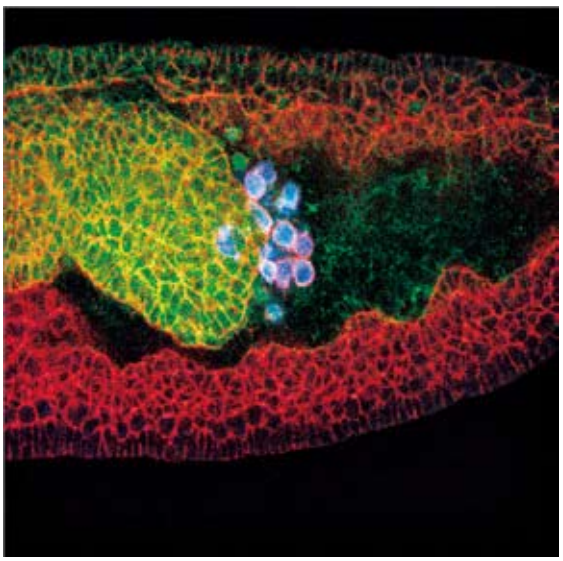
In the developing mouse brain, neural stem cells normally maintain epithelial structure during asymmetric division. Oblique cleavages occur stochastically and generate a different type of self-renewing neurogenic progenitors (white arrows) outside of the germinal zone. Divisions occur at 1h:46min, 21:39,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

Germline Development

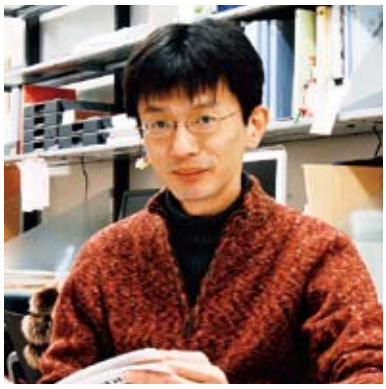
Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the *Drosophila* fruit fly, the formation and differentiation of germ cells is controlled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

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

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



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



Akira NAKAMURA Ph.D.

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

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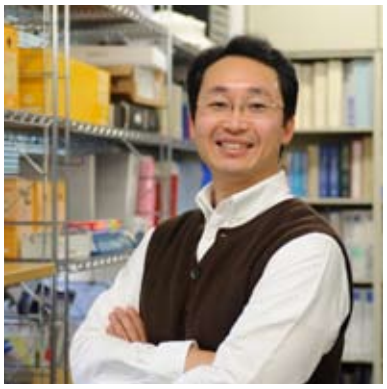
Chromatin Dynamics



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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Hiragami-Hamada K, et al. N-terminal phosphorylation of HP1 promotes its chromatin binding. *Mol Cell Biol* 31. 1186-200 (2011)

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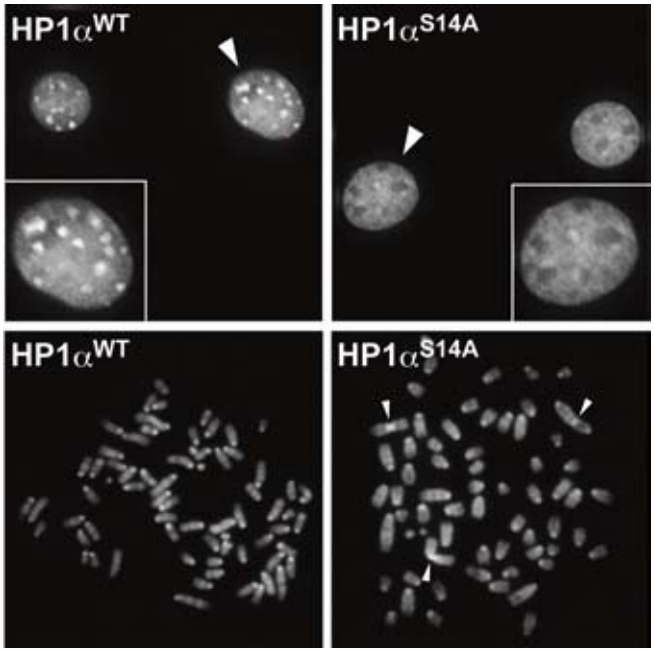
Multicellular organisms are made up of diverse populations of many different types of cells, each of which contains an identical set of genetic information coded in its DNA. Cell differentiation and the process of development itself depend on the ability of individual cells to maintain the expression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

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

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

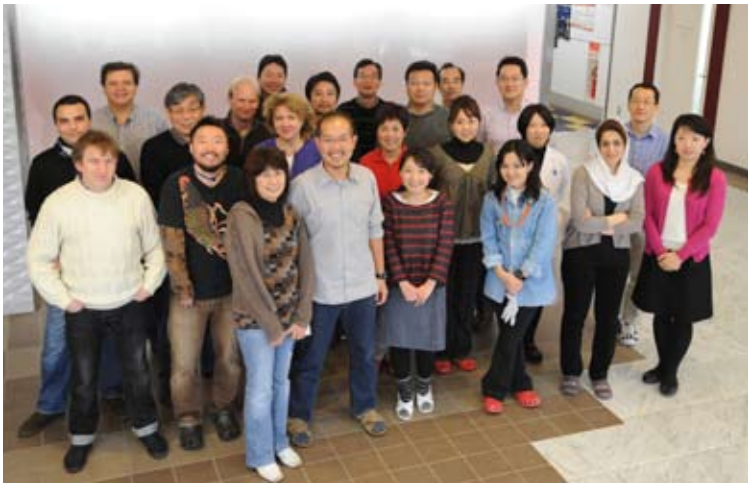


Disruption of HP1α N-terminal phosphorylation leads to defects in its heterochromatic localization (upper panels) and increased chromosomal instability (lower panels).

Stem Cell Biology

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

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



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

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Ban H, et al. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proc Natl Acad Sci U S A* 108.14234-9 (2011)

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Growth Control Signaling

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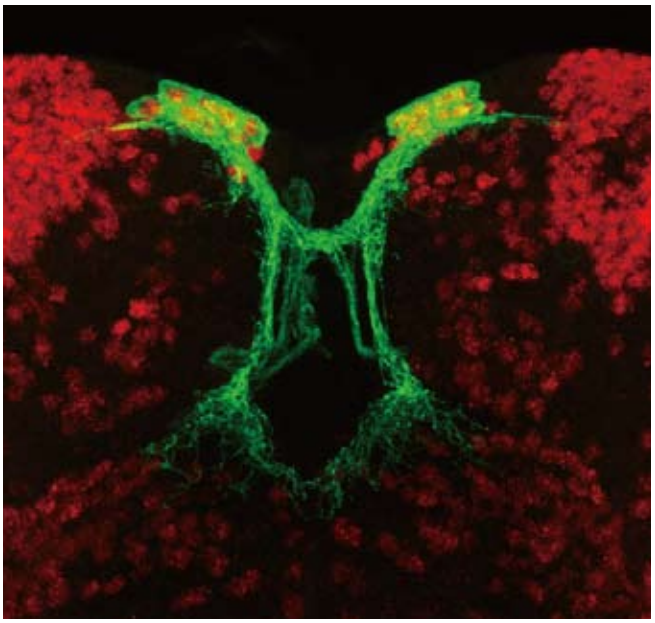
The processes of animal development, including organ size and body size, are genetically predetermined, but these processes are also influenced by environmental factors such as nutrition and temperature. The close link between cell and tissue growth control and environmental cues ensures that developmental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



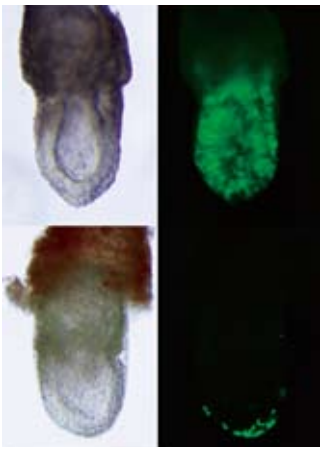
Cluster of neurosecretory cells in the larval brain called insulin-producing cells of the fruit fly *Drosophila melanogaster*, showing expression of *Drosophila* insulin-like peptide (Green) and nuclear protein Dachshund (Red).

Pluripotent Stem Cell Studies

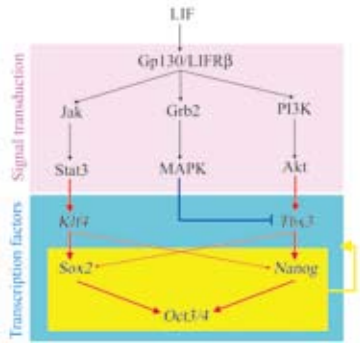
Pluripotency is a term used to describe the ability of certain types of stem cells that are capable of giving rise to at least one type of cell from all three germ layers – endoderm, mesoderm and ectoderm. Stem cells are also characterized by their ability to produce copies of themselves with similar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

Pluripotency can be primarily determined by a particular set of transcription factors, for as we now know, pluripotency can be induced by 4 transcription factors. These transcription factors should form a self-organizing network able to stabilize their expression and maintain pluripotency. At the same time, the network should have the sensitivity to signals that induce differentiation. How can the transcription factor network satisfy these contradictory requirements? What is the principle behind the structure of the transcription factor networks governing developmental processes? We are now trying to answer these questions by studying ES cells and the changes they undergo in their differentiation toward trophoblast stem cells, extraembryonic endoderm cells, and primitive ectoderm cells.

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



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



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

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

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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Mammalian Epigenetic Studies

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

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

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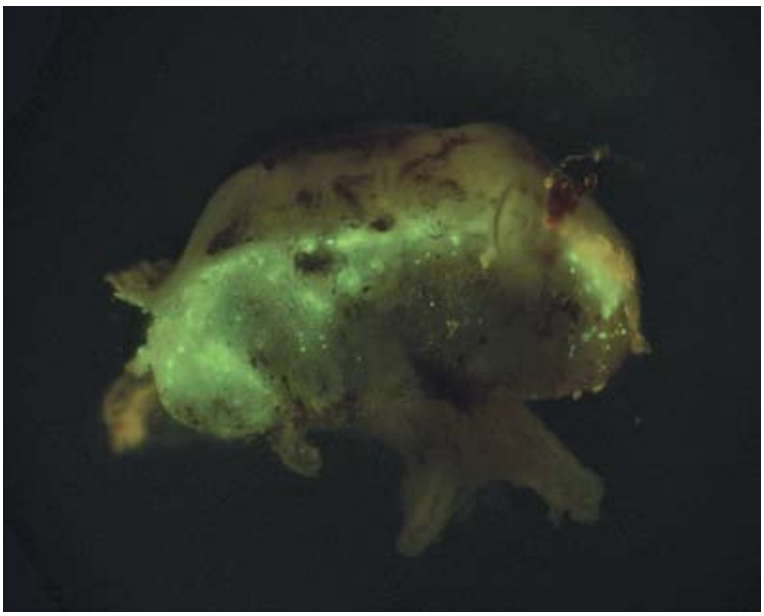
Oda M, et al. DNA methylation regulates long-range gene silencing of an X-linked homeobox gene cluster in a lineage-specific manner. *Genes Dev* 20. 3382-94 (2006)

Tsumura A, et al. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. *Genes Cells* 11. 805-14 (2006)

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

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



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

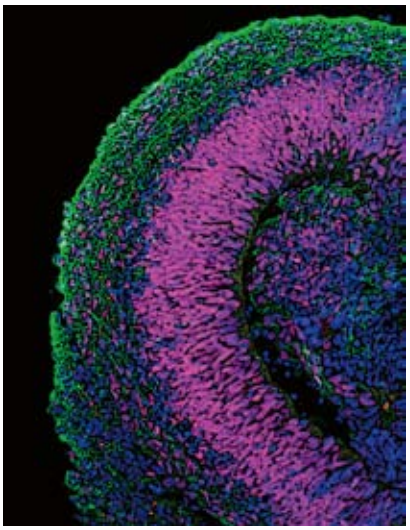
Organogenesis and Neurogenesis

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

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

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

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



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

Yoshiki SASAI M.D., Ph.D.

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

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Publications

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

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

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

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

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

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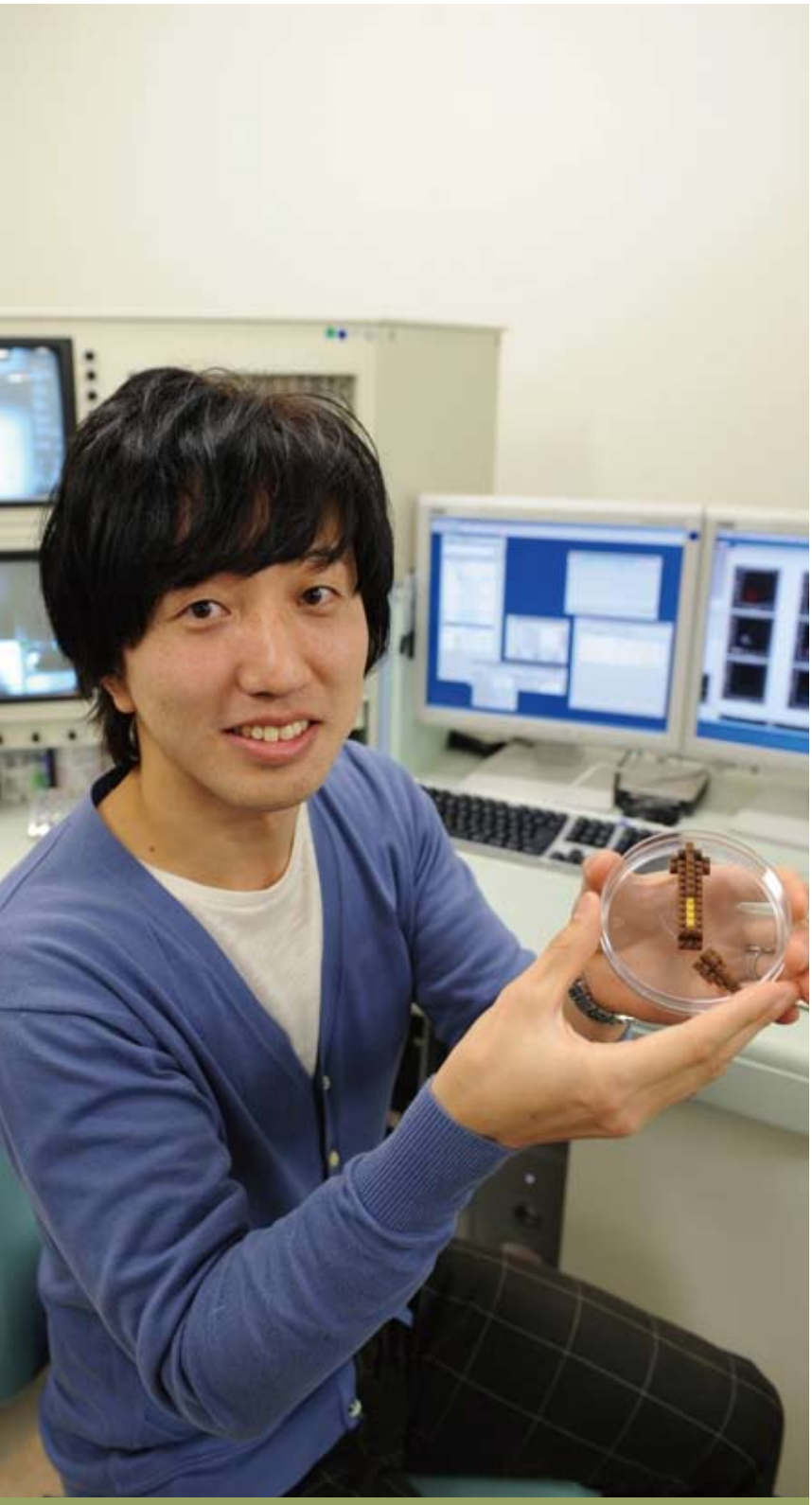
Planarian *Islet* homolog works in posterior differentiation



Tetsutaro HAYASHI

Planarians are a group of small flatworms with significant capacity for regeneration, which enables them to reform entire bodies from individual amputated fragments. To do so, however, each fragment needs to have a sense of where it should be in the body, so that missing head and tail regions form as needed and in the appropriate location with respect to the rest of the regenerating body. This positional information is thought to be conferred by gradients in the expression of various genes that activate, modulate or inhibit cell differentiation. The Wnt signaling pathway, for example, is known to be necessary for the specification of posterior regions. The molecular mechanisms that underlie this function, however, are poorly understood.

A new study by Tetsutaro Hayashi and others in the Genome Resource and Analysis Unit (Fumio Matsuzaki, Unit Leader) now reveals an additional factor, encoded by a planarian LIM- homeobox gene known as *Djislet*, is required for the posterior differentiation of Wnt-expressing cells. Published in Development, this reports yields new insights into the coordination that occurs between stem and differentiated cells in an important model of animal regeneration.

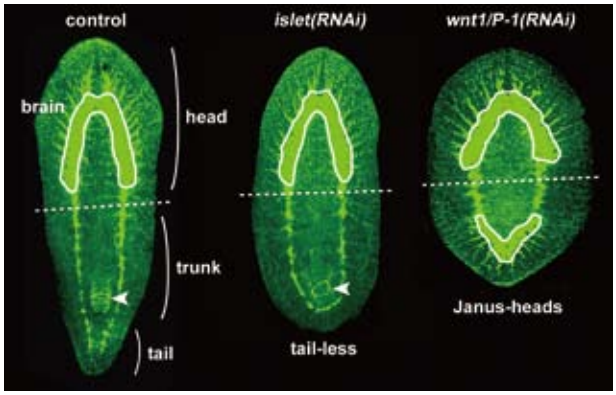
The study began with the isolation of an apparent homolog of the vertebrate gene Islet from an EST (expressed sequence tag) library for the planarian species *Dugesia japonica*, which is indigenous to Japan. In vertebrates, the Islet family of LIM-homeobox transcription factors is known to be involved in the proliferation, maintenance and migration of stem and progenitor cells in a range of tissues. When Hayashi knocked down the function of this gene, dubbed *Djislet*, using RNA interference (RNAi), he found that its loss of function resulted in a tailless regeneration phenotype in fragments from any part of the body (head or trunk), suggesting that the gene might function in posterior differentiation and regeneration.

This suggested in turn the possibility of a functional link to the Wnt signaling pathway, which had previously been shown to be required for posterior specification and regeneration in another flatworm species, *Schmidtea mediterranea*. Analysis of the expression of

Hayashi T, et al. A LIM-homeobox gene is required for differentiation of Wnt-expressing cells at the posterior end of the planarian body. *Development* 138.3679-88 (2011)

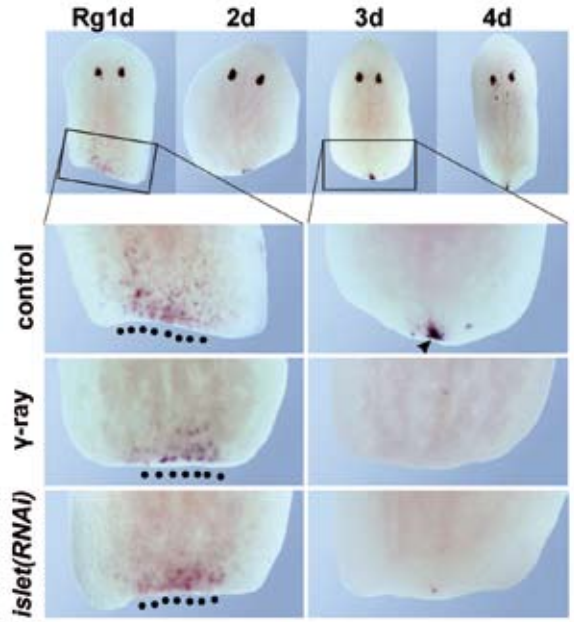
these genes, *Djislet* and *Djwnt1/P-1*, showed co-localization at the midline of the blastema, the locus of regeneration in planarians, at day 3 of regeneration. Moreover, in *Djislet(RNAi)* worms, the expression of *Djwnt1/P-1* disappeared from the posterior blastema, suggesting that this gene functions downstream of the Islet function.

Interestingly, the loss of Islet function had a more limited effect than that of Wnt, in that *Djwnt1/P-1(RNAi)* worms show both tailless regeneration and occasional formation of two-headed regenerants (known as the Janus-heads phenotype), while *Djislet(RNAi)* animals regenerated without tails, but never with two heads. Examining gene expression in the process of *Dugesia* regeneration more closely, the lab found Wnt is expressed in two phases, at days 1 and 3, with the Islet homolog required only for the second. Importantly, this second phase is senstitive to γ-ray irradiation, suggesting that it involves stem cell-derived cells.



Djislet(RNAi) animals show tail-less regeneration phenotype. *Djwnt1/P-1(RNAi)* animals show two-headed regenerants (Janus-heads phenotype). Arrowhead, pharyngeal neuron. Dashed lines indicate the amputation site.

Hayashi next checked the effect of *Djislet*(RNAi) on a panel of genes known to function in the posterior blastema, and was able to groups these into three classes: Class I, which includes genes expressed in γ-ray-insensitive cells not regulated by *Djislet*; Class II genes which are partly downregulated by loss of *Djislet* function; and Class III, which are mainly expressed in γ-ray-sensitive cells and strongly downregulated by *Djislet(RNAi)* at day 3 of regeneration. These findings collectively point to a role for Islet via Wnt signaling in the maintenance and activation of posterior genes in differentiating blastema cells for tail formation.



The expression patterns of *Djwnt1/P-1* mRNA in the head regenerants during posterior regeneration (from 1 to 4 days after posterior amputation). The expression of *Djwnt1/P-1* is eliminated by *Djislet(RNAi)* and γ-ray irradiation at day 3, but not day 1.

Hagfish show some backbone

Kinya G. OTA

The earliest roots of the phylum Vertebrata trace back more than 500 million years, to the appearance of the body plan characterized by the vertebrae for which it is named. Hagfish, cartilaginous jawless fishes that lack vertebrae, have thus sometimes been excluded from the vertebrate ranks, despite sharing many characters with other members of the taxon. In such schemes, the hagfish are technically invertebrates, but are classed together with vertebrates as 'craniates,' referring to their well-developed crania. The debate about hagfish's true taxonomic place has raged for more than a century, but is their lack of vertebral elements enough to leave them an evolutionary odd man out?

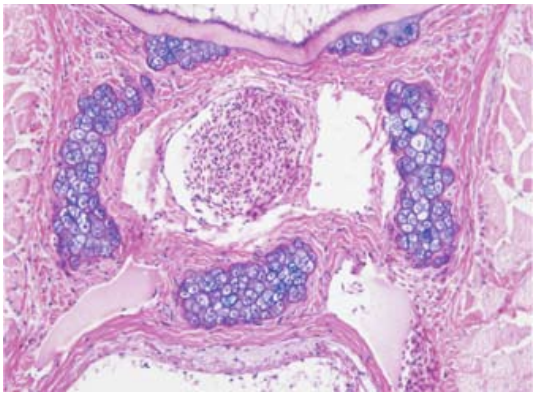
Vertebra-like cartilaginous nodules had in fact been found in hagfish over a century ago, but the existence of these has been neglected in the decades since that discovery. Kinya G. Ota and colleagues in the Laboratory for Evolutionary Morphogenesis (Shigeru Kuratani, Group Director) have now conclusively shown the presence of these elements and analyzed their anatomical features as genuine vertebrae in the Japanese in-shore hagfish, *Eptatreus burgeri*. They further identified true vertebra-like traits in embryonic hagfish developmental patterns and processes, although these are for the most part lost in the adult. Reporting in *Nature Communications*, the group suggests that the hagfish are, along with the lampreys, indeed true members of the vertebrate cyclostomes.

Ota's work was enabled by the lab's access to hagfish embryos for anatomical and histological analyses, something that only became possible when the Kuratani lab succeeded in breeding the animals in captivity several years ago. Building on this achievement by developing a long-term incubation system, Ota gained access to a sufficiently large number of fertilized eggs to investigate their development at multiple stages.

The early pharyngula-stage hagfish was found to develop just under 80 somites, the repeating structures along the anterior-posterior axis of the embryo that in most vertebrates serve as the primordia for many of the bones and

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

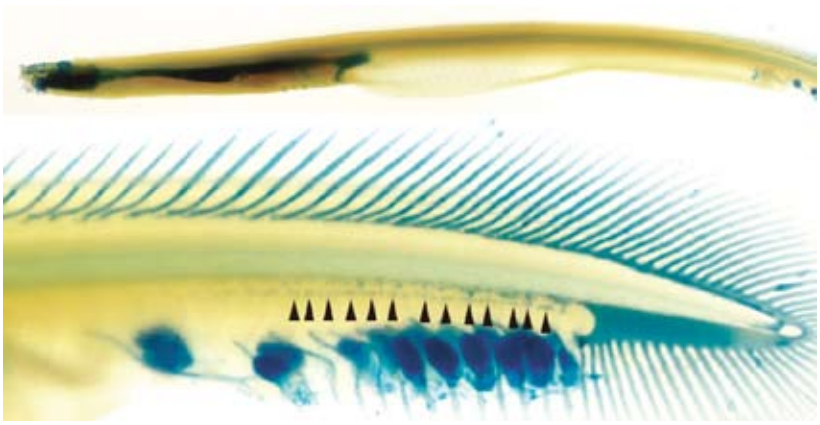
skeletal muscles in the trunk. Looking at gene expression in the somatic region of the trunk, Ota noted similarities in the expression of characteristic genes in patterns corresponding to those of jawed (gnathostome) vertebrates. Specifically, hagfish *Twist* and *Pax1/9* were expressed in segmentally arranged ventral mesenchyme derived from the medial part of epithelial somites, while *Pax3/7* and *MyoD* were respectively switched on dermomyotome and myotome, respectively, indicating that this stage of hagfish somitogenesis follows a similar routine for the formation of somite-derived muscle and skin to that in later vertebrates.



Histological view of the cartilaginous vertebra of the hagfish

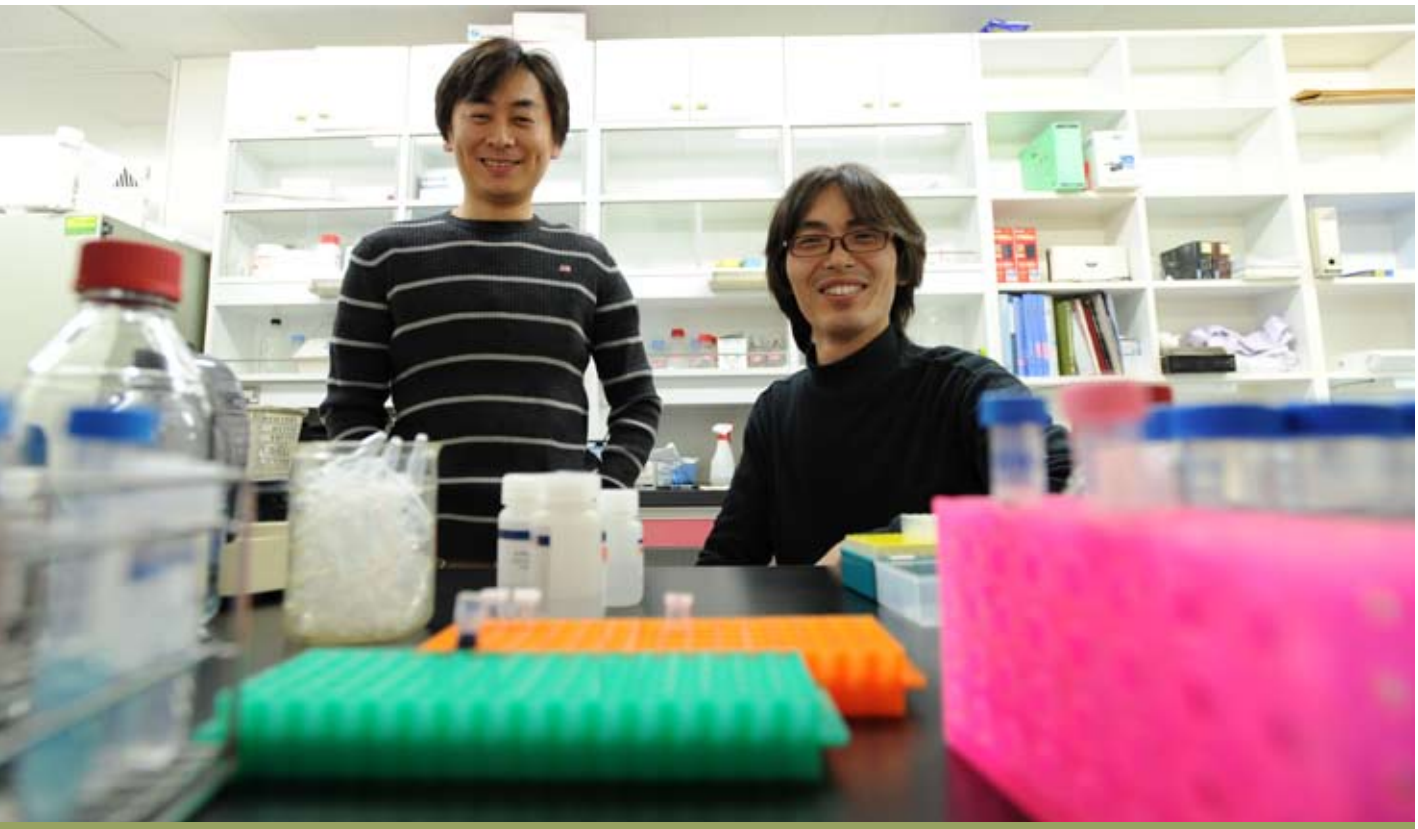
By the late pharyngula stage in hagfish, an additional dozen or so somites have formed, including those that give rise to vertebra-like elements at around somite 90. Ota found that the *Pax1/9*-expressing cells at this stage had become more broadly distributed, suggesting that some had migrated further ventrally, and might thus correspond to the sclerotome in later vertebrates, which is the somitic component that gives rise to the vertebrae.

Given these similarities, the group surmised that hagfish somites indeed form both dermomyotome and sclerotome, indicating homology with other vertebrates. And indeed close examination of the adult anatomy yielded cartilaginous axial elements to either side of the dorsal aorta that may represent the hagfish's vertebra-like equivalent. Although these cartilages lack the metameric quality of true vertebrae, their location and arrangement within the body are reminiscent of ventral components of gnathostome vertebrae known as the neural spine and neural arches.



Whole-mount stained adult hagfish showing tail section with cartilaginous nodules beneath the notochord

Out of the ventricular zone: OVZ cells arise from basal inheritance



Daijiro KONNO, Atsunori SHITAMUKAI

The developing mammalian brain is characterized by rapid proliferation and differentiation, needed to generate the diversity of neural cells and tissues that make up the adult organ. During this stage, one cellular population known as radial glia cells plays the part of neural stem cells, capable of both self-renewal and the generation of differentiated progeny. These cells feature processes at both their apical and basal ends, which are allocated asymmetrically to daughter cells in a context-dependent manner, but the relationship between such segregation and the ability to self-renew remains poorly understood.

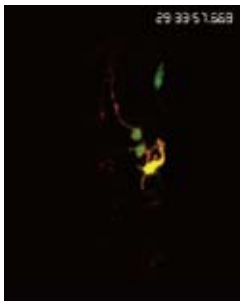
New research by Atsunori Shitamukai and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) shed new light on the angle of cell division as a crucial determinant of daughter cell fate, with progeny produced by oblique divisions assuming a new combination of differential fates depending on whether they inherit the apical or basal process. Published in *The Journal of Neuroscience*, these findings are in strong agreement with previous work in the primate nervous system, suggesting that the mouse can serve as a useful model for more detailed studies of this neurodevelopmental process.

The group began by observing the fates of daughter cells from apical and basal progenitors, using an electroporation system previously developed by the lab, which enabled them to distinguish between daughter cell types they called apical and other progenitors, after the location at which their subsequent cell division occurs. Apical progenitors, or radial glia cells, divide at the apical surface after interkinetic nuclear migration (INM) and form the apical junction, while basal progenitors divide a single time in the subventricular zone (SVZ), giving rise to a pair of neurons. The group tested whether the fates of daughter cells in such divisions were dependent on the size of the inherited apical membrane, as had been suggested by some previous studies, but found that in this context the question of self-renewal vs. differentiation was unaffected by differences in inherited domain size.

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

Shitamukai next examined whether the inheritance of the basal process played a role. All apical progenitors divided asymmetrically with regard to this structure, meaning only a single daughter inherits it. By monitoring subsequent changes in the morphology and behavior of daughter cells that had or had not inherited basal process, they were able to determine that those cells that inherited a process often maintained their ability to self-renew, becoming either apical or nonsurface progenitors; daughter cells that did not inherit the basal process showed no such proliferation.
Expression of genetic indicators of Notch activation, a hallmark of self-renewal ability in these cells, again showed that inheritance of the basal process is associated with the generation of self-renewing progenitor cells.

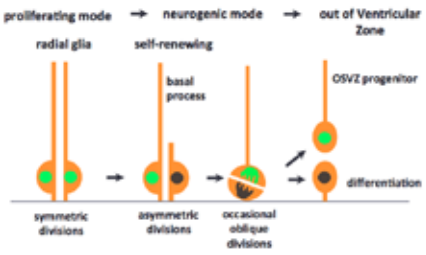
Shitamukai et al. then employed a strategy previously developed by the Matsuzaki lab for genetically perturbing the orientation of the cleavage plane, to examine the respective roles of the apical and basal domains on daughter cell fate. They found that such misorientation at apical divisions induces progenitors outside the ventricular zone (outer VZ progenitors) that retain the basal process and, unlike basal progenitors, are capable of self-renewal. Analysis of marker genes for differentiation and self-renewal further supported these findings.



In the developing mouse brain, neural stem cells normally maintain epithelial structure during asymmetric division. Oblique cleavages occur stochastically and generate a different type of self-renewing neurogenic progenitors (white arrows) outside of the germinal zone.
(See CDB website for video)

Previous work by the lab had shown that even in normal mouse brain slices, there are occasional progenitor cell divisions in which only a single daughter inherits the apical domain. To determine whether these basal-only daughters in wildtype slices become outer VZ progenitors as well, the group observed unmodified both live brain slices and fixed brain sections and found that, in this context as well, there is a small population of outer VZ progenitors that retain the basal process and proliferative capacity. Interestingly, the survival of these progenitor cells depends on Notch signaling with their own progeny cells.

Neural progenitor cells that are equivalent to outer VZ progenitors, known as either outer subventricular zone (OSVZ) progenitor or outer glial cells, have been identified in the developing brain of gyrencephalic mammals, including ferrets and primates. In primates in particular, these OSVZ progenitors predominate among self-renewing neural progenitor cells from mid-neurogenic stages onwards, and generate most of the neurons in the upper layers of the neocortex. Therefore, the appearance of OSVZ progenitor cells has been thought to be important in the evolution of the brain, as a cause of the expansion in brain size and the consequent emergence of brain gyration in these species. Some have speculated that rodents, which are agyrencephalic, might lack OSVZ progenitors as well. Shitamukai et al., however, have shown that the OSVZ type of progenitor cells had already evolved in the rodent taxa, although their population is smaller than that of radial glial cells. The group also proposes how OSVZ progenitor cells are generated in primates, suggesting that they may be born from radial gilal cells by oblique apical divisions in which one daughter cell loses the apical domain, as happens in mice.



Scheme showing transitions in neural stem cell modes. The first transition is from symmetric to asymmetric division, while the second is from radial glia (apical progenitor) to OVZ progenitor.

Matsuzaki notes, “A number of human hereditary diseases are thought to be caused by abnormal behaviors of neural progenitors. Some of these may be due to abnormalities in OSVZ progenitor cells, which would have made them difficult to study experimentally, as this is a minor population in mice. Our LGN mutant mice generate a number of the OSVZ progenitors comparable to that of radial glial cells, which makes it possible to analyze some properties and genetic defects of the OSVZ progenitors using this mouse strain as a model.”

Dual role for AIR-1 in mitotic spindle assembly

Mika TOYA

Mitotic cell division involves a critical process in which replicated chromosomes separated by spindle fibers are pulled apart into separate daughter cells. In the roundworm *C. elegans*, such mitotic spindles, comprising microtubules and associated proteins, are assembled through both γ-tubulin-dependent and -independent processes. Previous studies have shown that γ-tubulin-independent microtubule assembly relies on the function of AIR-1, the *C. elegans* homolog of Aurora A kinase, a key regulator of numerous mitotic events. But just how AIR-1 works in this aspect of microtubule assembly remains unclear.

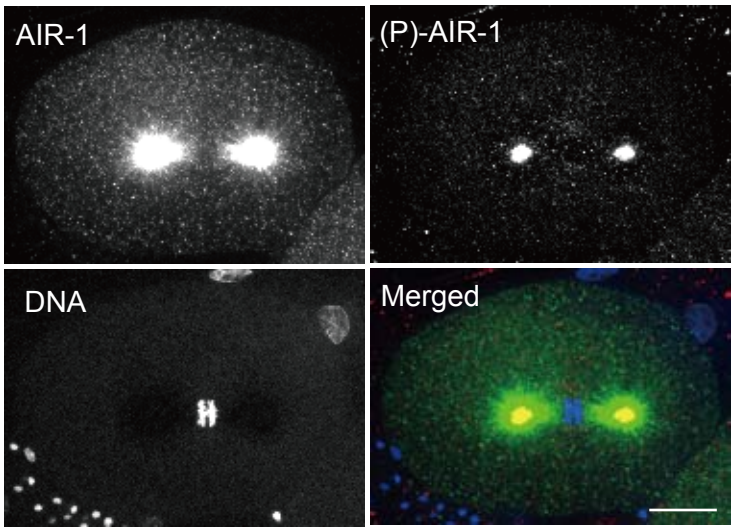
Mika Toya and others in the Laboratory for Developmental Genomics (Asako Sugimoto, Team Leader) have succeeded in deciphering AIR-1's role. In a report published in *Nature Cell Biology*, the team used live cell imaging and RNAi approaches to show that AIR-1 stabilizes the spindle in a non-kinase-dependent fashion in addition to its kinase-dependent role at the centrosomes.

Toya, who has since joined the CDB Laboratory for Cellular Adhesion and Tissue Patterning, began by observing microtubules in mitotic cells in which the expression of *air-1* or *tbg-1* (the *C. elegans* γ-tubulin gene) were knocked down by RNA interference in living embryos. They found that while microtubule numbers and lengths remained roughly constant in the *air-1(RNAi)* embryos, these increased significantly during chromosome condensation in the *tbg-1(RNAi)* cells, suggesting the two mechanisms are different.

In other organisms, in addition to centrosomes (the major microtubule organizing centers), condensed chromatin can also trigger microtubule assembly. To determine whether this is the case in *C. elegans* as well, they tested *air-1* and *tbg-1* knockdown in embryos in which chromatin-stimulated microtubules were made readily visible, and found that while γ-tubulin appeared to play no role, loss of AIR-1

Toya M, et al. A kinase-independent role for Aurora A in the assembly of mitotic spindle microtubules in *Caenorhabditis elegans* embryos.
Nat Cell Biol 13.708-14 (2011)

function caused microtubules to grow to shorter lengths and in fewer numbers. Immunofluorescence also showed that AIR-1 localized around condensed chromatin, but γ-tubulin was not seen in these regions.

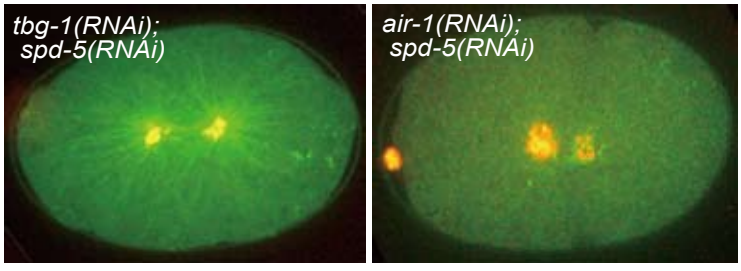


Kinase-active form of AIR-1, (P)-AIR-1, detected at the centrosomes as a subpopulation of the pan-AIR-1 signal

The kinase activity of AIR-1 depends on phosphorylation of the protein at a specific site. To monitor such activity, the team developed a phospho-specific AIR-1 antibody, allowing them to specifically visualize the subpopulation of AIR-1 proteins showing kinase activity. Interestingly, the phospho-AIR-1 signal was not detected in proximity to condensed chromatin, suggesting that its kinase activity was not involved in this aspect of its function.

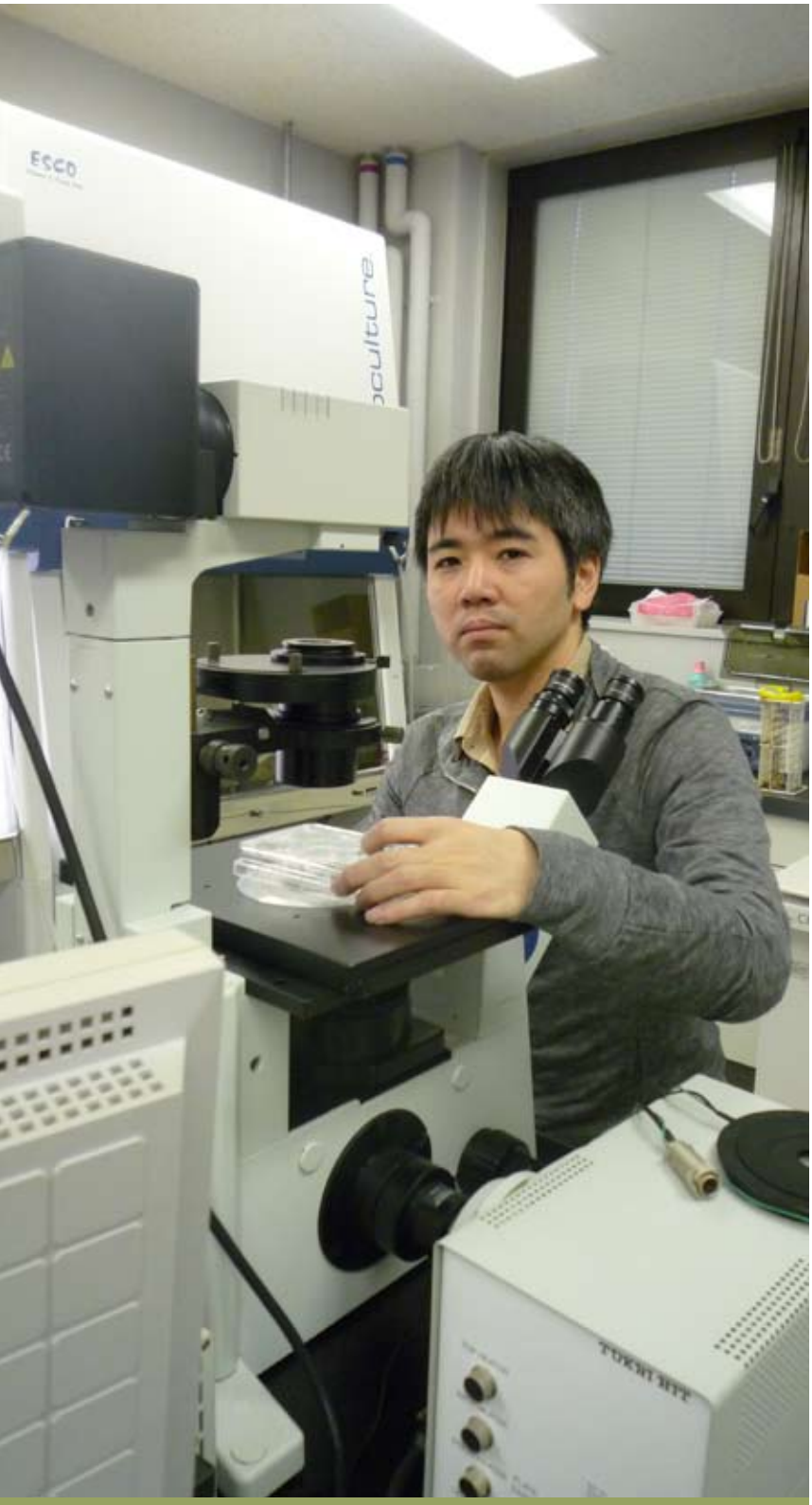
The team tested this idea by generating transgenic embryos that expressed the kinase-inactive form of AIR-1. Toya et al. found that while AIR-1 association with microtubules was unaffected by the absence of kinase activity, its kinase-inactive form appears to be critical for stabilizing microtubules once assembled. Kinase activity was likewise found to be dispensable for the chromatin-stimulated microtubule assembly. The association of the kinase-inactive form of AIR-1 with microtubules was regulated by levels of the wildtype protein – in the presence of wildtype AIR-1, microtubule localization of the kinase-inactive construct was reduced. Given these findings, the team speculates that the balance between active and inactive forms of AIR-1 may be important in controlling mitotic spindle assembly and behaviors.

“The next step will be to find out whether Aurora A in other organisms, including mammals, has similar kinase-independent functions,” says Sugimoto, now at Tohoku University. “We speculate that kinase-independent roles of protein kinases may be more widespread, although these have been overlooked in previous studies.”



Assembly of chromatin-stimulated microtubules. Chromatin-stimulated microtubules were assembled in the absence of γ-tubulin (left); this was dramatically reduced in the absence of AIR-1 (right).
(See CDB website for video)

Mon2 tethers plasm to pole



Tsubasa TANAKA

The uneven distribution of cellular components is a fundamental process in development and differentiation, as can be seen in the sequestration of maternal RNAs and proteins in a form of cytoplasm known as germ plasm at the posterior pole of the *Drosophila* oocyte, which is allocated only to daughter cells that go on to form the germ lineage. Failure to maintain this germ plasm at the egg's polar region results in defects in germ cell formation, highlighting its critical importance to the fly's reproductive viability. The means by which the germ plasm is anchored to the oocyte pole, however, remain incompletely understood.

Tsubasa Tanaka and colleagues in the Laboratory for Germline Development (Akira Nakamura, Team Leader) have now worked out at least part of this mystery, finding that the protein Mon2 works with a second factor, Oskar (Osk), to remodel actin and hold the germ plasm in its proper place. Published in *Development*, this work may shed light on more general principles of the generation of cellular polarity.

The role of Osk in germ plasm assembly is well established – *osk* RNA is carried to the oocyte's posterior pole where it is translated, forms complexes with other polar components, and triggers the remodeling of F-actin at the cell cortex. The resulting actin fibers serve as anchor points in the polar region. The Nakamura team previously found that Osk appears to accomplish this feat through the activation of the endocytic pathway, a mechanism by which cells absorb and recycle molecules on the cell membrane, but the mechanistic link between this process and actin remodeling remained uncertain.

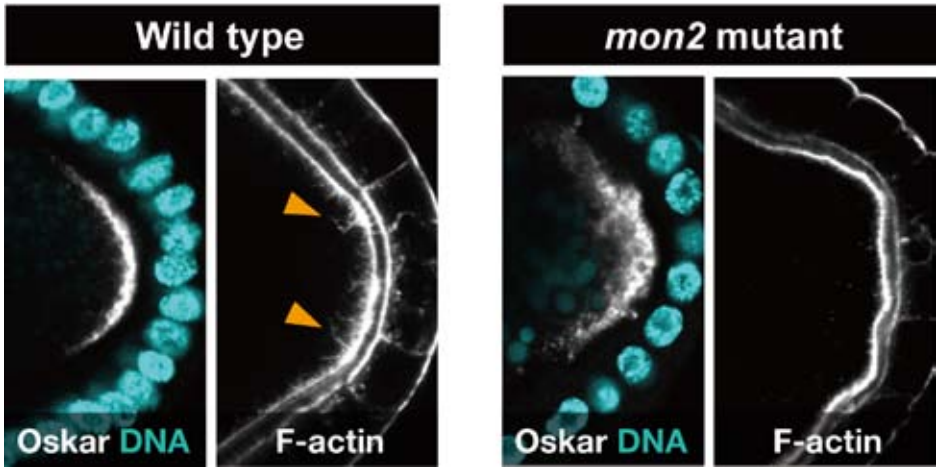
To investigate more closely, Tanaka began by identifying six mutants in which polar factors such as Osk and Vasa failed to stay localized at the poles. Analysis of the underlying genetic changes revealed that all six were caused by mutations in the gene encoding Mon2. When they looked at the patterns of Mon2 expression in the oocyte, they found that while it began as a punctate expression throughout the egg, it gradually shifted to the cortex, resembling the localization of Golgi and some endosomes.

Tanaka T, et al. Drosophila Mon2 couples Oskar-induced endocytosis with actin remodeling for cortical anchorage of the germ plasm.
Development 138.2523-32 (2011)

The team next asked what function Mon2 might have in maintaining the germ plasm at the pole. In the mutants, they found no changes in microtubule assembly, needed to convey pole factors to their destinations, or in the ability of Osk to activate endocytosis. But when they looked at its effect on actin, they found that Mon2 loss of function interfered with the cytoskeletal component's remodeling. Analysis of genetic interactions between Mon2, Osk and the endocytic regulator Rab5 revealed that Mon2 operates downstream of both Osk and the endocytosis it triggers.

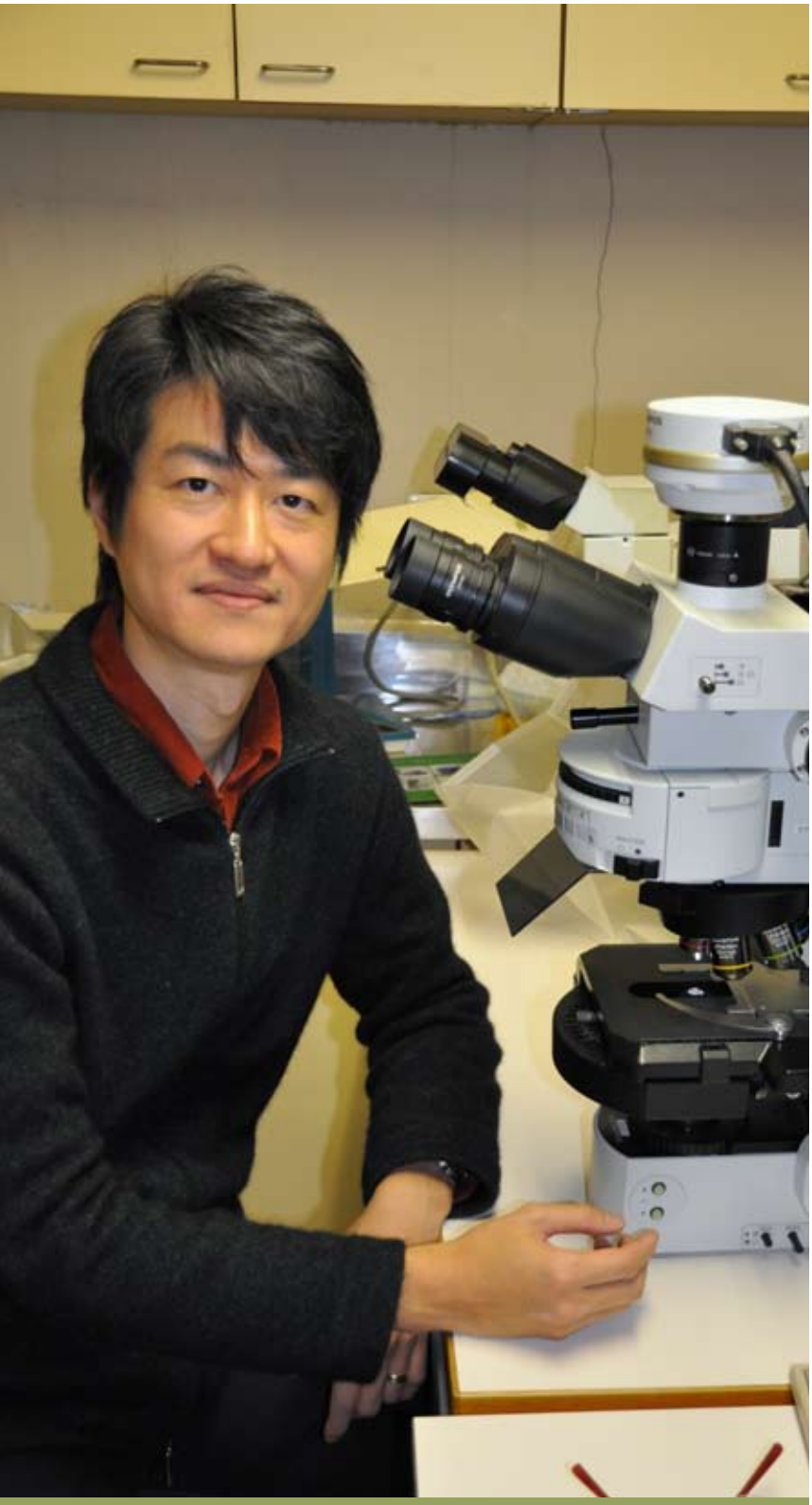
When Tanaka tested for protein binding partners for Mon2, he found that it physically associated with a pair of actin nucleators, Cappuccino (Capu) and Spire (Spir). Interestingly, Spir includes a Rab-binding domain, pointing to a possible interaction with endosomes. Loss of Capu and Spir function resulted in the loss of actin projections at the polar cortex; the actin fibers instead accumulated in fuzzy ball-like accretions, indicating a failure of the actin remodeling requisite for germ plasm tethering. Knowing that both Spir and Capu interact with Rho1, a protein known for its roles in actin regulation, the team next tried to copy the loss of Capu and Spir phenotype by interfering with Rho expression, which resulted in the expected failure of actin remodeling. They further found that in Mon2 mutants, Rho's normal localization at the oocyte posterior pole was lost. The picture that develops is one in which the activation of endocytosis by Osk spurs actin remodeling by Mon2 downstream of Spir, Capu and Rho, thereby providing an anchorage for the germ plasm.

"There have been a number of recent indications that the endosome serves as a platform for the assembly of molecular complexes, as well as other lines of evidence that show that intracellular vesicles, such as endosomes, play a role in ferrying molecules through the cell," says Nakamura. "Given the importance of these functions, what we have learned about Mon2 in this study may have implications for the generation of polarity in other contexts as well."



Loss of Mon2 function results in a failure to maintain pole plasm at the pole

Pushing in, crowding out



Yoichi KOSODO

The surface, or cortex, of the mammalian brain is formed from highly organized layers comprising distinct types of neural cells. This cellular diversity, and the underlying order that informs the overall cortical structure, are largely the result of routines that play out during the brains development in embryonic stages. Important among these is a process called asymmetric cell division, in which progenitor cells give rise to daughter cells of different fates. Interestingly, neural progenitors remain on the move during this process, shuttling their nuclei back and forth between the apical and basal sides of the developing cortex in an action known as interkinetic nuclear migration (INM). These movements are timed in sync with the cell cycle, but just how this is accomplished has remained enigmatic.

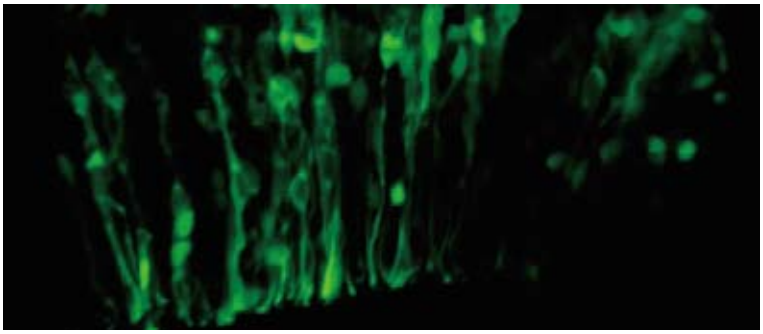
Yoichi Kosodo and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) have identified a pair of mechanisms that couple INM to the mitotic cycle. In an article published in *The EMBO Journal*, the group shows that cell-autonomous basal-to-apical nuclear migration proceeds under the control of the microtubule-associated protein Tpx2, which drives migration of the nucleus in the opposite direction via a displacement, or “crowding out,” effect.

The group began by developing a system to allow them to track nuclear movement during INM in living tissue, enabling them to identify a number of novel features of this behavior and its coordination with various stages of the cell cycle. To test whether interkinetic nuclear migration in fact depends on cell cycle progression, they used an inhibitor to arrest neural progenitors at the G1 phase, and found that this prevented the apical migration of nuclei at the start of INM. Previous studies had raised the possibility that this linkage might rely on the function of Tpx2, a microtubule nucleating protein, the expression of which is regulated by the cell cycle. Monitoring of a fluorescent-tagged version of the protein revealed that it associates with microtubules in the apical processes of neural progenitor cells.

Kosodo next interfered with Tpx2 function using RNAi to examine whether it is involved in apical nuclear migration, and found that its

Kosodo Y, et al. Regulation of interkinetic nuclear migration by cell cycle-coupled active and passive mechanisms in the developing brain. *EMBO J* **30.1690-1704** (2011)

knockdown resulted in a significant decrease in this aspect of INM; specifically, that loss of Tpx2 function caused a slowdown in the speed of nuclear movement toward the apical region. Observations of microtubule distribution in wildtype and Tpx2-inhibited cells indicated that the factor works to localize microtubules in the apical process of neural progenitor cells. Taken together, these results suggest that basal-to-apical nuclear migration during the G2 phase is dependent on the reorganization of microtubules by Tpx2.

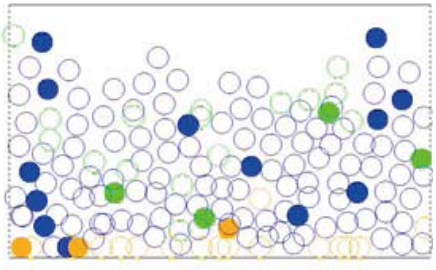


Neural progenitors undergo dynamic oscillations of nuclei, called interkinetic nuclear migration, and divide facing the ventricular zone (apical side). In this movie, cells with nuclear GFP migrate basally at G1 phase while those in G2 phase (cytoplasmic GFP) migrate apically. (See CDB website for video)

What then of nuclear migration in the opposite direction? In G1-arrested neural progenitor cells, the group observed that nuclei accumulated basally, raising the possibility of a separate mechanism behind this. Using a magnetic fluorescent microbead assay, they tracked the motion of apically-located beads in cultured brain slices, and found that this drew them to the basal side, from which they did not subsequently return to the apical region, even when nuclei continued to migrate in that direction. It appeared that their translocation was therefore non-cell-autonomous, but the mechanism remained in doubt.

One possibility was that the active migration in the basal-to-apical direction increases nuclear density in the apical region, which might lead to a "crowding out" effect. Kosodo et al. tested this by arresting the cell cycle at S phase by drug treatment and observing the effects on nuclear migration over time. Cell cycle arrest causes a drop in the number of apically migrating nuclei. They found that this consequently reduced the rate at which nuclei moved in the basal direction as well. The same phenomenon was observed in observations of microbeads, suggesting that it was not due to some unknown bioactive property of the mitotic inhibitor, but that basal movements of nuclei or beads are tightly linked with apically directed nuclear movements. To further test their hypothesis that apical-to-basal nuclear migration is the result of displacement, the group, in collaboration with Akatsuki Kimura at National Institute for Genetics, constructed a computational simulation of INM to model conditions that might influence nuclear migration in the apical-to-basal direction, and found that their in silico predictions jibed well with their in vivo observations, strongly suggesting that this aspect of INM is driven by physical displacement of nuclei from the basal region.

"In the developing brain, a huge number of neural progenitors must engage in highly dynamic movements in order to give rise to its myriad neurons," says Matsuzaki. "What we have discovered is that the independent but linked mechanisms that drive movements in opposing directions help to avoid extreme deviations or collisions of progenitors and maintain the structural order of the brain's morphology. Indeed, this may represent a fundamental strategy for maintaining order in dynamic cell populations."



A computer modeled simulation faithfully mimics the behavior of apical to basal nuclear migration of neural progenitors. The model assumes that G1 nuclei move such that the local nuclear density is minimized, whereas G1 nuclei apically migrate at a constant speed (See CDB website for video)

2011 Awards

As one of the world's leading institutes in the fields of developmental biology and stem cell research, the RIKEN CDB strives to maintain the highest levels of originality, impact and quality in its scientific contributions. The individual achievements and careers of many of its scientific staff have been recognized with awards from numerous organizations, including government ministries and agencies, private foundations and academic societies.

The CDB takes great pride in the achievements of its researchers, as individuals and as indispensable members of their laboratories.

Awardee	Position	Laboratory	Award	Organization
Shigeru Kuratani	Group Director	Evolutionary Morphogenesis	Alexander Kowalevsky Medal	Saint-Petersburg Society of Naturalists
Hiroshi Nagashima	Research scientist	Evolutionary Morphogenesis	2011 Achievement in Anatomy	The Japanese Association of Anatomists
Yukari Terashita	Junior Research Associate	Genomic Reprogramming	Prize for Academic Excellence	Japanese Society of Mammalian Ova Research
Sayaka Wakayama	Research scientist	Genomic Reprogramming	Prize for Academic Excellence	Japanese Society of Mammalian Ova Research
Chong Li	Research scientist	Genomic Reprogramming	Prize for Academic Excellence	Japanese Society of Mammalian Ova Research
Kazuo Yamagata	Visiting scientist	Genomic Reprogramming	Prize for Academic Excellence	Japanese Society of Mammalian Ova Research
Teruhiko Wakayama	Team Leader	Genomic Reprogramming	Prize for Academic Excellence	Japanese Society of Mammalian Ova Research
Hiroki R. Ueda	Project Leader	Systems Biology	JSPS Prize	Japan Society for the Promotion of Science
Shigeru Kuratani	Group Director	Evolutionary Morphogenesis	MEXT Prize	Ministry for Education, Culture, Sports, Science and Technology
Yosuke Tanaka	Research scientist	Stem Cell Biology	Poster Prize	Max Delbrück Centrum Berlin-Buch
Yasuhide Ohinata	Visiting scientist	Pluripotent Stem cell Studies	MEXT Prize	Ministry for Education, Culture, Sports, Science and Technology
Yukari Terashita	Junior Research Associate	Genomic Reprogramming	Award for excellent poster presentation	Asian Reproductive Biotechnology Society
Mototsugu Eiraku	Deputy Unit Leader	Four-dimensional Tissue Analysis	Mitsubishi Kagaku Prize	Molecular Biology Society of Japan
Yoshiki Sasai	Group Director	Organogenesis and Neurogenesis	Inoue Prize for Science	Inoue Foundation for Science

E14.5 mouse inner ear bone labyrinth, containing the cochlea and vestibular system, as visualized by injection of ink (paint filling).

Embryonic Induction

Hiroshi SASAKI Ph.D.

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

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

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Publications

Wada K, et al. Hippo pathway regulation by cell morphology and stress fibers. *Development* 138.3907-14 (2011)

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)

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

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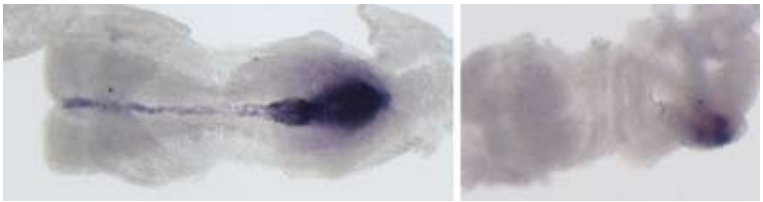
During vertebrate embryogenesis, simply structured regions of undifferentiated cells organize and cooperate to give rise to the anterior-posterior and dorsal-ventral body axes, the neural tube and other embryonic structures deriving from the three germ layers, following developmental patterns that can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



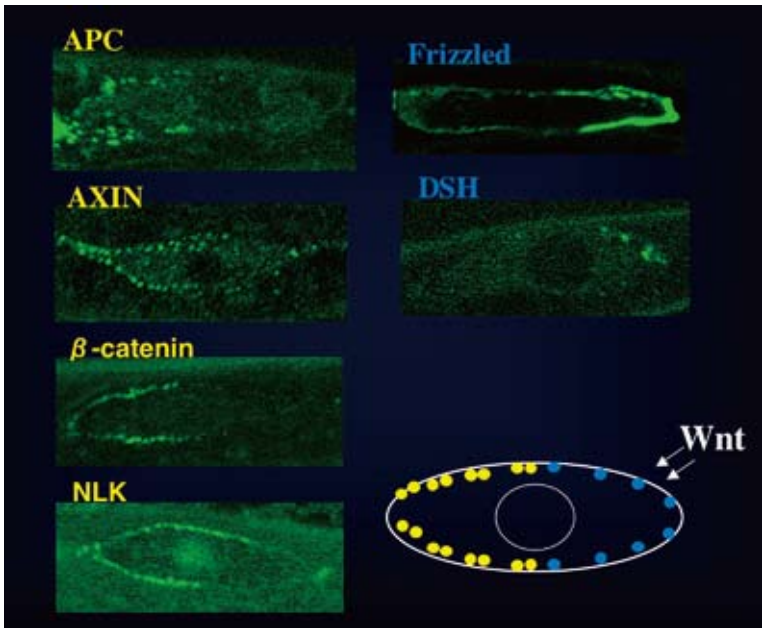
Day 8.5 mouse embryos showing that the notochord and node (signaling centers regulating trunk/tail development; stained purple on the left) is absent in the *Tead1;Tead2* double mutant (right; remaining staining is in the primitive streak). *Tead1;Tead2* double mutants also have defects in cell proliferation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

Cell Fate Decision

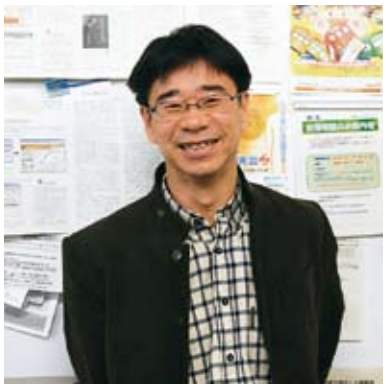
The development of multicellular organisms involves a single cell (such as a fertilized egg) which gives rise to diverse progeny through successive cell divisions. The process of cell division in which the resulting daughter cells are of different characters or "fates" is known as asymmetric cell division. 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-catenins acting in the Wnt signaling pathway. This pathway works to polarize cells fated to undergo asymmetric division; we seek to gain a better understanding of how it achieves this by studying the intracellular localization of the Wnt pathway's molecular components.

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



Asymmetric cortical localization of the Wnt pathway components



Hitoshi SAWA Ph.D.

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

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

Shibata Y, et al. Multiple functions of PBRM-1/Polybromo- and LET-526/Osa-containing chromatin remodeling complexes in C. elegans development. *Dev Biol* 361.349-57 (2012)

Yamamoto Y, et al. Multiple Wnts redundantly control polarity orientation in Caenorhabditis elegans epithelial stem cells. *PLoS Genet* 7.e1002308 (2011)

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Early Embryogenesis

Guojun SHENG Ph.D.

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

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

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Publications

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

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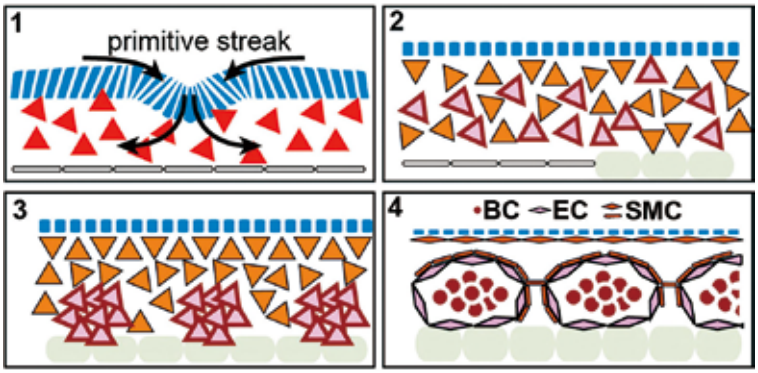
Nakazawa F., et al. Negative regulation of primitive hematopoiesis by the FGF signaling pathway. *Blood* 108.3335-43 (2006)

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

Mesoderm cells are derived from ectoderm cells during gastrulation through a process called epithelial to mesenchymal transition (EMT). During EMT, mesoderm cells leave epithelial-shaped ectoderm sheet and become migratory. We are interested in understanding the molecular and cellular mechanisms governing this EMT. We are also interested in understanding how this process is linked to mesoderm fate specification in the ectoderm and to mesoderm regionalization along the dorsal-ventral axis during gastrulation.

After their formation, mesoderm cells give rise to several well-defined lineages: axial, paraxial, intermediate, lateral plate and extraembryonic. The extraembryonic lineage contains three cell types: blood, endothelial and smooth muscle. We are investigating how these three cell types are specified and how they are organized to form functional hematopoietic and vascular systems.



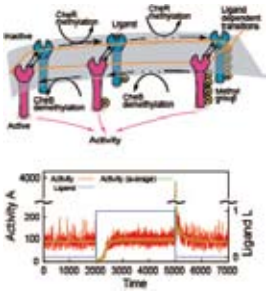
The circulatory system of early chicken embryos (E5 shown here) is complex and contains embryonic, allantoic, and yolk sac sub-systems. The diversity of blood cells during this period is revealed by the transcriptomic analysis of non-red blood cells in circulation.

Physical Biology

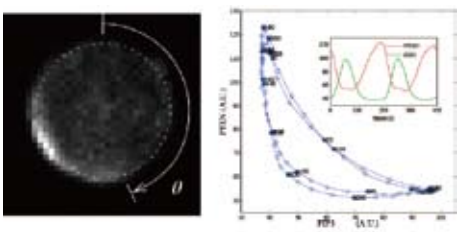
Advances in measurement technologies have afforded us glimpses into dynamic functional processes, such as morphogenesis and information processing, in cells and tissues involved in development and regeneration. The truly organic dynamism of the biological phenomena exhibited by living cells, individually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

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

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



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



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

Tatsuo SHIBATA Ph.D.

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

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Ooyama S. and Shibata T. Hierarchical organization of noise generates spontaneous signal in Paramecium cell. *J Theor Biol* 283.1-9 (2011)

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Shibata T. and Fujimoto K. Noisy signal amplification in ultrasensitive signal transduction. *Proc Natl Acad Sci U S A* 102.331-6 (2005)

Developmental Genomics

Asako SUGIMOTO Ph.D.

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

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Publications

Toya M, et al. A kinase-independent role for Aurora A in the assembly of mitotic spindle microtubules in C. elegans embryos. *Nat Cell Biol* 13, 708-14 (2011)

Hanazawa M, et al. PGL proteins self associate and bind RNPs to mediate germ granule assembly in C. elegans. *J Cell Biol* 192.929-37(2011)

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

Toya M, et al. Imaging of mitotic spindle dynamics in Caenorhabditis elegans embryos. Methods *Cell Biol* 97, 359-72 (2010)

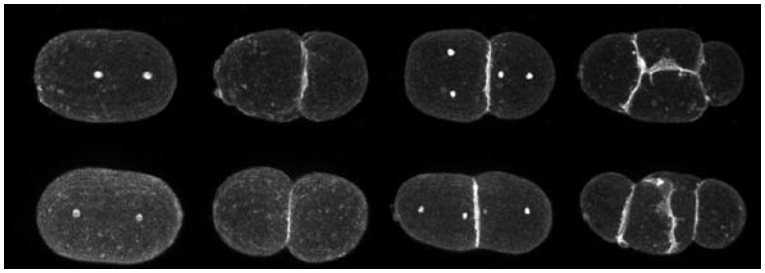
Motegi F and Sugimoto A. Sequential functioning of the ECT-2 RhoGEF, RHO-1 and CDC-42 establishes cell polarity in Caenorhabditis elegans embryos. *Nat Cell Biol* 8. 978-85 (2006)

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

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

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

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



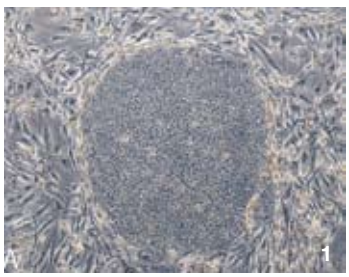
The first two rounds of cell divisions in *C. elegans* embryos. Centrosomes and cell membrane are labeled with GFP. Top: wildtype. Bottom: par-3 mutant, showing a defect in the cell division axis.

Retinal Regeneration

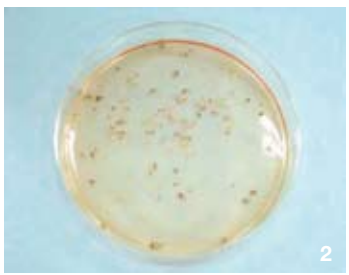
The retina has been called the “approachable part of the brain,” owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that in adult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

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

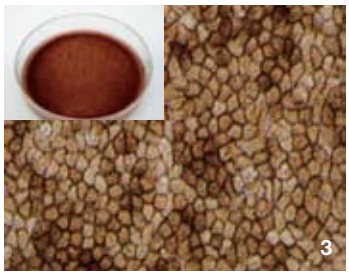
Therapeutic applications cannot be developed from basic research alone; the clinical approach – a thorough understanding of the medical condition to be treated is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily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



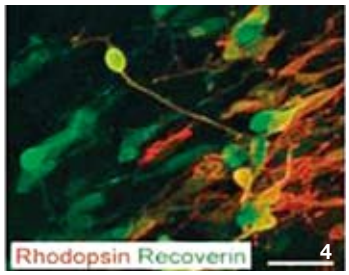
Human iPS cells



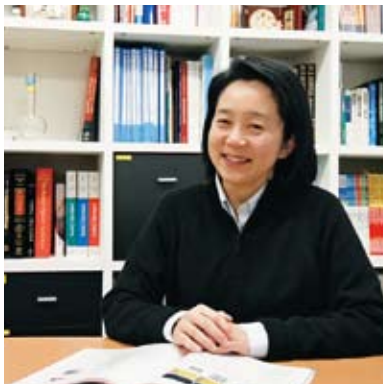
Retinal pigment epithelial cells differentiated from iPS cells



Purified retinal pigment epithelial cells



Photoreceptor cells differentiated from iPS cells



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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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Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph.D.

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

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Publications

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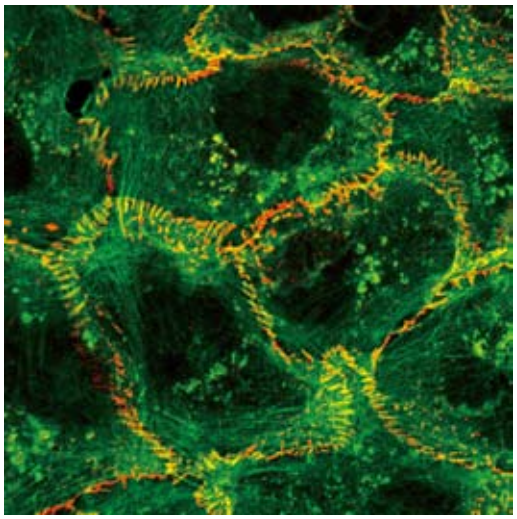
Animal cells organize into tissues with complex architecture. Our lab is exploring the molecular mechanisms by which individual cells assemble into a tissue-specific multicellular structure, such as epithelial sheets and neural networks. We are also interested in the molecular basis of how the tissue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

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

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



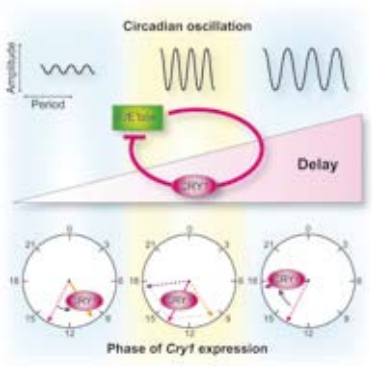
Double-immunostaining for F-actin (green) and Kusabira Orange-tagged E-cadherin (red) introduced into A431D cells. In these cells, E-cadherin dynamically moves along cortical actin filaments, resulting in the unique distributions shown here.

Systems Biology

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

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

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



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

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

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

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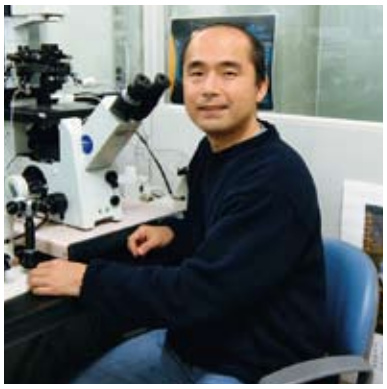
Genomic Reprogramming



Teruhiko WAKAYAMA Ph.D.

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

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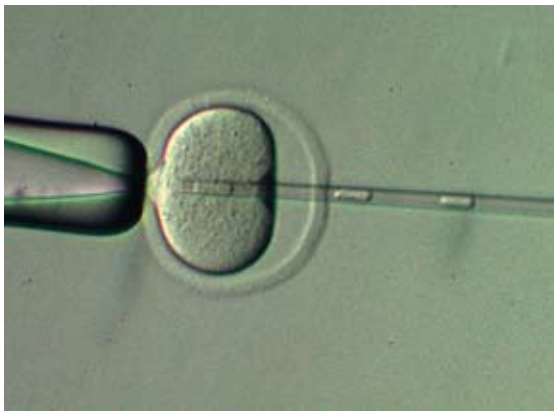
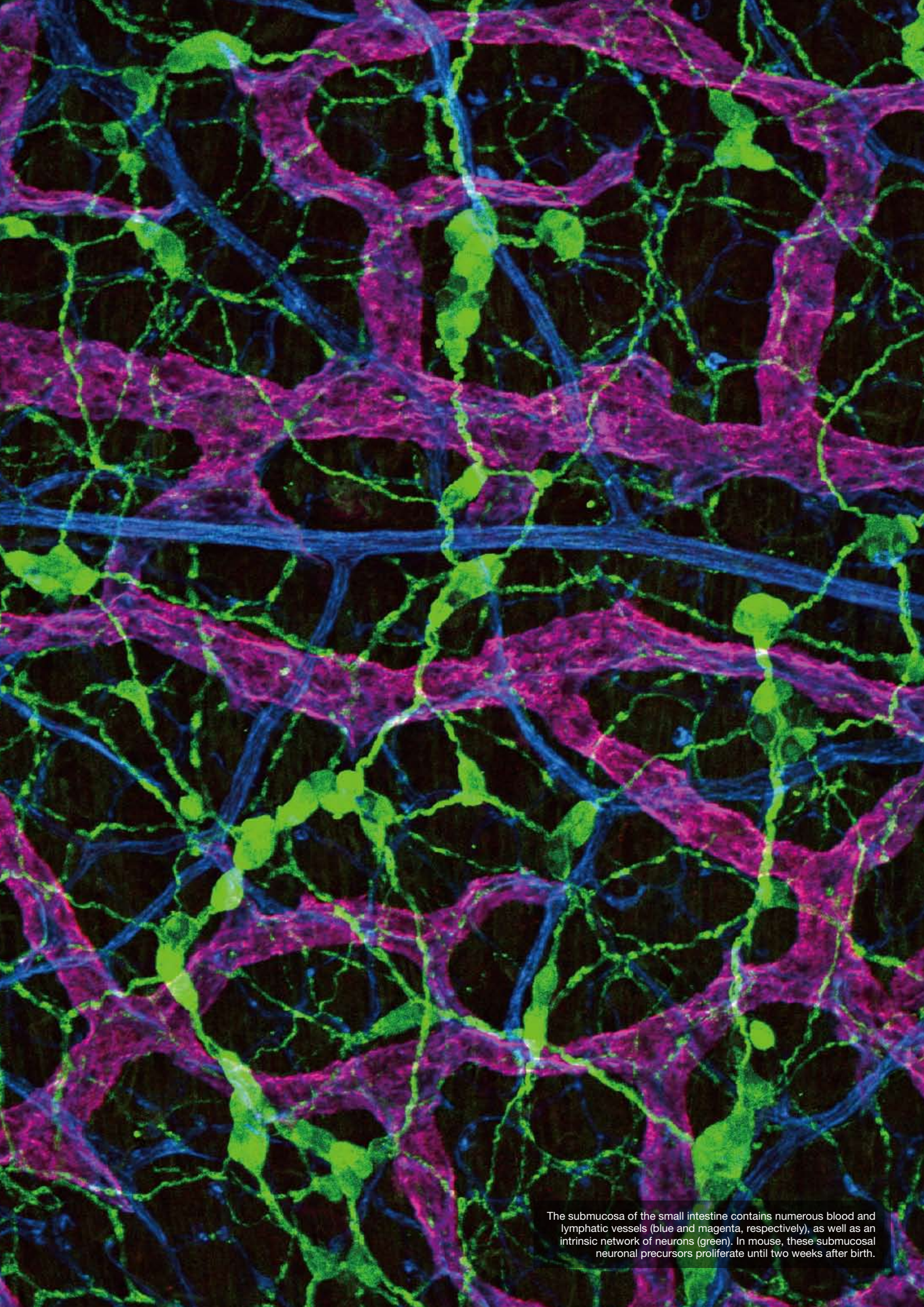
A limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual cells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



The submucosa of the small intestine contains numerous blood and lymphatic vessels (blue and magenta, respectively), as well as an intrinsic network of neurons (green). In mouse, these submucosal neuronal precursors proliferate until two weeks after birth.

First germline transcriptional repressor in ascidian found

Maki SHIRAE-KURABAYASHI

In most animal embryos, molecular measures are in place to prevent nascent germline cells from mistakenly differentiating into a somatic lineage. This is typically achieved by the repression of somatic gene expression by specific genetic factors in the germ plasm, such as Pgc in *Drosophila* and PIE-1 in *C. elegans*. It has been suspected that transcriptional repression is also at work in embryos of the ascidian *Ciona intestinalis*, which also has a germ plasm-like cytoplasmic compartment known as the postplasm, but the factors and mechanisms that might lie behind this process remain unknown.

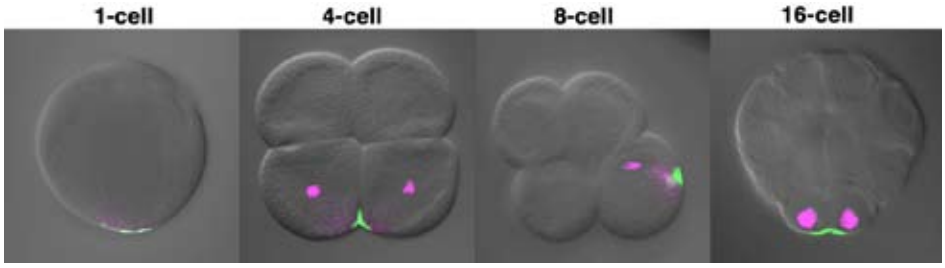
New work by Maki Shirae-Kurabayashi and colleagues in the Laboratory for Germline Development (Akira Nakamura, Team Leader) has identified what appears to be a key transcriptional repressor in the ascidian, a protein product of *Ci-pem-1* RNA, which is an ascidian specific component of the postplasm. This study, published in the journal *Development*, shows that Ci-Pem-1 interacts with another general co-repressor of mRNA transcription, lending further support to the knockdown findings.

Transcriptional repressors in other widely studied species, such as fruit fly and nematode, work by inhibiting the phosphorylation of the C-terminal domain in RNA polymerase II, a critical enzyme in mRNA transcription. Shirae-Kurabayashi began this study by examining such RNAPII phosphorylation in *C. intestinalis* blastomeres, at a stage of development in which the germline begins to emerge as a distinct lineage, as signaled by the inheritance of the postplasm. The team analyzed RNAPII phosphorylation in individual blastomeres using anti-phospho-CTD antibodies, and found that germline blastomeres showed lower levels of phosphorylation than did somatic cells.

Shirae-Kurabayashi M, et al. Ci-Pem-1 localizes to the nucleus and represses somatic gene transcription in the germline of *Ciona intestinalis* embryos. *Development* 138.2871-81 (2011)

Familiar with the expression of *Ci-pem-1* RNA from a previous study, Shirae-Kurabayashi used immunostaining to visualize the distribution of its protein product. They previously reported that the postplasmic *Ci-pem-1* RNA is ejected from the germline during gastrulation by an asymmetric cell division (Shirae-Kurabayashi et al., 2006). In the present study, the team found that the signal of its protein product also dropped in the germline by the tailbud stage. More intriguingly, they found that this protein localizes to the nuclei in the germline blastomeres in the most posterior region of the cleavage-stage embryo. This nuclear accumulation, however, fluctuated over the course of the cell cycle, appearing only in late telophase when the nuclear envelope reassembles following replication. To eliminate the possibility that this reaction was an artifact of cross-reactivity, they re-tested in blastomeres injected with a morpholino against *Ci-pem-1*, and found, in line with expectations, that MO knockdown prevented its nuclear localization.

Thinking that this protein might play a role in transcriptional control in germline cells, the team examined the effects of *Ci-pem-1* knockdown on embryonic gene expression. When they knocked down this factor, they detected ectopic expression of a number of genes ordinarily repressed in germline blastomeres. The expression of both beta-catenin and GATA-dependent genes was de-repressed in the knocked-down germline blastomeres, indicating that *Ci-pem-1* exerts a broadly repressive effect on somatic gene expression.



Ciona intestinalis embryos at 1, 4, 8 and 16-cell stage probed for *Ci-pem-1* mRNA (green) and Ci-Pem-1 protein (magenta). In the pair of germline blastomeres, *Ci-pem-1* mRNA is highly concentrated in the postplasm at the posterior cortex, while Ci-Pem-1 protein is enriched in the nuclei.

The structure of the Ci-Pem-1 protein includes a motif reminiscent of binding domains in other species that associate with the transcriptional co-repressor Groucho. Shirae-Kurabayashi performed a co-immunoprecipitation assay using tagged constructs for these two proteins, and found that Groucho associated with GFP-labeled Ci-Pem-1, but not with GFP alone, pointing to a physical interaction of potential functional significance.

“Unlike Pgc in *Drosophila* and PIE-1 in *C. elegans*, our findings suggest that Ci-Pem-1 appears to repress somatic gene expression without directly inhibiting RNAP II phosphorylation, indicating that distinct mechanisms and factors are responsible for transcriptional quiescence in different organisms,” says Nakamura. “Interestingly, recent work by Mitinori Saitou, a former CDB team leader, has shown that the silencing of somatic transcriptional programs also occurs during the establishment of mouse germ cells, which are induced through cell-cell signaling without the germ plasm, meaning the active repression of somatic transcriptional programs during the establishment of the germline is a conserved hallmark in animal development, regardless of the mode of germ cell formation.”

Hippo signaling follows form

Ken-Ichi WADA

Cells have a well-developed sense of their surroundings, identifying and interacting with many diverse components of their environment. This is clearly seen in the proliferation of cells in culture – a population will grow steady until the cell density crosses a given threshold, triggering a shutdown. This phenomenon, known as “cell contact inhibition of proliferation,” has been tied to the Hippo signaling pathway, but the molecular mechanism remains mysterious. Specifically, it is unknown whether the key cue behind this process is cell morphology or cell-cell adhesion, or some combination of the two.

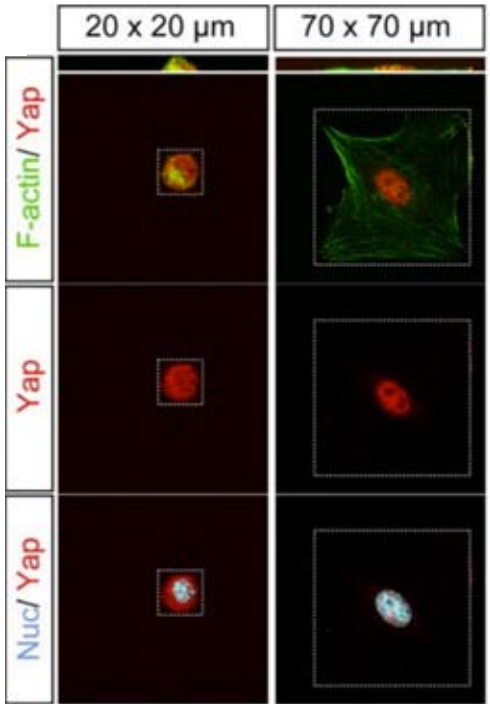
A study by Ken-Ichi Wada and others in the Laboratory for Embryonic Induction (Hiroshi Sasaki, Team Leader) looked at individual cells to examine the effects of cell morphology on Hippo signaling in isolation from other influences. Published in *Development*, the report of this work describes how cell shape and underlying cytoskeletal proteins, such as F-actin, regulate the Hippo pathway in vitro. Wada now works at the RIKEN Advanced Science Institute, while Sasaki has moved to the Kumamoto University Institute of Molecular Embryology and Genetics.

In a previous study, the Sasaki team had shown how changes in cell density control Hippo activity; Hippo is down-regulated at lower densities, resulting in the nuclear accumulation of a coactivator protein, Yap, increasing activity of Tead, a transcription factor that promotes proliferation. Conversely, at higher densities, Hippo is activated, causing the phosphorylation and cytoplasmic retention of Yap, preventing Tead activity and, as a result, stoppage of proliferation. This report, however, left open the question of how Hippo signaling is able to detect the density of cells in an environment.

Numerous studies have shown links between cell-cell adhesion and regulation of the Hippo pathway, and other reports have described the effect of cell morphology on proliferation, which led Wada to speculate that cell shape might also play be a missing piece in the Hippo–proliferation puzzle. In order

Wada K, et al. Hippo pathway regulation by cell morphology and stress fibers.
Development 138.3907-14 (2011)

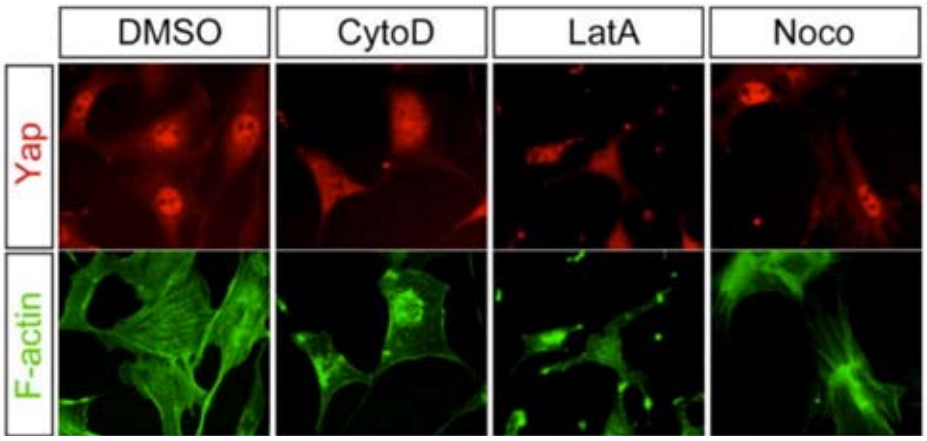
to study this, however, he first needed an experimental system that would allow him to examine cells in isolation from contact with other cells. By culturing cells in microdomains of different sizes, he was able to grow single cells in spatial constraints that affected their morphology. In smaller 20 × 20 μm domains, the cells assumed a compact, rounded form, while in roomier 70 × 70 μm quarters, they became flattened and spread out. Looking next at the expression of the Hippo pathway factor Yap, he noted that in cells grown in the smaller domains, Yap remained outside the nucleus, while in those cultured in the larger domains it was localized in the nucleus, suggesting that morphology can act as a regulator of the distribution of this protein.



In smaller, rounded cells (left), Yap (red) accumulates outside the nucleus (blue), while in flattened cells (right) with extensive F-actin (green), Yap localize within the nucleus.

But how is this regulatory control achieved? The key may lie in a form of F-actin known as stress fibers. In cells cultured in the 50 × 50 μm domains, as a model of lower cell density, stress fibers were abundant and present throughout the cells, while in the 20 × 20 μm domains their distribution was punctate and only faintly detectable. To determine whether the apparent correlation between stress fiber quantity and cell density works in Hippo regulation, the team next disrupted stress fiber formation using an actin-inhibiting agent. As expected, this caused Yap to localize in the cytoplasm, as occurs in high-cell-density environments.

This latest work form the Sasaki team cements a role for cell morphology in the regulation of proliferation mediated by the Hippo pathway. “It will be interesting to tease out how Hippo signaling integrates diverse information inputs relating to a cell’s shape and its adhesive contacts with neighboring cells,” says Sasaki. “We know that actin is also involved in cell-cell adhesion, so that may be one key to understanding the link to Hippo. We are also very interested in the role that Hippo plays in controlling differentiation in the preimplantation mammalian embryo, and whether these latest findings will prove to be important to our understanding of early development as well.”



Stress fibers (green) are found throughout untreated cells, in which Yap (red) accumulates in the nucleus, while in cells treated with an actin-inhibitor (CytoD, LatA), there are fewer stress fibers and Yap is cytoplasmic.

BrainStars project maps regional gene expression in brain

Takeya KASUKAWA, Koh-hei MASUMOTO

The mammalian brain is one of the pinnacles of evolutionary achievement; myriad interconnecting neurons in discrete functional domains give rise to an organ of astonishing complexity. Numerous efforts have been made to profile the molecular signatures of the brain's many regions, typically using a technology known as in situ hybridization to visualize gene transcripts at specific sites. Other more comprehensive techniques for analyzing gene expression, such as DNA microarrays, also exist, but these have yet to be used in measuring the central nervous system's genetic activity across a wide range of different regions.

Takeya Kasukawa and Itoshi Nikaido of the Functional Genomics Unit, Koh-hei Masumoto of the Laboratory for Systems Biology (both headed by Hiroki R. Ueda) and others have done just that, profiling genome-wide expression across nearly 50 brain regions in the mouse. Published in *PLoS ONE*, this work identified a large number of genes showing interesting characteristics such as multi-state expression, control properties, and putative ligand-receptor interactions. The results have been compiled into an open, searchable database at http://brainstars.org.

The group sampled 51 brain regions associated with distinct functions, such as various sensory modalities, internal clocks, memory, fear, and feeding, at 4-hour intervals over the course of a day. They next used quantitative PCR (qPCR) to analyze these samples for the expression of region-specific genes, and used global clustering to identify developmental, evolutionary and anatomical groupings, noting a high degree of consistency with expectations, but a few notable exceptions as well. Retina, pituitary and pineal tissue, for example, were markedly distinct from the other 48 regions in this analysis, which may be due to differences in anatomy or cell-type composition.

Their next step was to identify and analyze multi-state patterns of gene expression, finding more than 8,000 genes showing multimodal expression across the sampled regions. Such multiple states may

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

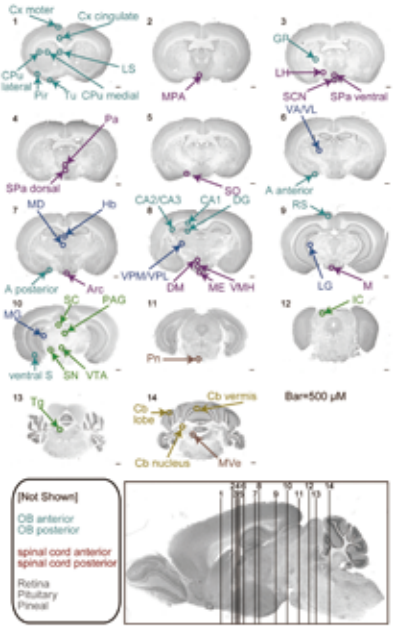
reflect either differential regulation in different cell types, or heterogeneity in the balance of various cell types in different brain regions. A closer examination revealed a number of interesting genes with from two to four distinct states, including various G-protein-coupled receptors (GPCRs) and a large proportion of homeobox and nuclear receptor genes.

Such multi-state expression data was then used to identify candidate marker genes, defined as those whose highest or lowest expression state occurs only in a single region of the central nervous system. More than 2,000 such genes were found, including several transcriptional regulator/target gene pairs. Not all genes are multimodal, however; the study also found a class of genes with single-state expression across multiple CNS regions, suggesting that they may function as stable internal controls.

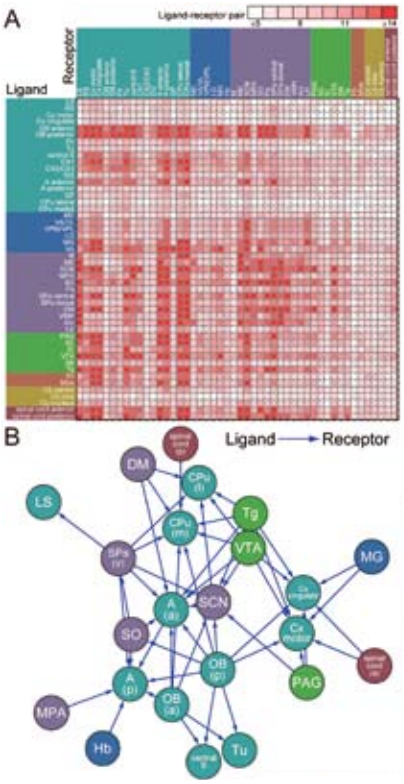
To test the utility of their dataset, Kasukawa, Nikaido and Masumoto focused on neurotransmitter (NT) and neurohormone (NH) ligands and receptors as a means of measuring the interconnectivity between CNS regions, counting the number of NH/NT ligand-receptor expressions for every pairing of CNS regions, and plotted these by density. In examples of the revelatory power of this approach, they identified a high level of connectivity between the hypothalamic and olfactory bulb regions, and large number of inbound connections to the suprachiasmatic nucleus, the circadian clock center, suggesting the requirement for multiple inputs in keeping the body's internal clock running on time. A number of other intrinsic connections, described in detail in the article and the public database, were also uncovered.

The final validation of the group's findings came from a comparison to several previous studies of regional gene expression in the mouse brain. Despite the smaller sampling sizes of the BrainStars study, it showed good levels of correlation with existing in situ hybridization data sets, with some differences however in findings for smaller and more complex regions, such as the hypothalamus.

The BrainStars database provides arrange of information on gene expression in the mouse brain, including gene expression profiles the results of various analyses," says Kasukawa. "We are hopeful that this resource will make a contribution to CNS research, and would be delighted to see it used widely by others.



Map of 44 of the brain regions sampled in this study.



(A) Color-coded table of CNS regions expressing genes coding for NH/NT ligands in one region and cognate receptor genes in the other. (B) Map of putative strong connections among CNS regions.

EPLIN responds to force

Katsutoshi TAGUCHI

Epithelial cells stay connected in a number of ways, relying on combinations of adhesion molecules to recognize and maintain contact with their neighbors. One form of epithelial cell-cell adhesion involves the zonula adherens, a belt-like region at the cell border at which complexes of cadherins, catenins, and other molecules, including actin filaments in the cytoskeleton, accumulate. Sheets of epithelial tissue undergo rearrangements in the course of numerous developmental and disease processes, ranging from convergent extension to wound closure. In the latter, purse-string-like actomyosin cables linked to adherens junctions (AJs) contract around the leading edges of cell margins, closing the space between cells. It is not known, however, how actin cooperates with the cadherin machinery in the remodeling of the AJ.

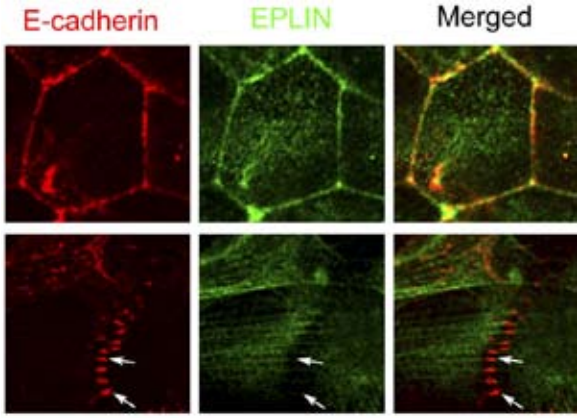
New work by Katsutoshi Taguchi and others in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) shows that the protein EPLIN plays a key role in the process, as a mechanosensitive regulator of junctional remodeling. Published in the *Journal of Cell Biology*, this research sheds new light on how physical forces, such as stretching, help determine cellular properties and behaviors.

Taguchi began by immunostaining a line of cultured epithelial cells for E-cadherin and F-actin, showing their accumulation at adherens junctions. Interestingly, this labeling revealed that these junctions took two different forms. In the interior of a cell colony, the proteins aligned linearly, whereas at the fringes, the junctions were punctate in form. These junction types appeared to be interconvertible, as both forms could be identified within single colonies.

In both the linear and the punctate adherens junctions, E-cadherin, β- and αE-catenins, and actin fibers play dynamic structural roles. Several proteins, including EPLIN and vinculin, have been identified as linkers between the cadherin-catenin complex and the actin cytoskeleton to date, prompting Taguchi to examine their possible roles. Through additional immunostaining, he found that while vinculin is

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

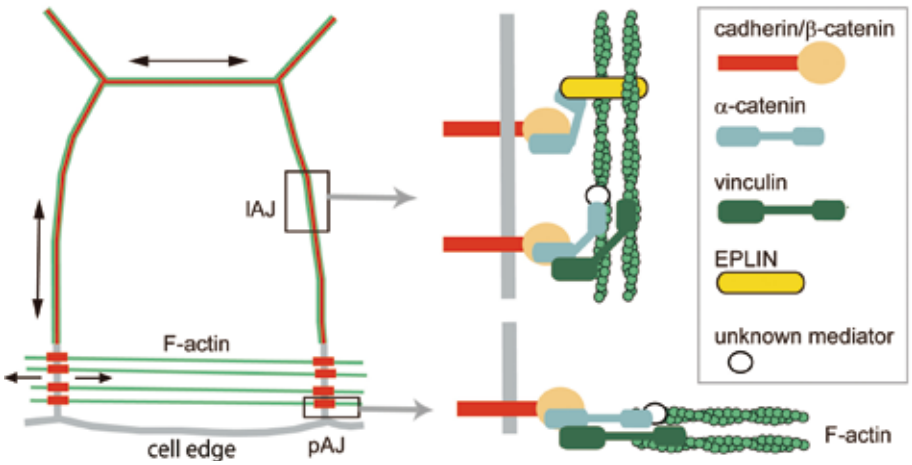
present in both linear and punctate AJs, EPLIN was only detectable in the linear type. Knockdown of the two proteins by RNAi further supported a role for EPLIN; loss of the function of this protein allowed the formation of punctate AJs only, suggesting that it is required in the formation of the linear adherens junction.



In interior cells, E-cadherin and EPLIN align to form robust linear adherens junctions (top), while in cells at the periphery, cadherin is distributed in a punctate fashion (bottom).

What then is the reason for the lack of EPLIN at punctate AJs? The group focused on F-actin as a candidate. In linear AJs, actin fibers run parallel to the cell membrane, while in the punctate they are perpendicular, suggesting a possible influence by mechanical forces, such as tension, in realigning the cytoskeleton. Taguchi tested this hypothesis by laser ablating anchor points targeted by F-actin in punctate AJs. Loss of this tension-generating structure resulted in immediate upregulation of both EPLIN and E-cadherin, and rearrangement of the adherens junctions to the linear form. In the converse experiment, when parallel actin fibers were ablated from linear AJs, the EPLIN signal was lost, suggesting that this molecule responds to "pulling" forces on cells by accumulating at linear junctions. A subsequent test of this idea using a stretching apparatus to exert tension on epithelial colonies showed that this form of stress stimulates the localization of EPLIN at the cell-cell junction.

The current study provides new insight into how EPLIN plays a mechanosensory role in the remodeling of adherens junctions in response to mechanical forces. The punctate adherens junction may play a stabilizing role by giving flexibility to otherwise rigid sheets of epithelium under stress. "Many types of epithelial tissue are subject to external mechanical stimuli, but they tend to be quite resilient and remain intact even when pulled and stretched," says Takeichi. "It seems that these sheets of cells must have some mechanism for detecting and responding to these kinds of stresses. It will be interesting to explore whether the function of EPLIN observed in vitro in this study plays roles in vivo as well."



EPLIN accumulates at linear AJs where the cell membrane is subject to tension, whereas it is absent from the punctate AJS that form in cells not exposed to such mechanical stress.

Self-organized pituitary-like tissue from mouse ES cells

Taisuke KADOSHIMA, Hidetaka SUGA, Mika SOEN, Maki MINAGUCHI

The possibility that functional, three-dimensional tissues and organs may be derived from pluripotent cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), represents one of the grand challenges of stem cell research, but is also one of the fundamental goals of the emerging field of regenerative medicine. Developmental biology has played a central role in informing such efforts, as it has been shown that stem cell differentiation can be directed to follow a given lineage pathway by culturing stem cells in conditions that recapitulate the specific cellular and molecular environment from which such cells normally emerge during embryogenesis. Intriguingly, recent work has shown that when ES cells are cultured under the appropriate conditions, they can be driven to self-organize into complex, three-dimensional tissue-like structures that closely resemble their physiological counterparts, a remarkable advance for the field.

New work by Hidetaka Suga of the Division of Human Stem Cell Technology, Yoshiki Sasai, Group Director of the Laboratory for Organogenesis and Neurogenesis, and others has unlocked the most recent achievement in self-organized tissue differentiation, steering mouse ESCs to give rise to tissue closely resembling the hormone-secreting component of the pituitary, known as the adenohypophysis, in vitro. Conducted in collaboration with Yutaka Oiso at the Nagoya University Graduate School of Medicine, this work was published in *Nature*.

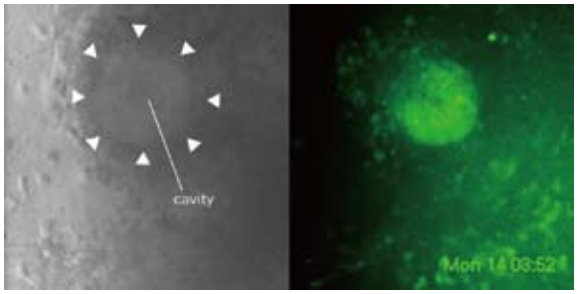
The Sasai group has complied an impressive list of achievements in induced differentiation using an ES cell culture technique dubbed SFEBq (shorthand for “serum-free floating culture of embryoid body-like aggregates with quick re-aggregation”), including high-efficiency methods for the differentiation of dopaminergic, cerebral cortex, cerebellar Purkinje, and other neuronal cell types. In recent years, refinements of this approach have enabled the first tantalizing insights into the developmental capacity of pluripotent stem cells, showing their ability to give rise to multi-cell-type populations of cortical and retinal neurons that spontaneously self-organize into stratified tissue nearly identical to that of the developing embryo.

In the group’s most recent work, Suga sought to use SFEBq to derive the secretory component of the pituitary (hypophysis) from mouse ES cells. In embryonic development, the pituitary emerges from a region of the non-neural (rostral) head ectoderm, adjacent to the anterior neural plate. Guided by molecular interactions,

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

this placodal region forms an indentation, known as Rathke's pouch, in the developmental predecessor of the roof of the mouth, and eventually gives rise to the anterior section of the pituitary, the source of hormones involved in growth, reproduction and modulating the physiological response to stress.

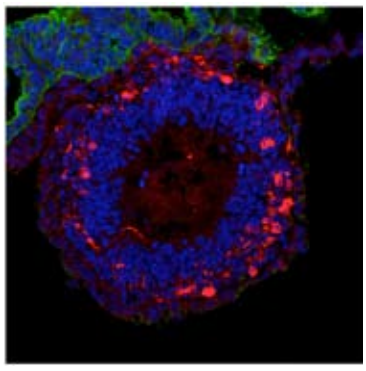
The Sasai lab had previously reported how a modified version of the SFEBq approach lacking extrinsic growth factors could spur ES cells to give rise to hypothalamic neurons. Building on this finding, Suga found by further tweaking the conditions he could steer populations of such stem cells to differentiate simultaneously into the neighboring rostral head ectoderm and hypothalamic neuroectoderm, both of which are required to development of the adenohypophysis. Interestingly, the cells in these clusters spontaneously organized into distinct layers resembling those of the corresponding embryonic tissues. This effect appears to be attributable to the increase of BMP signaling in larger cell aggregates, leading to differentiation into non-neural ectoderm. To test whether co-culture of this ectoderm with hypothalamic tissue would lead to the formation of Rathke's pouch-like structures, Suga observed the ESC-derived aggregates for nearly two weeks, and found that by adding the signaling factor Sonic hedgehog, he was able to induce the self-directed formation of this vesicular tissue.



Rathke's pouch forms from ES cells cultured using the SFEBq approach (left: bright field image; right: Lim3::Venus expression)
(See CDB website for video)

The next important test was whether this in vitro anterior pituitary anlage would show similar functionality to the physiological activity of the adenohypophysis. Of the many critically important pituitary hormones, they chose adenocorticotropic hormone (ACTH) for their first assay. Previous research had suggested that Notch signaling interferes with the development of ACTH-secreting cells, so the group added a Notch blocker to the culture medium and found that this triggered the generation of such cells at high efficiencies. Following a similar principle, they showed that by adding Wnt, glucocorticoid and insulin to the culture at appropriate doses and stages, they could obtain growth hormone-secreting cells in quantity. By varying the recipe of the growth factor cocktail, they were able to induce other pituitary hormones as well. And, critically, the group was able to show that in vitro hormone secretion could respond to requisite signals and engage in regulatory feedback just as in the body.

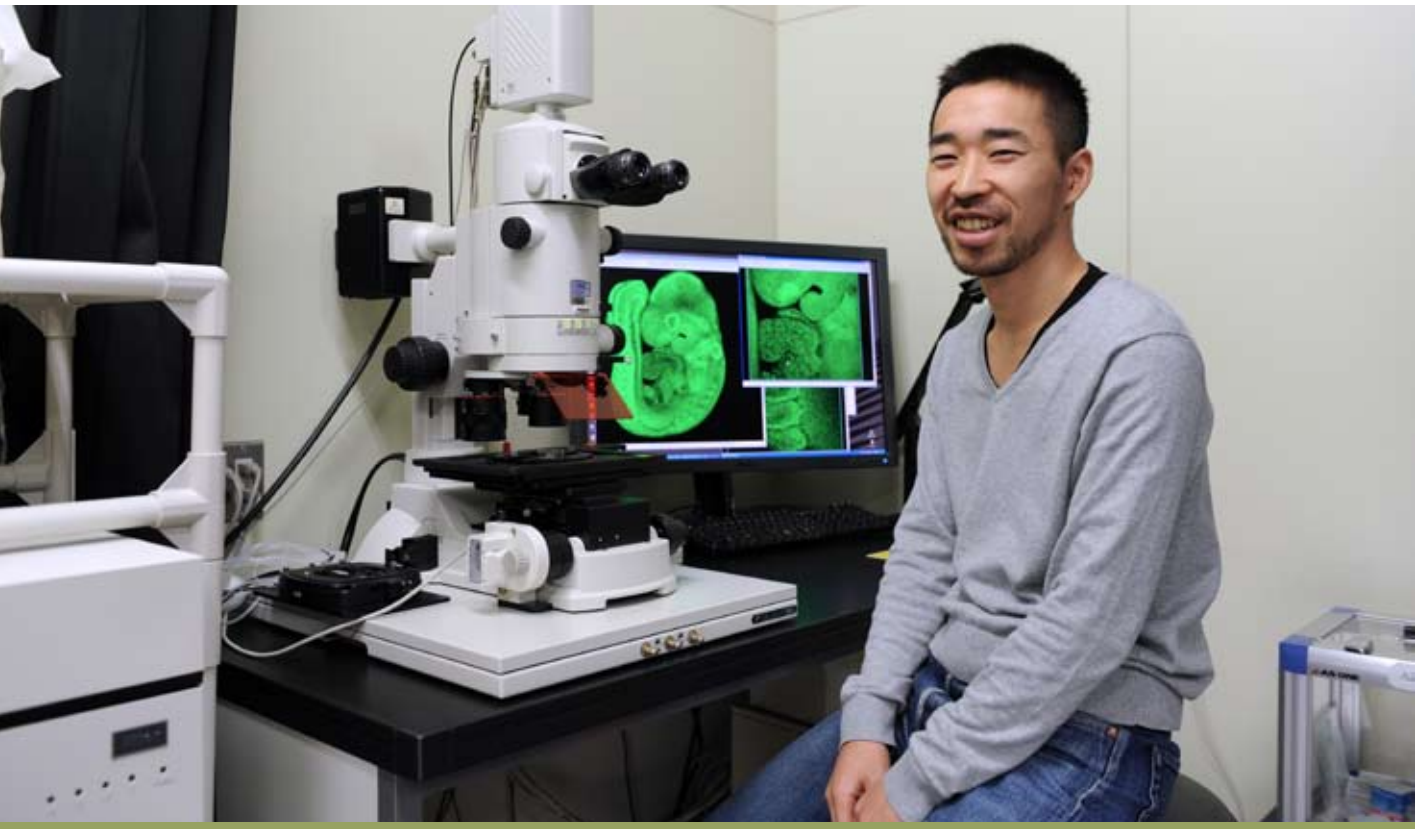
In a final series of experiments, Suga et al. transplanted the ESC-derived ACTH-secreting tissue into the kidneys of adult mice whose own pituitary glands had been ablated, to see whether it would be capable of compensating for pituitary function in this model system. Within a week of transplantation, these mice showed strong overall survival, a marked rise in ACTH levels and a concomitant increase in corticosterone (a glucocorticoid hormone stimulated by ACTH) over untransplanted controls, which uniformly weakened and died within eight weeks of hypophysectomy.



ACTH-secreting cells (red) generated from ESC-derived Rathke's pouch

Yoshiki Sasai, leader of the study, commented on this most recent demonstration of the remarkable self-organizing capabilities of embryonic stem cells in vitro. "We have previously shown how ES cells can give rise to self-organized, three-dimensional neuronal and sensory tissues, and in this report we describe for the first time how this principle can be used to generate to an endocrine tissue, suggesting our approach is of general applicability. Suga, himself an endocrinologist, remarks, "We currently treat pituitary deficiencies by hormone replacement, but achieving the correct dosage is not a straightforward problem, given the naturally fluctuating levels secreted within the body. I am hopeful that this new finding will lead to further advances in regenerative medicine in the endocrine system."

New mutant mice for live imaging



Takaya ABE

The study of developmental processes frequently demands the ability to study how individual cells look and act. This has been bolstered in recent years by the development of technologies for fluorescence live imaging, which have made it possible to visually examine and monitor cells in living embryos of various species. Applications of live imaging techniques have been introduced in the mouse as well, but these are complicated the inability to simultaneously label multiple tissues using discrete markers, and the random integration of fluorescence genes, which makes it difficult to constrain sites and dosages of expression.

In a step forward for the field, the Laboratory for Animal Resources and Genetic Engineering (LARGE; Shinichi Aizawa, Laboratory Head) has developed 12 new mutant strains of mice engineered to conditionally express fluorescent markers at seven different cellular sites, such as nucleus or cell membrane. These new mice, engineered using conditional Cre/loxP recombination, express fluorescent proteins at levels sufficient for strong visualization, and enable dual labeling by different fluorescent signals at user-determined developmental sites and time-points. Published in *Genesis*, these novel mouse resources are now available for distribution from LARGE.

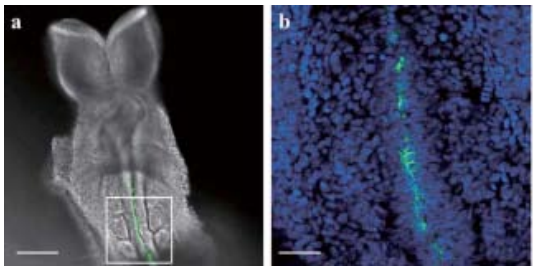
In tackling the challenges posed by mouse biology for live imaging techniques, the lab focused on the ROSA26 locus, which enables introduced genes to be expressed nearly ubiquitously in mouse tissues, and can be mutated on both sites in a pair of chromosomes without causing abnormal phenotypes. They took advantage of these properties by introducing genes for fluorescent proteins into the ROSA26 locus and expressing them conditionally using the Cre/loxP system, which made it possible to engineer a series of mice specially adapted for use in live imaging studies.

Shioi G, et al. A mouse reporter line to conditionally mark nuclei and cell membranes for in vivo live-imaging. *Genesis* 49.570-8 (2011)

Abe T, et al. Establishment of conditional reporter mouse lines at ROSA26 locus for live cell imaging. *Genesis* 49.579-90 (2011)

They kicked off the effort by fusing genes for any of six fluorescence signals, such as EGFP (green) and mCherry (red), with genetic markers of subcellular sites, such as nucleus and mitochondria, yielding a total of 28 reporter genes. Testing these for specificity and strength of expression narrowed the field to 16 candidates, which were then introduced by homologous recombination into the ROSA26 locus in mouse embryonic stem cells (ESCs) accompanied by a stop sequence flanked by loxP sequences, making it possible to conditionally switch on their expression only in cells expressing Cre.

They next crossed these 16 reporter mouse lines with mice designed to express Cre constitutively, and examined their patterns of expression in E7.5 embryos. Twelve of the 16 expressed the reporter genes at appropriate sites and sufficient quantities to enable their use in imaging applications. For their next step, the team crossed mice that heterozygously expressed Venus (red) and mCherry (green) in the cell membrane and nucleus, respectively, thereby generating dual-labeled embryos, and making it possible to study the detailed movements of nuclei in from stage E6.5 to E7.5.



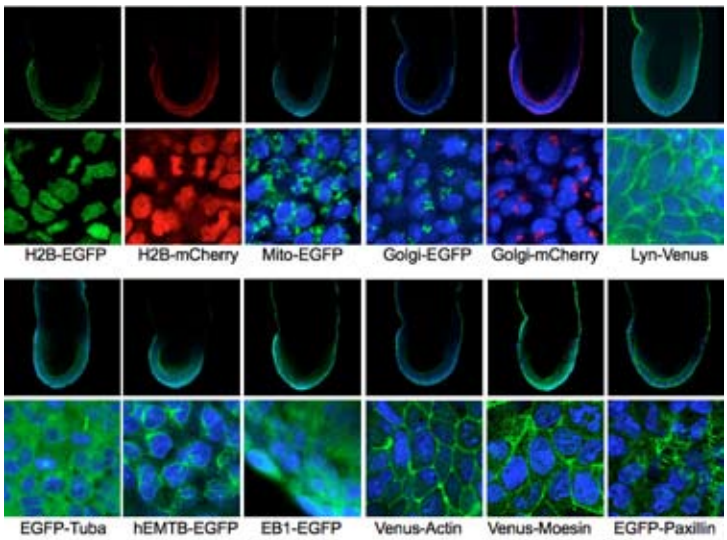
Conditional expression of Venus (green) at the notochord (left); the image at right shows the same site at higher magnification, nuclei stained blue by DAPI

As a final test of the specificity of expression, they crossed mice expressing Venus (green) in the cell membrane with other designed to express Cre only in the notochord. Observing the resulting embryos at E8.5 of development showed clear membrane expression of Venus in a tissue-specific manner.

Localization	Reporter gene (Marker + fluorescence signal)
Nucleus	H2B-EGFP, H2B-mCherry
Mitochondria	Mito-EGFP
Golgi apparatus	Golgi-EGFP, Golgi-mCherry
Cell membrane	Lyn-Venus
Microtubules	EGFP-Tuba, hEMTB-EGFP, EB1-EGFP
Actin cytoskeleton	Venus-Actin, Venus-Moesin
Focal contact	EGFP-Paxillin

List of 12 fluorescent reporter mouse lines

The group published a second report in the same issue of *Genesis*, describing a proof-of-concept application of a dual-labeled line expressing mCherry in nucleus and EGPF in the plasma membrane. Again using the Cre/loxP system, they were able to achieve the specific expression of the genetic reporters for these two organelles only in fetal Sertoli cells, indicating the usefulness of these new resources in live imaging studies.



Expression patterns in E7.5 embryo in sagittal sections (top) and visceral endoderm (bottom)

Spindle mechanism behind nuclear asymmetry

Kenji SUGIOKA

A host of factors, both internal and external, are involved in determining whether a mitotic cell division will give rise to daughter cells of like or different types. Extrinsic signals can exert a variety of effects within the cell, from directly regulating the transcription of genes to remodeling the cytoskeleton. But how structural changes such as the latter translate to downstream effects on cell symmetry remains only poorly understood.

Recent work by Kenji Sugioka and others in the Lab for Cell Fate Decision (Hitoshi Sawa, Team Leader) has revealed a new mechanism by which external Wnt signaling generates mitotic spindle asymmetry, and thereby promotes asymmetric localization of ß-catenin within the nucleus. Published in *Cell*, this work reveals a novel means by which external signals can affect cellular properties in a cytoskeleton-mediated fashion in the roundworm *C. elegans*. Both Sawa and Sugioka have since moved to the National Institute of Genetics in Mishima, Japan.

Early in roundworm development, a cell referred to as EMS receives external Wnt signals from a neighboring cell located posterior to it. Previous work has showed that this asymmetric signal causes WRM-1 (a *C. elegans* homolog of ß-catenin) to accumulate selectively at the anterior cortex of the EMS cell, which subsequently sets up an asymmetry during mitosis resulting in the accumulation of WRM-1 in the posterior daughter cell's nucleus, and a second factor, POP-1, in the nucleus of the anterior daughter. The upshot of these allocations is that the anterior and posterior progeny take on different development fates, with the anterior daughter giving rise to muscle, and the posterior, gut.

Suspecting that the cytoskeletal component known as microtubules might play a role in establishing this nuclear asymmetry, Sugioka treated EMS cells with a microtubule-disrupting chemical, and found that this indeed appeared to affect asymmetric WRM-1 and POP-1 localization in treated nuclei. Looking more closely at the behavior of microtubules during EMS cell division, the team found that astral microtubules increased in the cells' anterior as

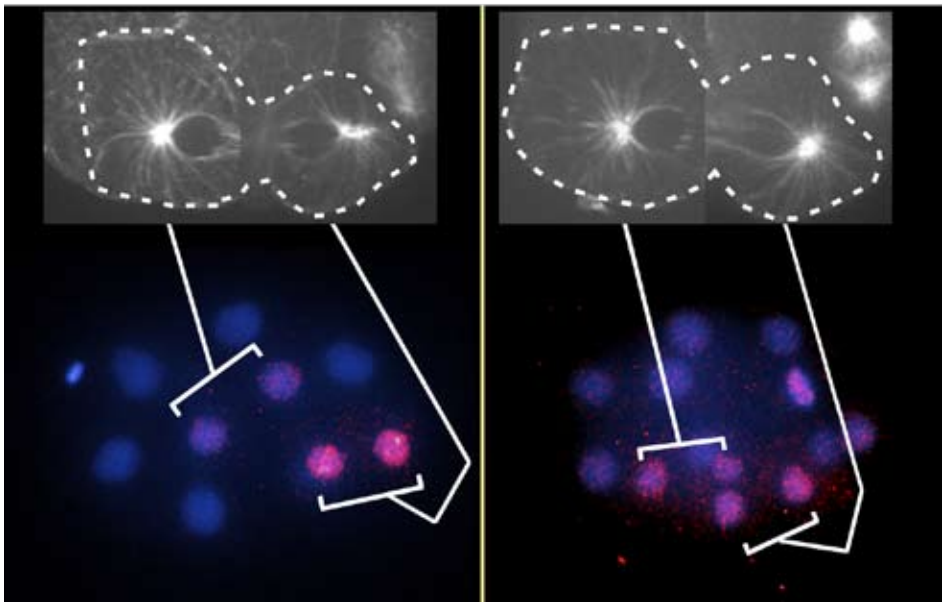
Sugioka K, et al. Wnt regulates spindle asymmetry to generate asymmetric nuclear beta-catenin in C. elegans. *Cell* 146.942-54 (2011)

mitosis progressed, with the greatest anterior-posterior differential occurring at the end stages of division. Inhibiting Wnt downstream factors WRM-1 and APR-1 by RNAi resulted in a decrease in anterior microtubules, indicating that their asymmetric localization is regulated by Wnt signaling.

APR-1 co-localizes with WRM-1 at the anterior cell cortex, and it has been suggested that its mammalian counterpart (APC) has a stabilizing effect on microtubules. Sugioka used a GFP fusion protein to examine the distribution of APR-1 in dividing EMS cells. Early in mitosis there was no apparent asymmetry in its localization, but it gradually accumulated in the anterior side of the cell at later stages, strongly reminiscent of the changes in microtubule distribution observed the previous experiment. Inhibition of Wnt also reduced or eliminated this asymmetry, strongly suggesting that this signaling pathway controls the development of asymmetry by inducing unilateral localization of APR-1, leading to the asymmetric accumulation of microtubules. A subsequent experiment showed that APR-1 binds to and stabilizes the plus-ends of microtubules at the anterior cortex, suggesting a mechanistic basis for this effect.

The team next used an optical (laser) disruption system to directly disrupt microtubule formation and observe the effects on the asymmetric nuclear localization of WRM-1 and POP-1. Perturbing anterior microtubule formation resulted in loss of asymmetric accumulation of these factors in nuclei, while dsirupting posterior microtubules conversely increased this asymmetry. Interestingly, when they used the same technique to induce microtubule asymmetry in Wnt mutants in which microtubules do not form asymmetrically, they found that the nuclear asymmetry of WRM-1 and POP-1 was restored. This set of findings makes a strong case for the role of microtubules in establishing the asymmetric nuclear accumulation of cell fate determinants in dividing *C. elegans* cells.

“It will be interesting to find out exactly what role microtubules are playing in conveying nuclear factors and establishing asymmetry,” says Sawa. “We will also be studying the question of how general this regulatory mechanism is to other biological contexts.”



Microtubules increase in anterior side of asymmetrically dividing EMS cell (top left); asymmetry is lost in Wnt mutant (top right). Only the posterior daughter in EMS cell division expresses gut marker *end-1* (red), but again this asymmetry is lost in the Wnt mutant.

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.

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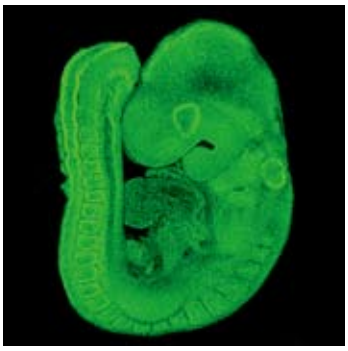
Genetic Engineering Unit

Shinichi AIZAWA Ph.D.

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



A mouse line expressing H2B-EGFP ubiquitously

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Animal Resource Unit

Kazuki NAKAO Ph.D.

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

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

Publications

Abe T, et al. Establishment of conditional reporter mouse lines at ROSA26 locus for live cell imaging. *Genesis* 49.579-90 (2011)

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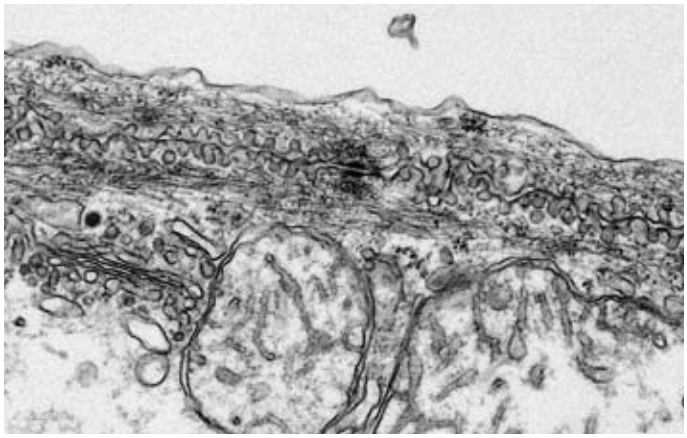
Electron Microscope

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

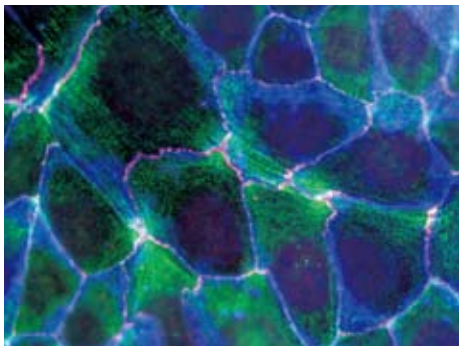
Shigenobu YONEMURA Ph.D.

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

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



Cells organizing the notochord of zebrafish embryo



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

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Publications

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

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Bio-imaging

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

Shigeo HAYASHI Ph.D.

Staff

Unit Leader
Yuko KIYOSUE

Research scientist
Togo SHIMOZAWA

Technical Staff
Tomoko HAMAJI

Part-Time Staff
Satoko NAKAMURA

Agency Staff
Emiko MAEKAWA

Publications

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

Optical Image Analysis Unit

Yuko KIYOSUE Ph.D.

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

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

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

Inverted microscope maintained by the Optical Image Analysis Unit

Genomics

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

Fumio MATSUZAKI Ph.D.

Genome Resource and Analysis Unit

Fumio MATSUZAKI 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 sequencers, making it possible to apply it to a wide range of genomic and epigenomic studies. 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
Fumio MATSUZAKI

Hiroshi TARUI
(~ MARCH 2011)

Technical Staff
Tetsutaro HAYASHI
Kazu ITOMI
Asuka MOMIYAMA
Kaori TATSUMI

Visiting Scientist
Osamu NISHIMURA

Assistant
Chiharu TANEGASHIMA

Publications

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

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

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

Eto K, et al. Reduced expression of a RNA-binding protein by prolactin leads to translational silencing of programmed cell death protein 4 and apoptosis in newt spermatogonia. *J Biol Chem* 284. 23260-71 (2009)

Functional Genomics Unit

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

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

Staff

Unit Leader
Hiroki R. UEDA

Special Postdoctoral Researcher
Yohei SASAGAWA

Research Specialist
Takeya KASUKAWA
Itoshi NIKAIDO

Technical Staff
Junko SAKAI
Kenichiro UNO

Assistant
Yuka TOKUMINE

Publications

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

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

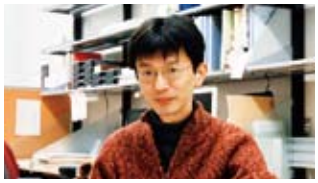
Isojima Y, et al. CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc Natl Acad Sci U S A* 106. 15744-9 (2009)

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

Proteomics

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

Shigeo HAYASHI Ph.D.



Staff

Lab Head
Shigeo HAYASHI

Unit Leader
Akira NAKAMURA

Technical Staff
Kaori SHINMYOZU

Publications

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

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

Hiragami-Hamada K, et al. N-terminal phosphorylation of HP1 promotes its chromatin binding. *Mol Cell Biol* 31. 1186-200 (2011)

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

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

Shimada A, et al. Phosphorylation of Swi6/HP1 regulates transcriptional gene silencing at heterochromatin. *Genes Dev* 23. 18-23 (2009)

Mass Spectrometry Analysis Unit

Akira NAKAMURA Ph.D.

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

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

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

Human Stem Cell Technology

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

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

Human Stem Cell Technology Unit

Yoshiki SASAI M.D., Ph.D.

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

Staff

Unit Leader
Yoshiki SASAI

Deputy Unit Leader
Masatoshi OHGUSHI

Research Specialist
Hiroyuki KITAJIMA

Visiting Scientist
Tokushige NAKANO

Technical Staff
Michiru MATSUMURA-IMOTO
Maki MINAGUCHI

Part-Time Staff
Yoshinori NAKAI

Publications

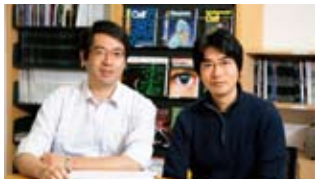
Ohgushi M. and Sasai Y. Lonely death dance of human pluripotent stem cells: ROCKing between metastable cell states. *Trends Cell Biol* 21.274-82 (2011)

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

Four-dimensional Tissue Analysis Unit

Yoshiki SASAI M.D., Ph.D.

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



Staff

Unit Leader
Yoshiki SASAI

Deputy Unit Leader
Mototsugu EIRAKU

Research Scientist
Hidetaka SUGA

Technical Staff
Eriko SAKAKURA

Junior Research Associate
Yuiko HASEGAWA

Publications

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

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

Science Policy and Ethics Studies Unit

Douglas SIPP

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

Staff

Unit Leader
Douglas SIPP

Publications

Cheng T. et al. Stem cell research on the rise in China. *Cell Stem Cell* (2012)

Sipp D. Global challenges in stem cell research and the many roads ahead. *Neuron* 70.573-6 (2011)

Sipp D. The unregulated commercialization of stem cell treatments: a global perspective. *Front Med* 5.348-55 (2011)

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

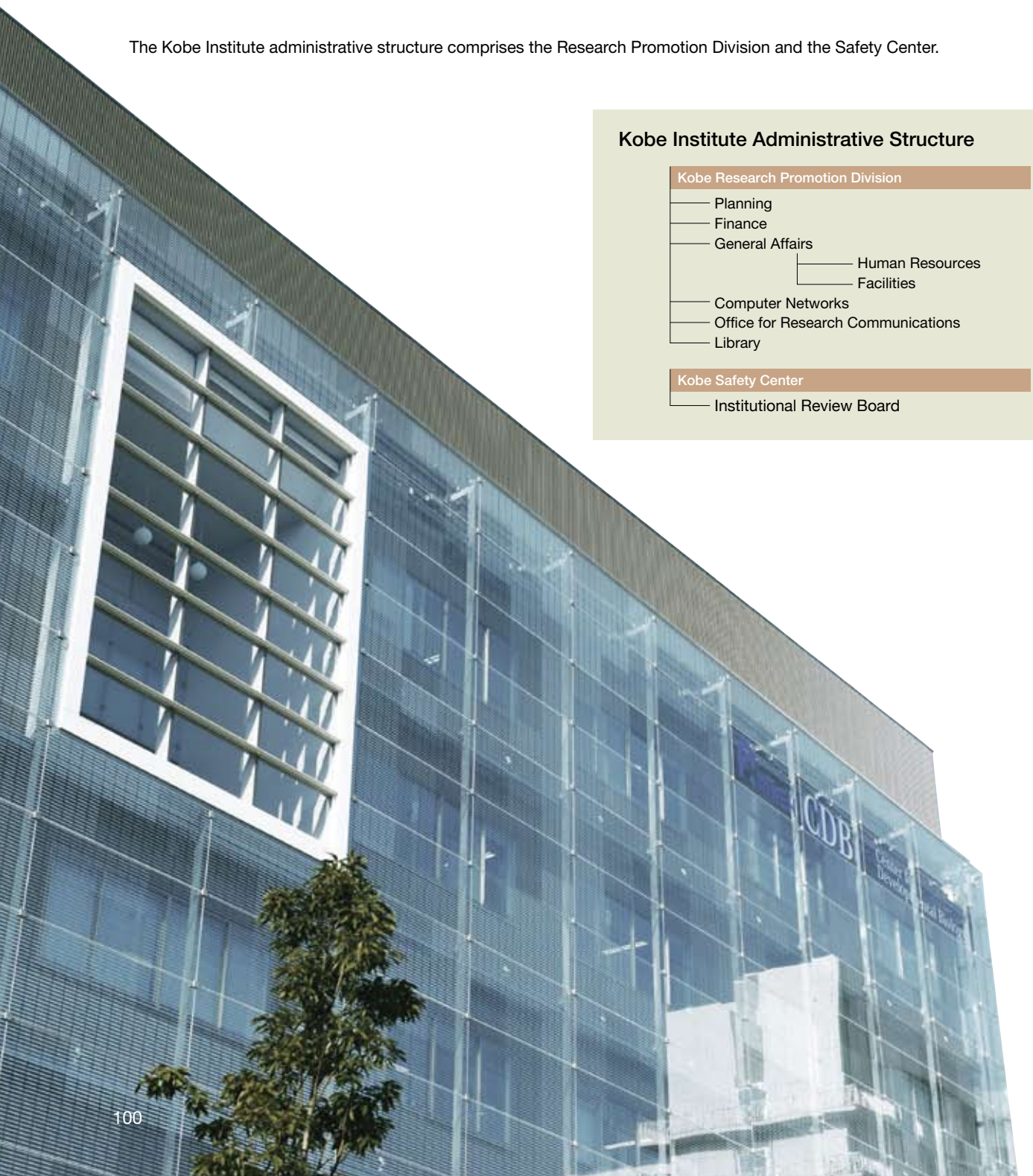
The RIKEN Kobe Institute was established in April 2000 as an organizational framework for the newly launched Center for Developmental Biology (CDB), which conducts a wide range of research, from fundamental studies of development and stem cells, to cutting-edge work with the potential to make a contribution 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). In April 2011, the Kobe Institute welcomed its newest research center, the Quantitative Biology Center, which will focus on systems, simulation, and computational approaches to the life sciences phenomena.

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

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

Kobe Institute Administrative Structure

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



Center for Molecular Imaging Science

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

Quantitative Biology Center

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

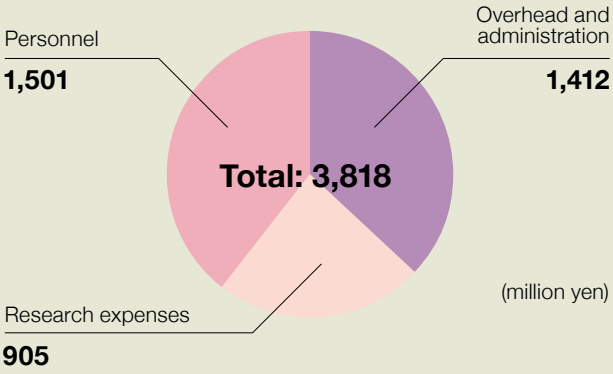
Research Promotion Division

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

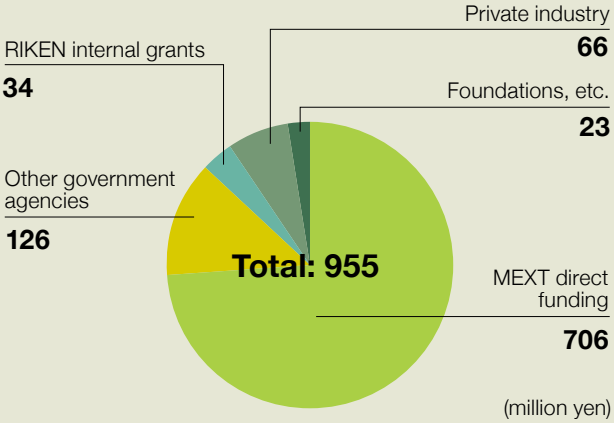
Safety Center

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

2011 CDB Budget



In additional to the dedicated funds outlined above, individual labs and investigators are encouraged to compete for external funding as well, from sources such as the Japan Society for the Promotion of Science (JSPS), the Japan Science and Technology Agency, and other governmental and private sources. These external funds represent a significant component of the Center' s total funding every year, and were particularly significant in the fiscal year ending March 2011, representing an additional 955 million yen in research, facilities, and operating funds.



2011 CDB Staff

Laboratory heads	25
Deputy unit leaders	2
Research scientists	105
Research associates	6
Technical staff	97
Assistants	21
Visiting scientists	113
Student trainees	39
Part-time staff	34
Other	22
Total	464
Research Promotion Division*	83

*Administrative staff for Kobe Institute


2011 CDB Symposium

Epigenetic Landscape in Development and Disease

March 14 - 15, 2011

The RIKEN CDB hosted its ninth annual symposium, “Epigenetic landscape in development and disease” on March 14–15. This year’s symposium was held on an abbreviated two-day schedule in the aftermath of the Great Tohoku Earthquake and tsunami that struck northeastern Japan on March 11, and opened with a moment of silence for those affected by the disaster.

Although a number of speakers and attendees were forced to cancel their participation due to travel disruptions and other concerns, more than 180 people were able to gather to share and discuss the latest developments in the study of epigenetics as it relates to ontogeny and pathology. In addition to the 21 talks, each day included a poster session in which nearly 70 poster presenters discussed their work.

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

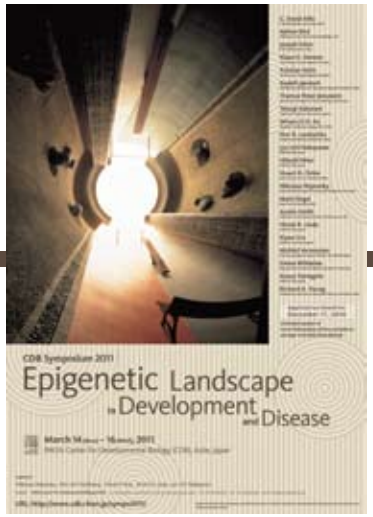
Session 1
Jun-ichi Nakayama (RIKEN CDB, Japan)
Klaus Hansen (University of Copenhagen, Denmark)

Session 2
Tetsuji Kakutani (National Institute of Genetics, Japan)
Rudolf Jaenisch
(Whitehead Institute for Biomedical Research & MIT, USA)

Session 3
Ichiro Hiratani
(National Institute of Genetics, Japan)
Kazuo Yamagata
(RIKEN Center for Developmental Biology, Japan)
Tetsuya J. Kobayashi (The University of Tokyo, Japan)

Session 4
Mark L. Siegal (New York University, USA)
Richard A. Young
(Whitehead Institute for Biomedical Research & MIT, USA)
Hiroki R. Ueda (RIKEN CDB, Japan)

Session 5
Minoru S.H. Ko (National Institutes of Health, USA)
Emma Whitelaw
(Queensland Institute of Medical Research, Australia)
Shahragim Tajbakhsh (Institut Pasteur, France)



Session 6
Hitoshi Niwa (RIKEN CDB, Japan)
Berthold Göttgens (University of Cambridge, UK)

Session 7
Rudolf Jaenisch
(Whitehead Institute for Biomedical Research & MIT, USA)
Michael Kagey
(Whitehead Institute for Biomedical Research, USA)
Yanina Tsenkina (University of Edinburgh, UK)
Yan Fung Wong(RIKEN CDB, Japan)

Session 8
Kiyoe Ura (Osaka University, Japan)
Kristian Helin (University of Copenhagen, Denmark)

Session 9
Austin Smith
(Wellcome Trust Centre for Stem Cell Research, UK)

2012 CDB Symposium

Quantitative Developmental Biology

March 26 – 28, 2012

The tenth annual symposium "Quantitative Developmental Biology" will be held on March 26 – 28, 2012 in the CDB Auditorium. We plan to discuss experimental, computational, and theoretical approaches toward understanding principles of development. By facilitating communication between the leaders in these fields and promising younger scientists at a collegial forum, we hope to create a unique atmosphere that provides a comprehensive perspective on how we can apply quautitative approaches to elucidate principles of development, which we hope will generate fresh perspectives and new interactions.

Invited Speakers

Buzz Baum
MRC Laboratory for Molecular Cell Biology, UK

Christopher Chen
University of Pennsylvania, USA

Cheng Ming Chuong
University of Southern California, USA

Lance Davidson
University of Pittsburgh, USA

Suzanne Eaton
Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Darren Gilmour
European Molecular Biology Laboratory, Germany

Shigeo Hayashi
RIKEN Center for Developmental Biology, Japan

Frank Jülicher
Max Planck Institute for the Physics of Complex Systems, Germany

Shigeru Kondo
Osaka University, Japan

Edwin M. Munro
The University of Chicago, USA

Eugene W. Myers
HHMI Janelia Farm Research Campus, USA

Stuart A. Newman
New York Medical College, USA

Shuichi Onami
RIKEN Quantitative Biology Center , Japan

Przemyslaw Prusinkiewicz
The University of Calgary, Canada

Satoshi Sawai
The University of Tokyo, Japan

Ralf Schnabel
TU Braunschweig, Germany

James Sharpe
Centre for Genomic Regulation, Spain

Tatsuo Shibata
RIKEN Center for Developmental Biology, Japan

Tadashi Uemura
Kyoto University, Japan

Cornelis Weijer
University of Dundee, UK

Orion Weiner
University of California, San Francisco, USA

CDB Seminars

The RIKEN CDB makes special efforts to provide a full and diverse series of invited seminars by scientists from around the world. To date, the Center has hosted more than 600 such talks, in addition to numerous meetings, distinguished lectures, internal forums, and colloquia. The following speakers presented CDB Seminars in the period from January to December 2011.

Date	Title	Speaker
01-11	Evolutionary design of robust oscillatory genetic networks	Yasuaki KOBAYASHI
01-24	Physical approach to the origin of life: How nature separates molecules	Yusuke T. MAEDA
01-25	Building the vertebrate body: molecular mechanisms of progenitor cell maintenance and lineage specification	Benjamin L. MARTIN
01-26	Transcriptional control of midbrain dopaminergic neuron development	Siew-Lan ANG
02-01	Towards a gene regulatory network for sense organ progenitors	Andrea STREIT
02-02	Molecular insights into the mammalian circadian clock: A link to nuclear receptor-mediated transcription and flexibility of its transcriptional networks	Jürgen A. RIPPERGER
02-08	Mathematical approaches to developmental biology; geometrical analysis of organ deformation and coding design of positional information	Yoshihiro MORISHITA
02-08	Hydroxylation regulates chromatin demethylation	Yuichi TSUKADA
03-09	Using patient-derived iPS cells to investigate cellular phenotypes in Timothy syndrome	Masayuki YAZAWA
04-11	Analysis of germ cell immortality	Shawn AHMED
04-14	Estimating the dynamics of forces during morphogenesis	Shuji ISHIHARA
04-21	How do cell-surface molecules specify synaptic-layer targeting in the visual system?	Takashi SUZUKI
05-16	Mechanical forces due to cell destruction: Biophysical investigation of the role of apoptosis in tissue dynamics during embryogenesis	Yusuke TOYAMA
05-17	Polarity is destiny	Henrik SEMB
06-01	The role of chromatin orchestra in circadian rhythms	Satchidananda PANDA
06-09	Stem cells and the origin of prostate cancer	Michael M. SHEN
06-09	Complete 3D kinetochore tracking reveals error-prone homologous chromosome biorientation during mouse oocyte meiosis	Tomoya KITAJIMA
06-10	Genome-wide RNAi screen identifies novel genes required for the response to hypoxia	Pablo WAPPNER
06-13	Molecular mechanisms of small RNA pathways in *Drosophila*	Katsutomo OKAMURA
06-16	Introduction to CSH Asia/CSHL and the importance of effective communication in science	Maoyen CHI

Date	Title	Speaker
06-23	Synthetic cell biology: Visualizing and manipulating cell signaling	Takanari INOUE
07-11	Sleep/wakefulness control using optogenetics in mice	Akihiro YAMANAKA
07-20	Dissecting microRNA function using mouse genetics and genomics	Stefan A. MULJO
07-26	The contributions of palaeontology to the study of development in a molecular world	Marcelo SÁNCHEZ
08-01	What happened to the genome of vertebrate closest living relatives?	Daniel CHOURROUT
08-17	A matter of commitment: TGFβ signaling in the regulation of neural stem/ progenitor cells	Yasuhide FURUTA
09-13	Multifaceted analysis of the *Drosophila* epithelial barrier junction, the septate junction	Kenzi OSHIMA
09-13	The retina as a model to explore different pools of neuronal progenitors	Caren NORDEN
09-14	Immunoglobulin gene conversion and somatic hypermutation: regulation, evolution and biotechnology	Hiroshi ARAKAWA
09-15	Dynamics of cell cycle transitions	Attila CSIKASZ-NAGY
10-11	Inter-axonal communication defines presynaptic tiling in *C. elegans*	Kota MIZUMOTO
10-17	Non-conservation of developmental 'toolkit' genes	Shigehiro KURAKU
10-19	Breaking symmetry: Polarization of the *C. elegans* zygote	Fumio MOTEGI
10-24	Establishment of intestinal stem cell culture system: from mouse to human	Toshiro SATO
11-21	Modeling ablation experiments at the cell and tissue level	Philippe MARCQ
11-22	The zebrafish lateral line as a model to study cell migration, morphogenesis and regeneration	Tatjana PIOTROWSKI
12-09	The functional interplay of chromatin insulators and transcription	Olivier CUVIER
12-12	Neuronal mechanisms that induce memories in the fly brain	Hiromu TANIMOTO
12-28	Neuronal cilia : Regulatory mechanism of ciliary length and function in feeding behavior	Yoshihiro OMORI

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.

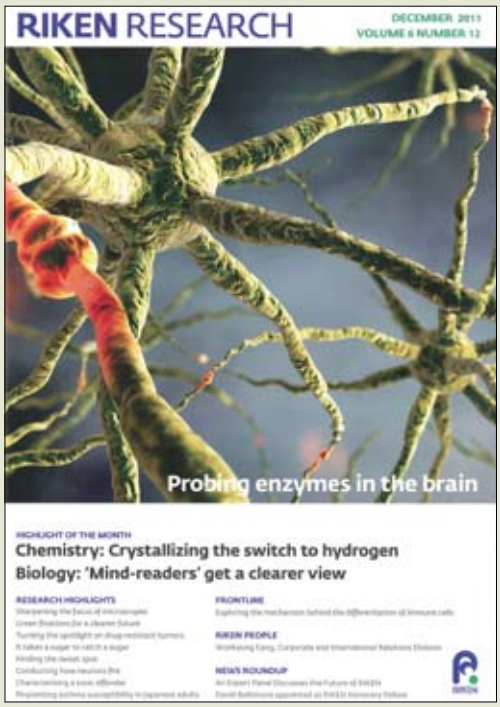
RIKEN was first organized in 1917 as a private research foundation, and reorganized in 2003 as an independent administrative institution under the Ministry of Education, Culture, Sports, Science and Technology.

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



Sendai

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

RIKEN 2012-033