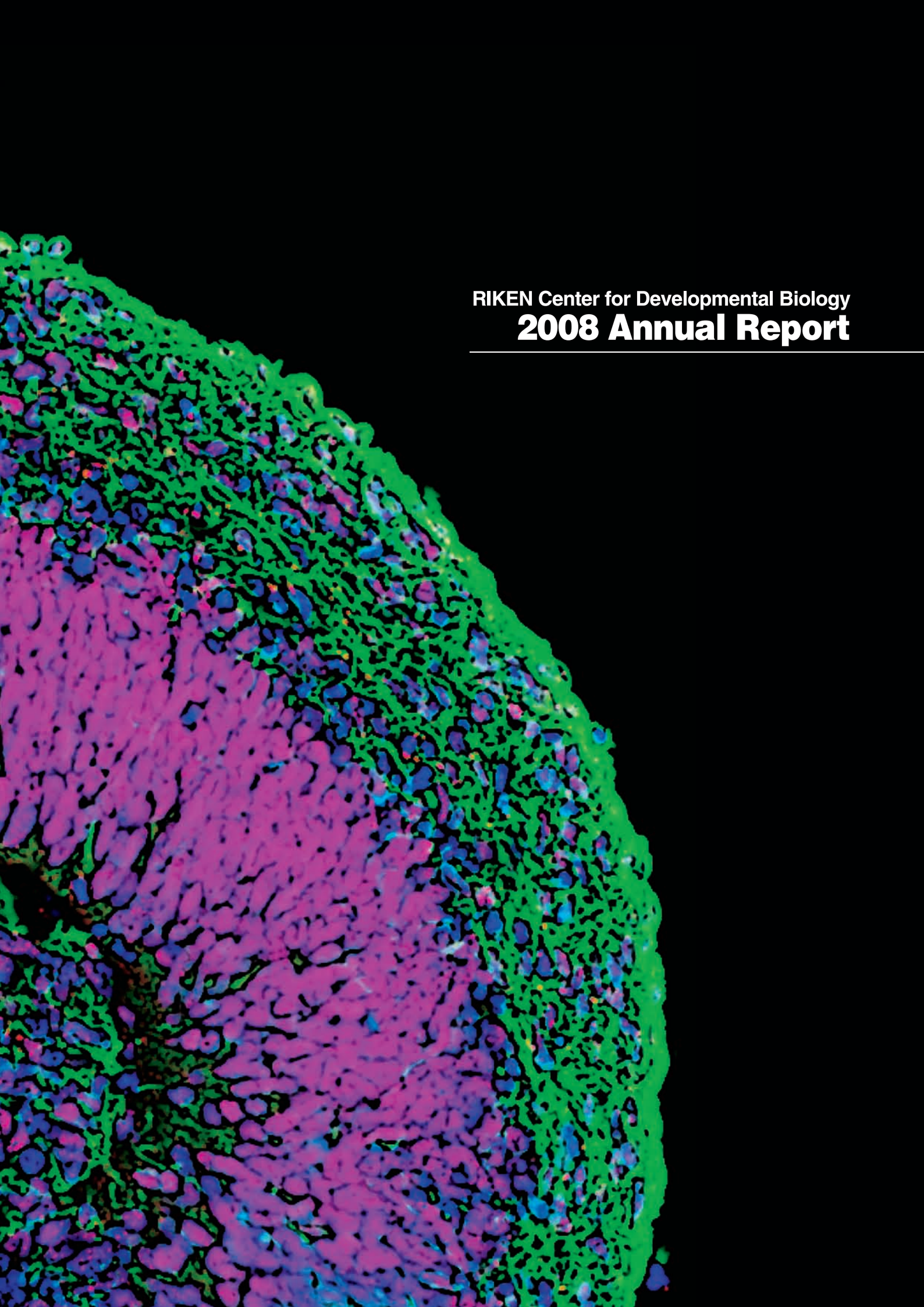

RIKEN Center for Developmental Biology

2008 Annual Report

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Contents

The Center for Developmental Biology was launched in April 2000 under the auspices of the Millennium Project research initiative that was established to drive research in the fields of information technology, environmental science and the study of aging, areas of vital importance to both Japan and the world in the 21st century. Located in Kobe, Japan, the Center's research program is dedicated to developing a better understanding of fundamental processes of animal development at the molecular and cell biological level, the even more complex phenomena involved in organogenesis, 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.

Greetings from the CDB Director

2008 marks a turning point for the RIKEN Center for Developmental Biology, with the reformulation of our research mission to keep pace with the rapid advances in the study of animal development and related fields and in concert with new five-year mid-term plan of the Japanese Ministry of Education, Sports, Culture, Science and Technology (MEXT). As in previous years, many of our labs will continue to focus on genetic, molecular and cell biological mechanisms of development, but we now also seek to broaden that scope to include greater research into organogenesis, defined as studies that explore the formation of higher-order, three-dimensional functional structures involving multiple cell types. And even as we pursue the understanding of development and organogenesis at a fundamental level, many CDB labs will also work to strengthen the scientific foundations for regenerative medicine through research into stem cells and regenerative processes.

With the CDB now in full operation for more than six years, many of the research programs have also entered into a second five-year term, and the past year has seen more publications than ever before. But even more than quantity, what has impressed is the breadth and diversity of CDB research across the board. Investigator-driven research has always been the hallmark of our organization, freeing our scientists to follow wherever nature and their curiosity may lead. In 2008, CDB labs took advantage of that freedom to develop new insights into transcriptional repression in the germline, the migration of neuronal progenitors, the embryonic origins of the blood system, and the genetic underpinnings of circennial biological rhythms. Several reports even drew international media attention, including the cloning of a mouse that had been dead and frozen for 16 years, and the in vitro differentiation of organized cortical tissue from human embryonic stem cells. Our labs also contributed to their fields through the development of research technologies from a method for purifying monoclonal antibodies to low-abundance antigens, to software for bisulfite sequencing analysis, to an improved mouse model of Hirschsprung's disease.

The CDB continues to contribute to the international research community as well, maintaining close involvement with and administrative support for the International Society of Developmental Biologists (ISDB), the Asia-Pacific Developmental Biology Network (APDBN), the Asia Reproductive Biotechnology Society (ARBS) and the Stem Cell Network: Asia-Pacific (SNAP). Our support for Asia-Pacific regional initiatives further underscores our commitment to building and fostering relationships with researchers throughout the region, such as through the sharing of transgenic and knockout mouse resources under the auspices of an APDBN partnering program.

In Japan, we also collaborate widely with scientists in national universities and research centers, and host many graduate students doing the bench work for their theses. We support students and the educational community as well through workshops and courses targeting participant groups from high school students and teachers, to graduate students, to working scientists. Two new highlights for 2008 were the launch of a workshop series for Japanese scientists wishing to learn basic laboratory techniques and ethical procedures for the research use of human pluripotent cells, and a short course organized in conjunction with the Japanese Society for Developmental Biology (JSDB) to provide local high school teachers with instruction in developmental biology concepts and techniques. We continued in our public outreach activities as well with an "Evening of Science and Music" hosted by the Japan Science and Technology Agency (JST) in Kyoto last summer.

A sampling of the full spectrum of CDB activities over the past twelve months is collected in the Annual Report you hold in your hands. Every year we strive to make this report as informative and lively as possible, and to convey through it the sense of excitement we have at confronting the new challenges and opportunities of the years ahead. I hope you find it to be a useful reference and wish you all the best for 2009.

Masatoshi TAKEICHI
Director, RIKEN Center for Developmental Biology

RIKEN Kobe Institute

Center for Developmental Biology

The Center for Developmental Biology is a research center within the RIKEN Kobe Institute, which also comprises the Kobe Research Promotion Division, which provides administrative services and support, the institutional Safety Center and the Molecular Imaging Research Program. The CDB is home to 31 laboratories in its Core Research (7 groups), Creative Research Promoting (19 teams) and Supporting Laboratories (5 labs) programs. The CDB Director is assisted by two deputy directors and advised by the Center's Advisory Council and Institutional Review Board.

Director

Deputy Directors

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 and organogenesis, and the scientific bases of regenerative medicine through the study of stem cells and regeneration.

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 allowed a great deal of flexibility in regard to projects, budget, and lab size. The program also places great emphasis on cooperation and international participation.

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 into new technologies and analytical software, and provide training and instruction on the use of research technologies.

Research Promotion Division

Safety Center

Center for Molecular Imaging Science

Institutional Review Board

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

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.

Neuronal Differentiation and Regeneration
Hideki ENOMOTO M.D., Ph. D.

Neocortical Development
Carina HANASHIMA Ph. D.

Vertebrate Axis Formation
Masahiko HIBI M.D., Ph. D.

Cell Lineage Modulation
Toru KONDO Ph. D.

Sensory Development
Raj LADHER Ph. D.

Germline Development
Akira NAKAMURA Ph. D.

Chromatin Dynamics
Jun-ichi NAKAYAMA Ph. D.

Cell Migration
Kiyoji NISHIWAKI Ph. D.

Pluripotent Cell Studies
Hitoshi NIWA M.D., Ph. D.

Mammalian Epigenetic Studies
Masaki OKANO Ph. D.

Mammalian Molecular Embryology
Tony PERRY Ph. D.

Mammalian Germ Cell Biology
Mitinori SAITOU M.D., Ph. D.

Embryonic Induction
Hiroshi SASAKI Ph. D.

Cell Fate Decision
Hitoshi SAWA Ph. D.

Early Embryogenesis
Guojun SHENG Ph. D.

Developmental Genomics
Asako SUGIMOTO Ph. D.

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

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

Genomic Reprogramming
Teruhiko WAKAYAMA Ph. D.

Animal Resources and Genetic Engineering Laboratory Shinichi AIZAWA Ph. D.
- **Genetic Engineering Unit** Shinichi AIZAWA Ph. D.
- **Animal Resource Unit** Kazuki NAKAO

Electron Microscope Laboratory Shigenobu YONEMURA Ph. D.
Genomics Laboratory Fumio MATSUZAKI Ph. D.
- **Genome Resource and Analysis Unit** Hiroshi TARUI Ph. D.
- **Functional Genomics Unit** Hiroki R. UEDA M.D., Ph. D.

Proteomics Laboratory Shigeo HAYASHI Ph. D.
- **Mass Spectrometry Analysis Unit** Akira NAKAMURA Ph. D.

Division of Human Stem Cell Technology Yoshiki SASAI M.D., Ph. D.

Advisory Council

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

Austin SMITH
(DBAC Chair) University of Cambridge, UK

Margaret BUCKINGHAM
Institut Pasteur, France

Stephen COHEN
Temasek Life Sciences Laboratory, Singapore

Hiroshi HAMADA
Osaka University, Japan

Haifan LIN
Yale University, USA

Yo-ichi NABESHIMA
Kyoto University, Japan

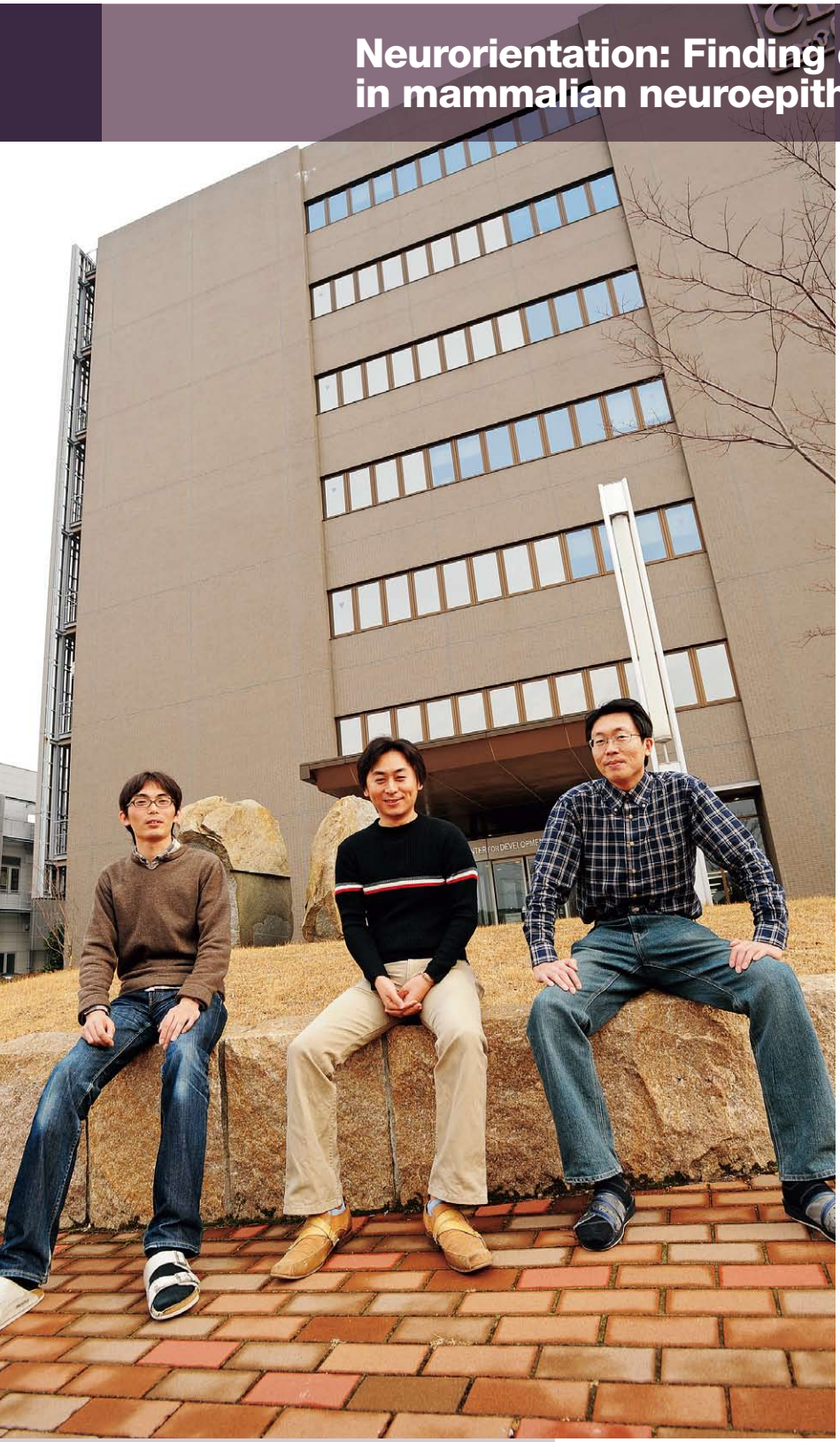
Toshio SUDA
Keio University, Japan

Yoshimi TAKAI
Osaka University, Japan

Patrick TAM
Children's Medical Research Institute, Australia

Chris WYLIE
Cincinnati Children's Hospital Research Foundation, USA

Neurorientation: Finding direction in mammalian neuroepithelial cell divisions



Atsunori SHITAMUKAI, Daijiro KONNO, Go SHIOI

When a cell divides, mitotic spindles form to segregate the replicated chromosomes and allocate the contents of the parent cell to the two daughters. The orientation of these spindles is therefore important, as it impacts directly on the cellular components that each daughter will inherit, and can influence or determine whether the division will be symmetric (producing daughters of identical cell fate) or asymmetric. Asymmetric cell division is particularly key to stem cell biology, as many types of stem and progenitor cell self-renew by generating one self-copy along with a daughter of another fate. This is true of the cells of the neuroepithelium in mammals, which serve as progenitors that both self-renew and give rise to neurons, suggesting that the orientation of the spindle apparatus may play a significant role in ensuring asymmetric mitosis, but its contribution, if any, remains unclear.

Daijiro Konno, Go Shioi and colleagues in the Laboratory for Cell Asymmetry (Fumio Matsuzaki; Group Director) shed light on the control of spindle orientation in neuroepithelial cells, and its function in maintaining the neuroprogenitor population. This work, published in *Nature Cell Biology,* identified the gene *LGN* as a critical component for orienting the mitotic spindle so as to allow neuroepithelial cell division to proceed asymmetrically.

Mitotic spindle orientation has been studied extensively in the fruit fly *Drosophila melanogaster,* revealing an elaborate network of genes that steers the spindles and links them to the cell cortex. Recent work from a number of labs has suggested that similar genetics may underlie this process in mouse neuroepithelium as well. Shioi and Konno began by creating a mutant mouse lacking the gene *LGN,* a murine counterpart to the *Drosophila* gene, *Pins (Partner of Inscuteable),* which participates in the cortical complex responsible for spindle orientation in the fly. Looking at dividing cells in the brain preparations from the *LGN* knockouts, they found that their orientations had become randomized, rather than occurring parallel to the epithelial surface as they do in wildtype animals. A similar spindle misorientation phenotype could also be produced by overexpression of *mInsc,* the mouse ortholog of *Inscuteable,* a *Drosophila* gene that functions to rotate the mitotic spindle perpendicular to the epithelial surface.

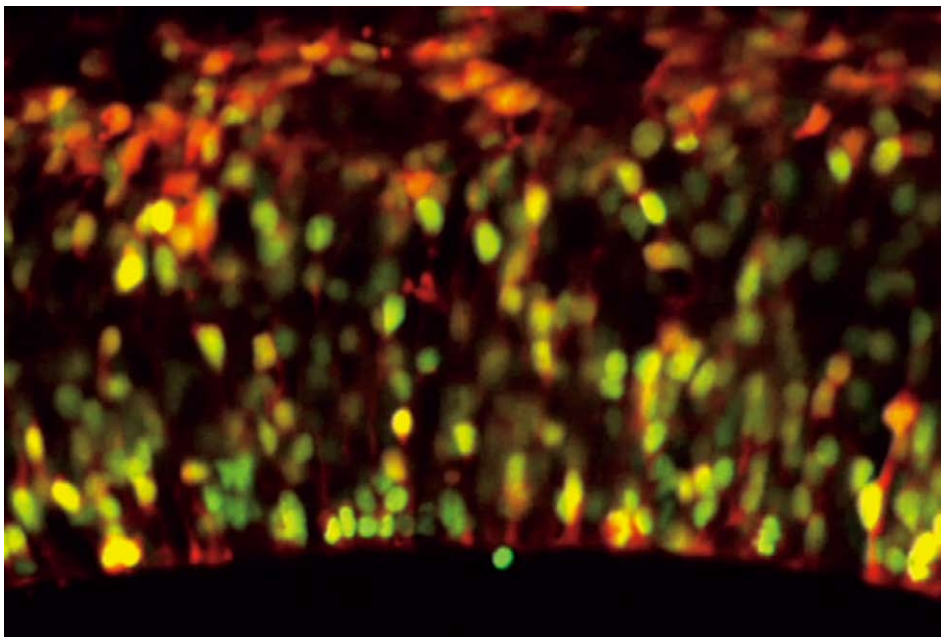
Konno D, et al. Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. *Nat Cell Biol* 10. 93-101 (2008)

To determine whether such misorientation had an effect on daughter cell fate, the Matsuzaki group next examined the distribution of LGN mutant daughter cells expressing the gene *Pax6,* which normally marks apical progenitors. They found that, rather than remaining in the ventricular zone (the region nearest the apical aspect of the neuroepithelium, where cell division generally occurs), *Pax6* cells were scattered more basally. This was also the case for cells overexpressing *mInsc,* suggesting linkage between the failure of the spindles to orient properly and the basal scattering of progenitors.

Apical progenitor cells are normally polarized, extending processes called “feet” from both their apical and basal surfaces. In wildtype, most progenitors divide in such a way that both daughters inherit part of the mother cell’s apical foot. But a third member of the group, Atsunori Shitamukai, found that in the orientation mutants, the apical foot was segregated to only one of the daughters at a much higher frequency. In both cultured brain slice preparations and in vivo, the progenitors produced fewer apical and more non-surface progenitors on division, and as it is the non-surface progenitors that primarily generate neurons, the rate of neurogenesis was unaffected despite this change in composition.

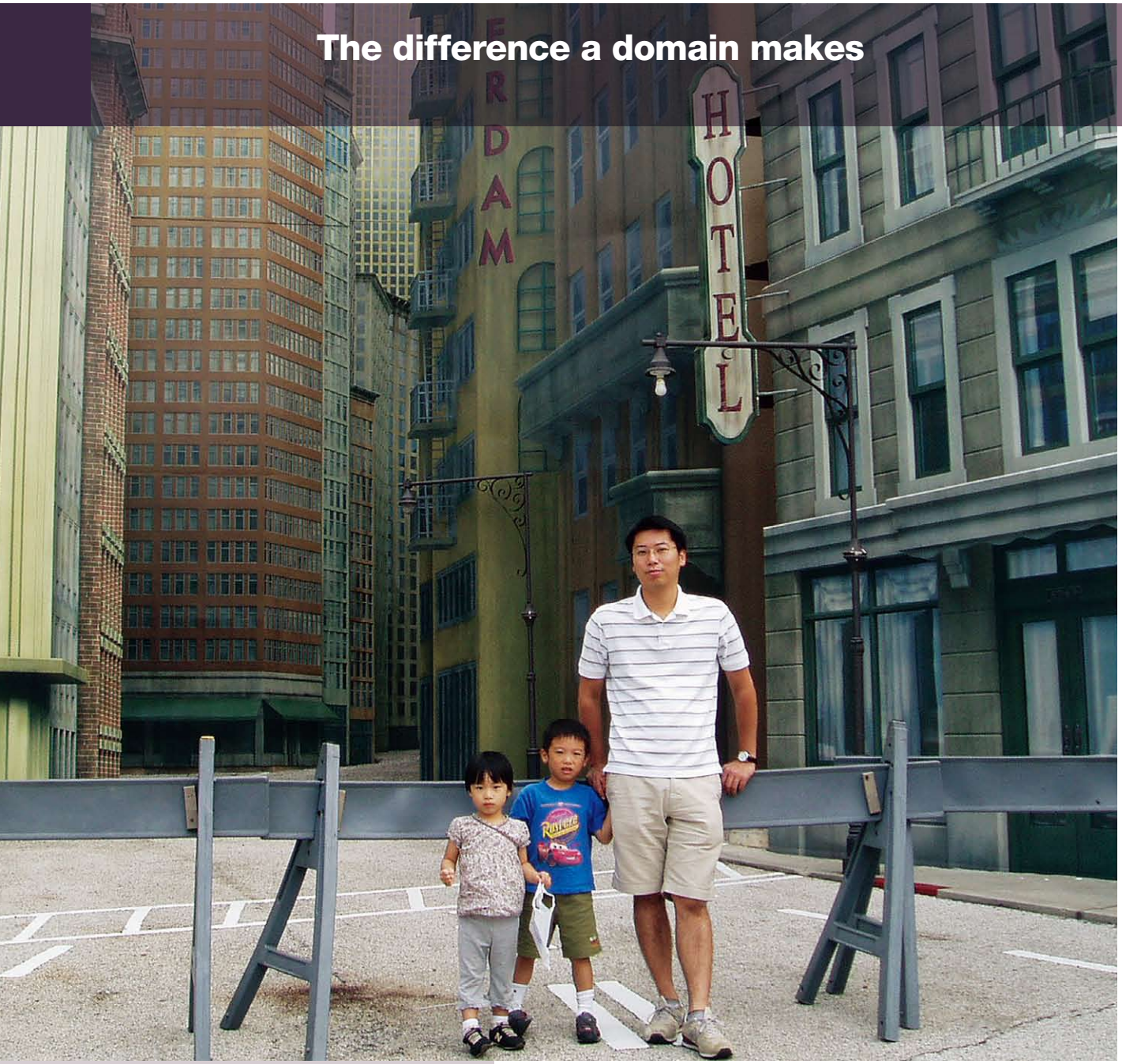
The larger picture that emerged from these findings was intriguing for its subtlety, as it suggests that the inheritance of the basal or apical compartment alone is not sufficient to determine cell fate of the apical progenitors; the failure to acquire an apical foot also seems to play a role in the generation of non-surface progenitors. The group’s observations also call into question some widely held views on what constitutes the normal orientation of neurogenic cell divisions, suggesting that such divisions take place within, rather than perpendicular or oblique to, the epithelial plane.

“It has been predicted that randomizing the orientation of cell divisions should affect epithelial morphogenesis and other phenomena,” notes Matsuzaki. “So the finding that LGN knockout mice, in which the orientation is indeed random, are viable came as something of a surprise to us, and highlights the robustness in the face of perturbations of mammalian development.”



Apical view of neural progenitor cell division. Both daughter receive apical components in the majority of divisions. Boundary between apical cells shown by ZO1:EGFP fusion protein (green). Centrosomes labeled with PACT domain-KO1 (red).

The difference a domain makes



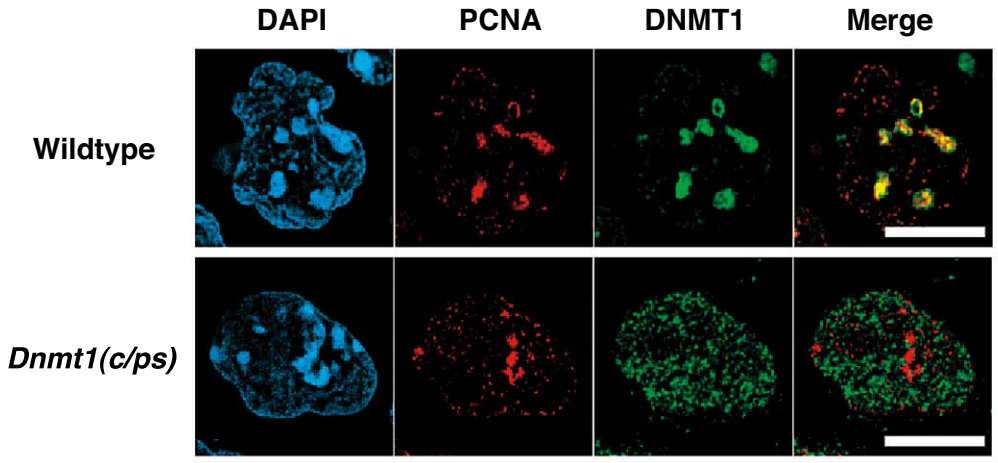
Shin-ichiro (with Rin and Juntaro) TAKEBAYASHI

DNA methylation is an important form of epigenetic marking that generally leads to the formation of heterochromatin, in which DNA is packed securely away from the transcription machinery. The vertebrate genome is stripped of nearly all of its methylation immediately following fertilization, after which different cells reacquire the distinct patterns of methyl residues appropriate to their differentiated state. The establishment and maintenance of DNA methylation is therefore a critical function in the regulation of gene expression. Three DNA methyltransferases have been identified in mammals: DNMT3A and -3B, which work in the establishment of DNA methylation patterns, and DNMT1, which functions in their maintenance during replication. DNMT1 is recruited to the site of DNA replication during the synthesis stage of cell division. Interestingly, although mice carrying a null mutation for *Dnmt1* exhibit very early embryonic lethality, indicating that this methyltransferase is required for development to proceed, the protein's large number of transcription factor-binding domains suggests that it may have multiple functions, which have remained unclear.

Recent work by Shin-ichiro Takebayashi in the Laboratory for Mammalian Epigenetic Studies (Masaki Okano; Team Leader) has brought an additional level of detail to our understanding of just how the DNMT1 enzyme achieves its developmentally important maintenance function. His work, published in *Molecular*

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

and Cellular Biology, reveals an essential role for the DNMT1 catalytic activity in mouse development, as well as the requirement for preexisting DNA methylation for the localization of DNMT1 to replicating DNA. Takebayashi began exploring the possibilities by making a subtle, single amino acid substitution in the catalytic center of the protein encoded by the *Dnmt1* gene, and used this to generate mutant embryonic stem cells and mouse embryos. He confirmed that in both the cells and the embryos DNA methylation levels were greatly reduced, similar to the case in *Dnmt1* null mutants. Even more tellingly, the catalytic defective mutant embryos were arrested shortly after gastrulation, failing to form somites and to undergo closure of the neural tube, phenotypes reminiscent of the null mutation. These findings point to a pivotal role for the catalytic domain in enabling embryonic development.



Nearly total loss of methylation of genomic DNA in nuclei *Dnmt1*^{ps} mutant cells, in which DNMT1 fails to localize to replication sites. Immunofluorescence shows DNMT1 and PCNA (a replication foci marker; scale bar 10 µm) at E9.5.

The team examined the expression of multiple genes in their mutant mice and discovered a range of defects in the regulation of multiple genes, including imprinted genes and retrotransposons, as well as cell growth arrest. Looking at the localization of heterochromatin marks, they found that these too were globally affected; heterochromatin recruitment to pericentromeric regions was lower in all somatic cells. They also saw a small percentage of cells that exhibited hyperacetylation (a marker of transcriptionally active chromatin), a phenomenon that was never observed in wildtype cells. The picture that emerged was one of chromatin structural and gene regulatory abnormalities on the loss of DNMT1 catalytic function, leading to early embryonic lethality.

Curious about how their mutant allele might affect DNMT1 recruitment to replication foci (where it is found in wildtype), they used immunofluorescence to track the mutated DNMT1 enzyme and discovered that it was instead diffusely present throughout the nucleus, suggesting that this defect was specifically attributable to the loss of the catalytic activity, rather than to some overarching functional change to the protein's structure.

Using a replication labeling with 5-methyldeoxy-CTP in embryonic stem cells without the all three active DNA methyltransferases and endogenous DNA methylation, they found that preexisting DNA methylation in a hemi-methylated state is a major determinant for the localization of DNMT1 to the replication foci.

Tead4 triggers trophectoderm

Noriyuki NISHIOKA

The first few steps of mammalian development see a single fertilized egg divide several times to form a compact sphere of cells known as a morula, which subsequently develops into a fluid-filled ball called a blastocyst. It is in the period surrounding the emergence of the blastocyst that the first cellular differentiation processes take place, notably the segregation of the inner cell mass (ICM), which gives rise to the embryo proper, from the trophectoderm, a tissue that contributes to extraembryonic tissues, such as placenta. Recent studies have indicated an important role for the mutual inhibition between a pair of genes, *Oct4* and *Cdx2,* which regulate the ICM and trophectoderm, respectively. The question of potential upstream regulation of these genes, however, remains open.

Noriyuki Nishioka, Shinji Yamamoto and others in the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) showed that the transcription factor Tead4 is required for trophectoderm development, in a manner that suggests it functions upstream of *Cdx2.* Their findings, published in the journal *Mechanisms of Development,* shed new light on the genetic regulation of the earliest cell specialization event in mammalian embryogenesis.

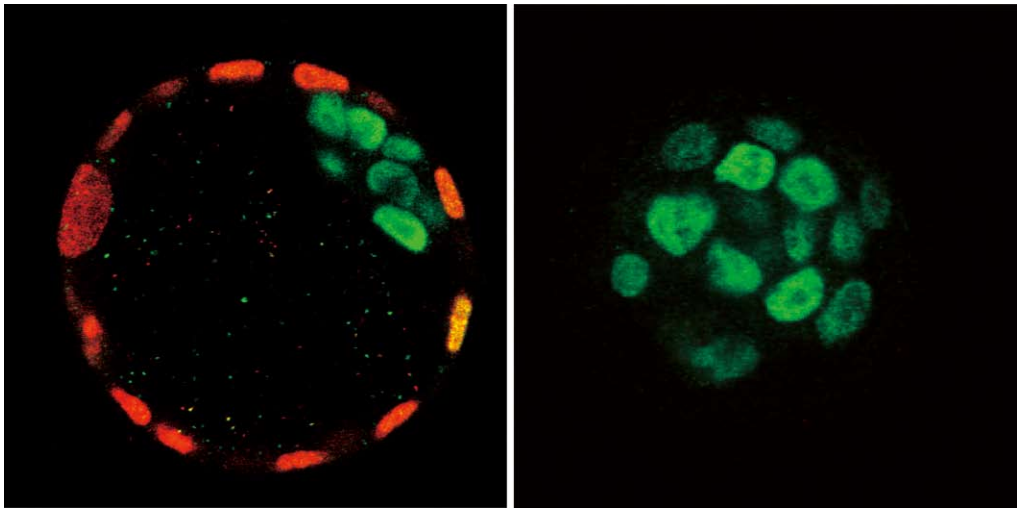
The Sasaki lab's study began with the generation of mice carrying a homozygous deletion of the Tead4 gene, following on their previous work that showed roles for Tead-family genes in the regulation of *Foxa2,* a transcription factor known to regulate the development of the midline signaling centers controlling post-implantation development. They were surprised to find that the knockout embryos died at extremely early stages in development, prior to the implantation of the blastocyst into the uterine wall.

This unexpected early lethality prompted Nishioka and Yamamoto to re-examine the expression of *Tead4,* and the related genes, *Tead1, -2,* and *-3,* in pre-implantation mouse embryos. Using RT-PCR to detect *Tead* transcripts, they found that *Tead4* expression switched on by the 4-cell stage; two other Tead genes, *Tead1* and *Tead2,* were also expressed. They next used immunohistochemistry to attempt to detect the various Tead proteins, and found both Tead1 and Tead4 in all blastomeres (as the individual cells of the early embryo are called), as well as in the cells of trophectoderm and the inner cell mass slightly later in development.

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

Using time-lapse videos of developing embryos, the team determined that, in *Tead4*$^{-/-}$ mutants, although the embryos developed apparently normally up to the morula stage, they failed to form a blastocoel (the cavity of the blastocyst) even as cell proliferation proceeded apace. The blastocoel forms when fluid seeps between gaps in the surface of the embryo and fills the interior, and it is known that this process can be disturbed by defects in cell adhesion. But on studying pathways involved in the formation of adherens and tight junctions, cell polarity and various forms of signaling, they found no abnormalities that could account for the blastocoel failure.

They turned next to the trophectoderm, as this tissue is also a critical requirement for normal blastocyst development. Testing for the expression of *Cdx2,* which is upregulated in the trophectoderm in wildtype embryos and required for TE lineage specification, they found that the gene was only faintly expressed in the *Tead4* mutant embryo after the first several rounds of cell division, and not at all in subsequent stages.



Tead4 is essential for trophectoderm development. (Left) Wildtype blastocyst showing trophectoderm (Cdx2, red) and inner cell mass (Oct3/4, green), respectively. (Right) A Tead4 homozygous mutant embryo at the comparable developmental stage. The blastocoel fails to form, and all cells assume inner cell mass fate, as revealed by the expression of Oct3/4 (green).

This was true of other TE-specific genes, such as *Eomes* and *Fgfr2,* as well. *Cdx2* functions in the pre-implantation embryo by repressing the expression of *Oct4*. When Nishioka and Yamamoto checked *Oct4* expression, they found that it was expressed throughout the late blastocyst, indicating that the entire embryo had adopted an inner cell mass fate.

Given that the ICM can serve as a source for embryonic stem (ES) cells, the team tried to establish an ES cell line using *Tead4* mutants, and succeeded in establishing three ES-like cell lines, one of which was capable of forming colonies in culture and differentiating into all three germ layer lineages in vitro, suggesting that Tead4 is not required for development of embryo proper.

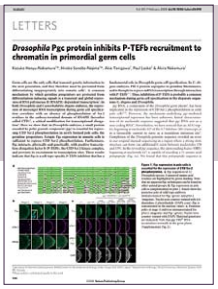
“The finding that trophectoderm completely fails to form in *Tead4* mutants provides an important clue for us to better understand early development in mammals,” says Sasaki. “But the important question of how this gene, which is expressed in every cell in the embryo, can prompt trophectodermal differentiation in only a subset of those cells still needs to be addressed.”

Keeping germline transcription under wraps

Kazuko Hanyu-NAKAMURA, Hiroko Sonobe-NOJIMA

The cells of the germline represent the future of a species, as they are the only cells capable of conveying genetic information from one generation to the next. Given this critical function, it is vital that they be protected from the influence of factors that might cause them to differentiate into somatic lineages. In many taxa, this protection is achieved by the transient and global repression of RNA polymerase II (RNAPII)-dependent transcription in germ cell progenitors, which keeps them from deviating from their purpose. The RNAPII enzyme is encoded in all eukaryotic genomes, and catalyzes the transcription of DNA into messenger RNAs (mRNAs). In some invertebrate species, such as the roundworm *C. elegans* and the fruit fly *Drosophila melanogaster,* the repression of transcription in fledgling germline cells has been linked to the absence of phosphorylation (a form of protein modification) of Ser2 residues in a specific domain in the RNAPII protein, but the means by which this is achieved has been obscure.

Kazuko Hanyu-Nakamura, Hiroko Sonobe-Nojima and colleagues in the Laboratory for Germline Development (Akira Nakamura; Team Leader) have identified the factor responsible for such transcriptional repression in *Drosophila* germ cell precursors. Their work, published in *Nature,* reveals that the product of the *polar granule component (pgc)* gene represses Ser2 phosphorylation of the RNAPII carboxy-terminal domain (CTD) in germline progenitors known as pole cells by interfering with the recruitment of a second factor, P-TEFb, which is known to play a role in CTD Ser2 phosphorylation in vivo.

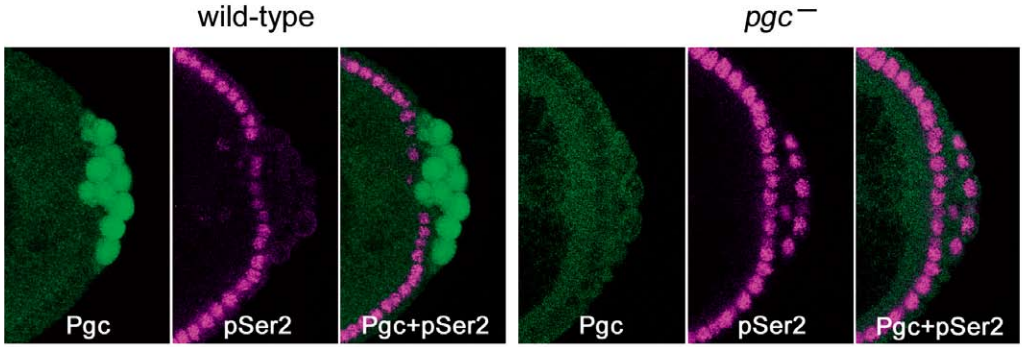

LETTERS

Drosophila Pgc protein inhibits P-TEFb recruitment to chromatin in primordial germ cells

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

The project began somewhat serendipitously when the Nakamura team noted that *pgc* RNA, which was assumed to be non-coding, in fact contains a sequence that suggests a translation start site. Going back to the completed *Drosophila* genome, they found that *pgc* should indeed be capable of encoding a small protein, which they verified by immunostaining; antibodies against the Pgc peptide showed immunoreactivity in early-stage pole cells, before tapering off later in embryonic development.

They next generated flies with various mutations causing losses of *pgc*-function and discovered that, while pole cells did form, there was no repression of Ser2 phosphorylation in the RNAPII carboxy-terminal domain, causing the cells to degenerate by mid-stage embryogenesis. This defect could be rescued by the expression of intact Pgc. Moreover, misexpression of *pgc* in somatic cells resulted in the repression of Ser2 phosphorylation, strongly suggesting that the newfound protein plays a central role in transcriptional repression.

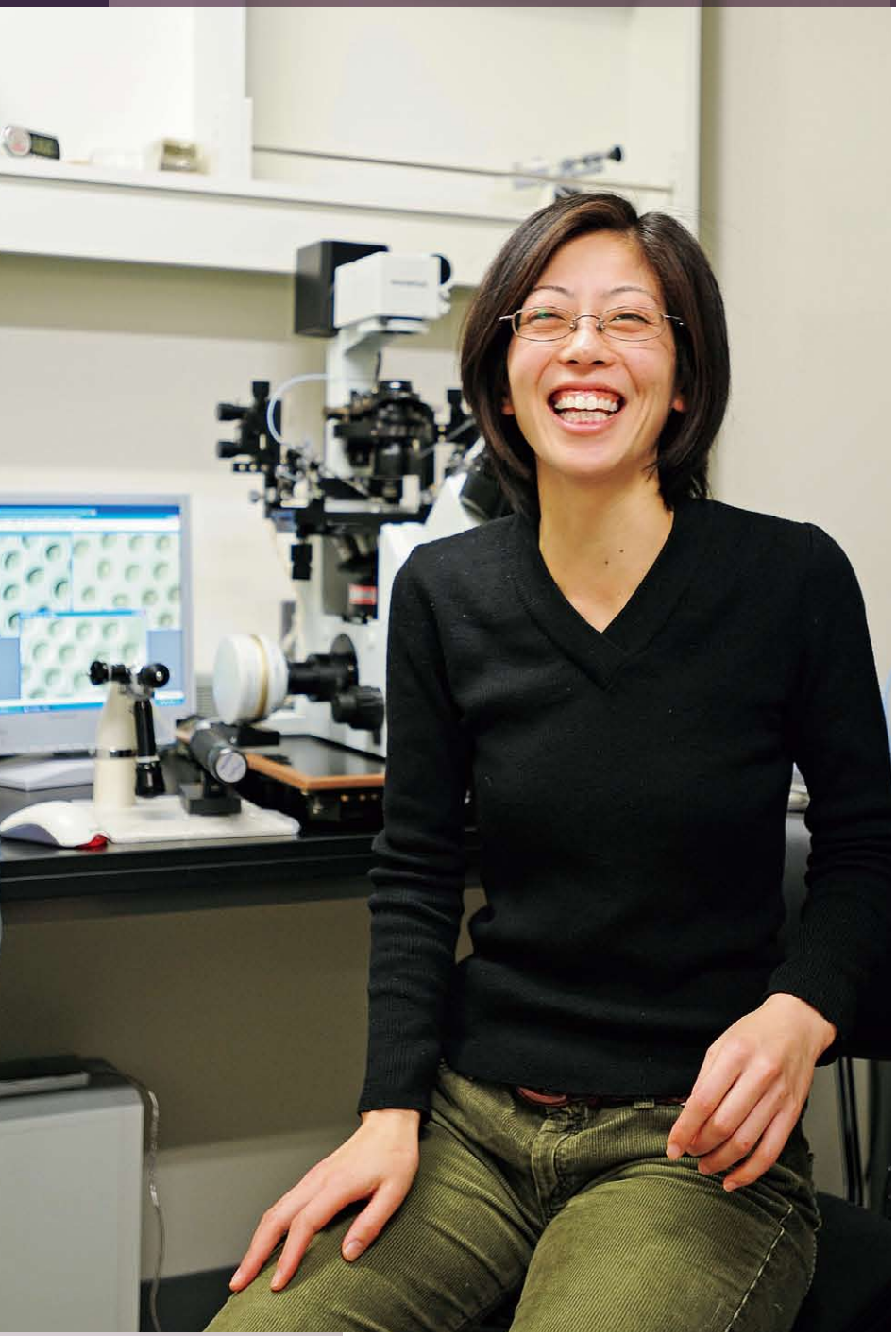


Posterior pole of the blastoderm-stage embryos immunostained for Pgc (green) and CTD phospho-Ser2 (magenta). Pgc is expressed in newly formed pole cells and CTD Ser2 phosphorylation is repressed in these cells (wild-type, left). In the absence of Pgc, pole cells fail to repress CTD Ser2 phosphorylation(*pgc*-,right).

The team next turned their attention to a second factor, P-TEFb (Positive transcription elongation factor b), which had previously been implicated in the phosphorylation of CTD Ser2 and promotion of productive transcription elongation. Suspecting that this might be a target of Pgc, they used co-immunoprecipitation and pull-down assays to establish a specific interaction between the two factors, both in vitro and in vivo, in which Pgc interacts with Cdk9, a catalytic subunit of P-TEFb. Overexpression of P-TEFb in pole cells produced a similar effect to that of loss of *pgc* function, suggesting that Pgc represses Ser2 phosphorylation in pole cells by interfering with the function of P-TEFb. But the finding that an MBP-Pgc construct, which interacted with P-TEFb, did not affect CTD phosphorylation by that factor in vitro indicated that Pgc's function was unlikely to regulate the catalytic activity of P-TEFb. They compared localization of P-TEFb in untreated somatic tissue and tissue in which Pgc was misexpressed, and found a dramatic reduction of their normal localization to active promoter regions on chromosomes. This suggested that Pgc may function by sequestering P-TEFb and preventing its recruitment to promoter sites, a view that was shored up by the finding that Pgc expression inhibited normal P-TEFb recruitment to heat shock genes following heat shock.

"Studies in *C. elegans* have shown that another germline protein, PIE-1, is also involved in regulating the phosphorylation of CTD Ser2 and that it appears to bind to the CyclinT subunit of P-TEFb," says Hanyu-Nakamura. "What's interesting is that Pgc and PIE-1 are non-homologous, which means these germline transcriptional repression systems may well have evolved independently."

Critical role for maternal nucleolus in nuclear transfer

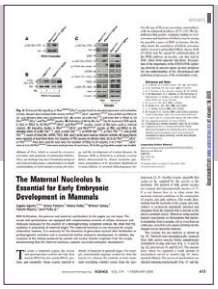


Sugako OGUSHI

Somatic cell nuclear transfer (SCNT), in which the nucleus from one cell (usually an unfertilized oocyte) is removed and replaced by one from another somatic cell, has been touted as a potentially powerful tool to enable personalized regenerative medicine, which has led to the process of human SCNT being called (somewhat prematurely), "therapeutic cloning." Despite its promise, enucleation imposes considerable stress on the egg, and the great majority of embryos created by nuclear transplantation fail to develop. Numerous hypotheses have been proposed to explain the high fail rate of cloning attempts, but the cause has remained tantalizingly out of reach after more than a decade of intense research.

New work by Sugako Ogushi in the Laboratory for Mammalian Germ Cell Biology (Mitinori Saitou; Team Leader) took a major step toward answering that question with the demonstration that the oocyte nucleolus is essential to early embryonic development in mammals. This study, done in collaboration with scientists from Kobe University, Italy, and the Czech Republic and published in the journal *Science,* shows for the first time the indispensable role played by the maternal nucleolus, possibly as the source of materials needed to form the zygotic nucleolus in the first stages of embryogenesis.

The nucleolus, which forms a part of the nucleus, is mainly responsible for assembling ribosomes, the protein factory organelles in which peptides are translated from mRNA. However, a fully-grown oocyte completely ceases transcription including ribosomal RNA synthesis. Its nucleolar structure apparently contains no DNA and shows highly compacted, monotonous morphology of uncertain composition and function. Although nuclear transfer generally involves the removal of the entire nucleus, Ogushi used a micromanipulation system to selectively pluck out only the nucleolus from oocytes taken from pigs and mice, a procedure called enucleolation. She found that, although these enucleolated oocytes were capable of normal maturation and could be activated parthenogenetically or by fertilization, the activated cells failed to develop nucleoli of their own. Interestingly, this defect could be rescued by reinjecting the maternal nucleolus into an enucleolated egg, but not by injection of nucleoli from somatic or ES cells.



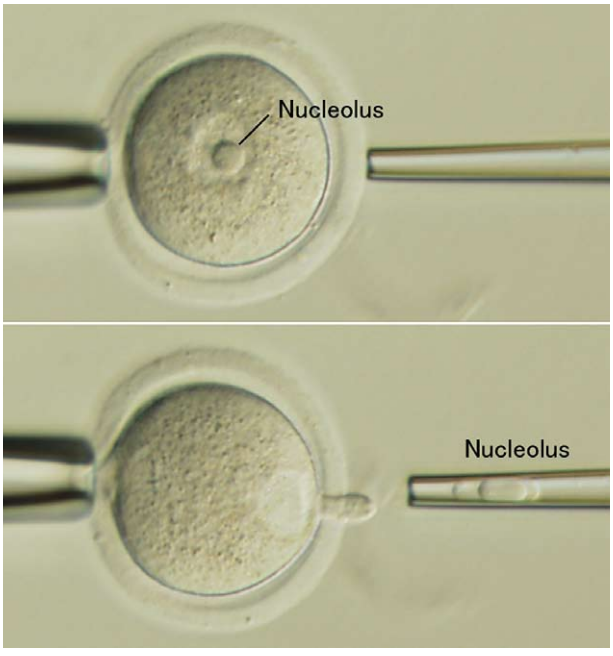
Ogushi S, et al. The maternal nucleolus is essential for early embryonic development in mammals. *Science* 319. 613-6 (2008)

Following the development of enucleolated embryos, Ogushi found that pig embryos produced from oocytes lacking nucleoli failed to develop past the first few cleavages, although their viability was restored by reinjection of the missing maternal nucleolar material. Oocytes that were sham-operated by aspirating a small amount of nucleoplasm but leaving the nucleoli intact developed zygotic nucleoli, and did not suffer the same embryonic arrest, suggesting that the enucleolated embryos' failure was not simply due to the trauma of micromanipulation.

Suspecting that the maternal nucleolus might be the missing ingredient preventing SCNT embryos from developing normally, the team next tried injecting maternal nucleoli along with somatic cell nuclei into oocytes that had had their own nuclei removed. This nucleolar coinjection alone, however, was insufficient to rescue development, indicating that the maternal nucleolus is a necessary, but not sufficient, component for mammalian embryogenesis. It is likely that additional factors in the germinal vesicle (as the oocyte nucleus is called) are also required, as oocytes in which the germinal vesicle has disintegrated, releasing its contents into the cytoplasm, support development after nuclear transfer, while those in which the germinal vesicle has not broken down, do not.

The discovery that nucleoli are inherited solely from the mother adds these structures to the list of organelles, such as mitochondria, that are passed on from only one of the parental lineages. It has been known for years that the early embryo relies on a stock of materials from the oocyte to drive development over the first few rounds of cell division. These new findings indicate that the nucleolus may be an important maternal contribution, needed by the embryo to build nucleoli of its own.

"In this study, we showed that the nucleolar structure in a fully-grown oocyte is essential for early embryonic development, and that it contributes to the nucleolar structure of the paternal pronucleus, demonstrating that the nucleolus of a zygote is exclusively of maternal origin" says Ogushi. "Although we still don't know its precise function and molecular components, we are now working to answer those questions. We hope these studies will lead to a better understanding of how a totipotent zygote is constructed."



Removal of the nucleolus from the oocyte. A mouse oocyte (upper panel) has a prominent nucleolus inside the nucleus. The nucleolus is gently aspirated by a very thin pipette (bottom panel).

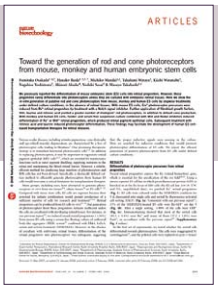
Breakthrough in photoreceptor differentiation from ES cells

Fumitaka OSAKADA

Visual impairment can be caused by loss of function in various parts of the eye, but the majority of currently incurable causes of vision loss, such as retinitis pigmentosa and age-related macular degeneration, involve the retina. Although retinal neurons regenerate only minimally in mammals under normal conditions, this region at the back of the eye represents one of the most promising targets for regenerative medicine in the nervous system. Such therapies may one day involve stimulating endogenous stem cells to regenerate damaged neural tissue or, alternatively, generating functional retinal cells in vitro and transplanting them into patients.

A report by Fumitaka Osakada, Hanako Ikeda and colleagues in the Laboratory for Retinal Regeneration (Masayo Takahashi; Team Leader) made an important stride toward realizing that dream. Their study, conducted in collaboration with clinicians and scientists from Kyoto University, and the CDB Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) and published in *Nature Biotechnology,* reports the high-efficiency generation of rod and cone photoreceptors and pigmented epithelium from mouse, primate and human embryonic stem cells under fully characterized culture conditions.

The Takahashi lab and others had previously developed methods for inducing the differentiation of retinal neural progenitors, but these relied on co-culture with embryonic retinal tissue or other confounding factors, and generally produced retinal neural cells at unacceptably low efficiencies. Working first with mouse embryonic stem (ES) cells, they sought to identify factors capable of spurring photoreceptor differentiation in a more reliable and efficient manner. After isolating progenitor cells and confirming their competence for further differentiation to retinal precursors (as shown by their expression of the marker gene *Crx*), the Takahashi team found that the γ-secretase inhibitor DAPT steered the mitotic progenitors to adopt a postmitotic precursor fate.


ARTICLES

Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells

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

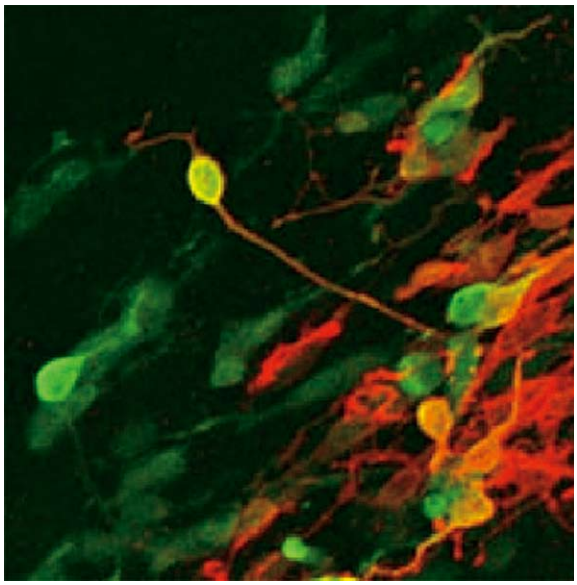
They next tested a panel of factors implicated in photoreceptor differentiation on the *Crx*$^+$ precursor cells, and found that a combination of aFGF, bFGF, taurine, Shh, and retinoic acid in retinal culture medium prompted nearly 30% of the precursors to begin expressing the light-sensitive opsins characteristic of either rod or cone photoreceptors.

One drawback of the method used for inducing differentiation from mouse ES cells is that it involves the use of fetal bovine serum, which problematizes its use for clinical applications. Seeking a workaround, Osakada turned next to a chemically defined medium for use with ES cells from cynomolgus monkeys and found that the serum free floating culture of embryoid body-like aggregates (SFEB) system worked for primate cells as well, without requiring the use of bovine serum. They next tested a pair of factors that had been shown to promote retinal-lineage differentiation – the Wnt-antagonist Dkk1 and the nodal-antagonist Lefty-AS – and found that in combination, these were highly effective in boosting the differentiation of retinal pigmented epithelium.

They further found that by treating retinal progenitors derived from monkey ES cells with retinoic acid and taurine, two of the factors used in the mouse ES study, they could strongly promote differentiation of *Crx*-positive postmitotic precursors, and subsequently of rod and cone-like cells expressing opsins appropriate to their putative cell types.

In a final set of experiments, the Takahashi lab assessed whether their findings using mouse and primate ES cells could be replicated using human embryonic stem cells as well. Using the SFEB culture (originally developed by the Sasai group) optimized for use with human ES cells, they found that as in the monkey ES cell tests, the combination of Dkk1 and Lefty-A caused a significant number of the undifferentiated cells to assume a retinal pigmented epithelial fate, as determined by morphology and gene expression. Differentiation of *Crx*$^+$ precursors into photoreceptor-like cells using taurine and retinoic acid also proved successful, although at slightly lower efficiency than in monkey cells, establishing this new technique as a potentially important tool for generating human photoreceptors for use in drug screening and future regenerative medical applications.

"Now we have the cells that might one day be useful for retinal cell transplantation therapy, but it needs to be remembered that these are only some of the raw materials. We will still need to establish methods for purifying them, and getting them to function in vivo after transplantation," cautions Takahashi. "We also need to recognize the differences in closeness to clinical application between retinal pigmented epithelial cells and photoreceptor cells. We now need to think practically about how to translate this basic research, and the hurdles to that lie ahead."



Photoreceptor cells differentiated from human ES cells.

ES cells show diversity

Yayoi TOYOOKA

The first few days of embryonic development in the mouse witness the zygote undergo a transformation from a single cell, to a raspberry-like cluster (morula), to a hollow ball (blastocyst) before implanting in the uterine wall. Cell differentiation begins by about the third day of embryogenesis, with exterior cells entering the trophectodermal lineage, a tissue that forms the placenta and other extraembryonic tissues, and interior cells, which take the first step of the path leading to various somatic fates. The inner cell mass (ICM) of the blastocyst is famous as the source of embryonic stem cells, although it has been shown that ES-like pluripotent cells can also be obtained from the epiblast, a transient structure that arises slightly later in development, after implantation. The epiblast in turn gives rise to primitive ectoderm, which is considered to be the source of the somatic lineages that yield the many differentiated cell types in the adult body. ES cells are of great interest because they show similar ability to give rise to all somatic lineages in culture, but it remains unclear whether all ES cells are truly created equal, or whether they show diversity among themselves.

A study by Yayoi Toyooka and colleagues in the Laboratory for Pluripotent Cell Studies (Hitoshi Niwa; Team Leader) came down clearly in favor of the heterogeneity theory, showing that subsets of ES cells exhibit gene expression variations corresponding to the ICM, epiblast and primitive ectoderm stages of development. In a report published in *Development,* Toyooka, revealed that while all ES cells express the pluripotency marker Oct*3/4,* they show fluctuations in the expression of another gene previously taken as an ES hallmark, *Rex1*.

The study began with the establishment of a system for visualizing the expression of these two genes using a pair of fluorescent proteins. When they cultured the cells using a method to select for Oct*3/4*-expressing cells, they found that some of these expressed *Rex1* while others did not, with predominantly *Rex1*$^{+}$ colonies showing compacted morphologies, and *Rex1*$^{-}$ colonies tending to be flatter.

They used quantitative PCR to examine gene expression in the two subpopulations in more detail, and found that the overall expression patterns in *Rex1*$^{+}$ cells was similar to that seen in inner mass cells, while *Rex1*$^{-}$ cells expressed genes in patterns reminiscent of primitive ectoderm. Interestingly, when they

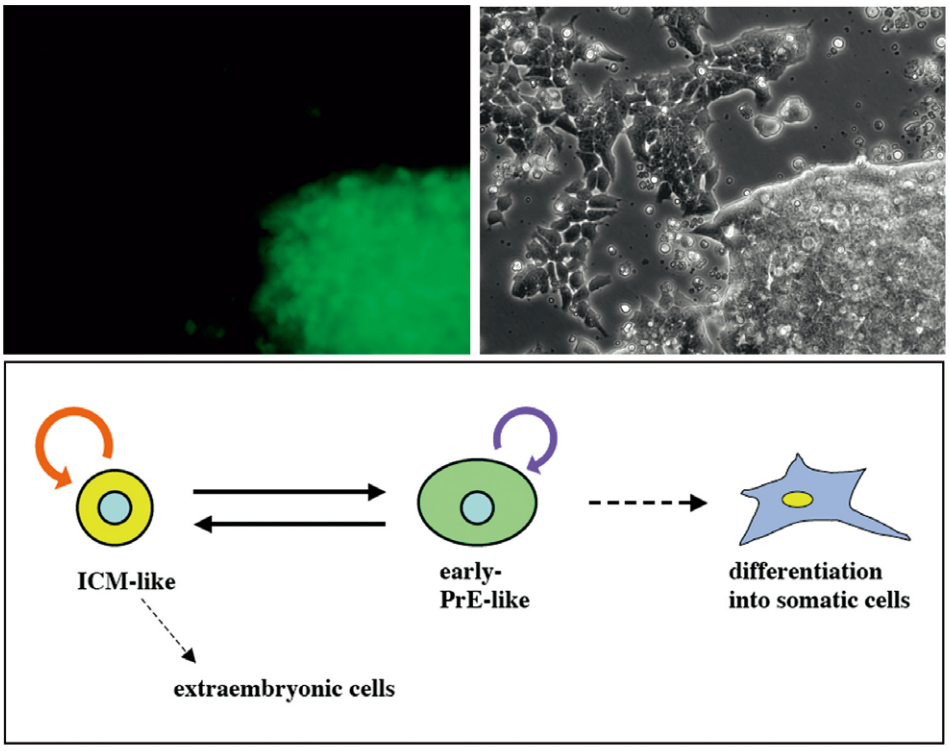
Toyooka Y, et al. Identification and characterization of subpopulations in undifferentiated ES cell culture. *Development* 135. 909-18 (2008)

separated these fractions into purified populations, they found that each was able to give rise to the other spontaneously; that is, *Rex1*$^+$ cells showed up in *Rex1*$^-$ colonies, and vice versa. This worked even in colonies derived from a single *Rex1*-positive or negative cell.

It has long been known that cells from the inner cell mass or epiblast can contribute to chimera formation when injected into blastocyst-stage embryos, but cells taken from primitive ectoderm cannot. Investigating whether this might be true of ES cells in which *Rex1* expression is switched on or off, Toyooka et al. injected either *Rex1*$^+$ or *Rex1*$^-$ cells into mouse blastocysts and found that, similar to ICM and epiblast cells, the *Rex1*$^+$ cells contributed strongly to chimeric embryonic tissues, but that, as is the case for primitive ectoderm, the *Rex1*$^-$cells did not.

Given this apparent correspondence of *Rex1*$^+$ and *Rex1*$^-$ cells to the inner cell mass and primitive ectoderm stage of development, respectively, the Niwa team next checked whether their differentiation would follow a similar pattern. When differentiation was induced in colonies enriched for *Rex1*$^-$ cells by the withdrawal of LIF (a factor that keeps mouse ES cells in an undifferentiated state) they showed a lower tendency to give rise to extraembryonic tissue than did a control group, indicating another similarity with primitive ectoderm cells in vivo. Conversely, *Rex1*$^-$ cells were comparatively easier to steer down somatic pathways, such as mesoderm or neuroectoderm, than were their *Rex1*$^+$ counterparts.

"I suppose that many people who have worked with ES cells already suspected that they were not entirely homogeneous," says Toyooka, "but now, by looking at *Rex1* expression, we've been able to show that there are definite subpopulations in terms of both gene expression and differentiative potential. Perhaps this work will serve as a model for studying the control of fluctuations in gene expression."



Upper panels show Rex1$^-$ positive cells labeled by GFP. Both Rex1$^+$ and Rex1$^-$ cells can be seen. The lower panel shows a cartoon of the two ES cell subpopulations.

Rbsn-5, a new factor in pole plasm assembly

Tsubasa TANAKA

The *Drosophila* oocyte is a polarized structure, with clearly demarcated anterior and posterior ends showing distinct patterns of RNA and protein localization. The posterior pole is home to the pole plasm, from which the cells of the germline originate, and whose assembly is mediated by the protein Oskar (Osk). Interestingly, osk RNA yields two isoforms, long and short Osk, each with distinct functions – short Osk serves as a recruiter of pole plasm components, while long Osk tethers them to the posterior cell cortex through interactions with the actin cytoskeleton governed by some as-yet unknown mechanism.

A study by Tsubasa Tanaka of the Laboratory for Germline Development (Akira Nakamura; Team Leader) has pointed to a hitherto unsuspected link between Oskar and the endocytic pathway that may help to explain how this protein fulfills its function. The report, published in *Development,* reveals that the endosomal protein Rabenosyn-5 is required, apparently downstream of Osk, for pole plasm assembly.

The study arose from a mutagenesis screen to identify mutations that affected the formation of pole plasm, a special form of cytoplasm found in the posterior end of the*Drosophila* oocyte. The screen netted one promising candidate factor that was identified as a homolog of the protein Rabenosyn-5 (Rbsn-5), a Rab-effector protein in mouse that functions in endocytosis, a process in which materials are absorbed from the extracellular environment and sorted for processing.

Given the failure of pole plasm assembly in the *rbsn-5* mutant, Tanaka checked for a possible link to Oskar, and found that although the initial recruitment of *osk* RNA to the posterior pole was unaffected, its accumulation was unstable and deteriorated at later stages. Knowing that *osk* RNA is carried to the posterior via a polarized network of microtubules, he investigated a the effects of loss of *rbsn-5* function on this process, and again found that while microtubule polarity was established, it failed to be maintained.

Although this opened up a plausible explanation for the pole plasm assembly defects in *rbsn-5* mutants, the close co-localization of Rbsn-5 and Osk in oocytes suggested that there might be something more to the story. Knowing the association of Rbsn-5 with endosomes in other species, Tanaka looked for

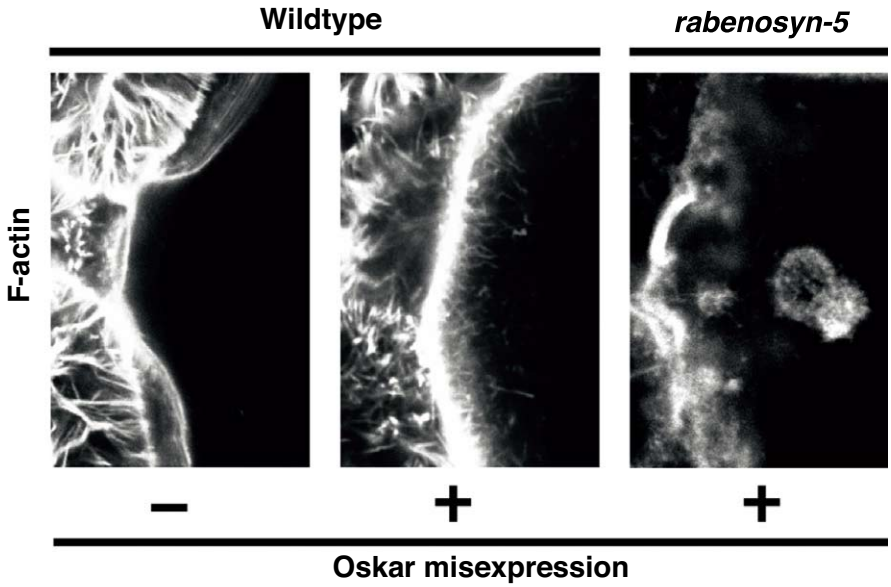
Tanaka T and Nakamura A. The endocytic pathway acts downstream of Oskar in *Drosophila* germ plasm assembly. *Development* 135. 1107-17 (2008)

correspondences with other endosomal markers, and found that all showed polarized (posterior) distributions similar to both Rbsn-5 and Osk. This posterior localization of endosomes appeared briefly but was subsequently lost in an *osk*-null mutant, suggesting that the maintenance of endocytic polarization is dependent on Osk. (The initial establishment of endocytic polarity appears rather to rely on the even earlier oocyte A-P polarization factor, Gurken.)

Experiments in which osk was misexpressed at the anterior pole further showed the involvement of Osk in the endocytic pathway. Anteriorly misexpressed Osk recruits endosomal proteins and stimulates endocytic activity. Wondering whether this might be a function specific to one of the two Osk isoforms, Tanaka tried expressing short and long Osk in the anterior and found that, while the ectopic expression of short Osk alone had no noticeable effect, misexpression of long Osk was sufficient to recruit endosomes to the anterior pole of the oocyte.

It has long been suspected that long Osk works by anchoring pole plasm components to the posterior cortex, but the involvement of Rbsn-5 in microtubule polarity in normal oocytes made it impossible to disentangle its known role in this process from a second possible role in Osk-mediated tethering. But as *rbsn-5*- oocytes maintain their competence to localize RNA to the anterior pole, they provide a unique environment for focusing on this question. Looking at Osk recruitment and maintenance in an *rbsn-5* mutant in which Osk was misexpressed at the anterior pole, Tanaka found that the anchoring of Osk and other pole plasm components to the cortex was weak, resulting in their diffusion into the cytoplasm. Although Osk promotes long F-actin projections from the cortex at both anterior and posterior ends of wildtype oocytes, the anterior misexpression of Osk in *rbsn-5* oocytes induced large aberrant F-actin aggregates, which diffuse along with pole plasm components into the cytoplasm, suggesting that long Osk stimulates endocytic activity, which in turn promotes proper F-actin reorganization to anchor pole plasm components to the oocyte cortex.

“Although the genetic approach used in this study reveals that the endocytic pathway acts downstream of long Osk to anchor pole plasm components,” says Nakamura, “further study of the molecular basis of long Osk function in endocytic activity is still needed to develop a better understanding of pole plasm assembly.”



F-actin at the anterior pole in wildtype or *rbsn-5* oocytes without or with misexpression of Oskar. Anterior misexpression of Oskar promotes long F-actin projections emanating from cortical actin bundles in wild type oocytes (middle image), but induces large aberrant F-actin aggregates in *rbsn-5* oocytes (right).

Cross purposes: Dual role for Src in control of cell-cell adhesion

Masayo SHINDO

Tissues in the embryo need to be capable of both stability and plasticity, which means that the connections between their component cells must be subject to dynamic controls. This regulation of intercellular adhesion at adherens junction is achieved through the action of multiple genetic pathways working on a range of adhesion and cytoskeletal molecules. Interestingly, the same regulatory pathways can be hijacked in cancer cells, allowing them to metastasize and form tumors. The Src family of oncogenes, for example, is known to downregulate the expression of E-cadherin, a major cell-cell adhesion molecule, but the wide range of related molecules with overlapping functions has made this relationship difficult to study in mammals.

A study by Masayo Shindo and colleagues from the Laboratory for Morphogenetic Signaling (Shigeo Hayashi; Group Director) took a new tack by looking at the function of Src in epithelial morphogenesis in the fruit fly *Drosophila melanogaster.* Their report, published in *Development,* suggests that Src has the somewhat paradoxical dual function of suppressing cell adhesion by E-cadherin while simultaneously upregulating its expression.

The study began with a gain of function screen for mutations producing altered epithelial morphology during tracheal development in the fly, which yielded the Src family members *Src42A* and *Src64B*, both of which caused defects in tracheal integrity on overexpression. Using antibodies to monitor the activity of these genes in vivo, the authors determined that these genes were upregulated in a range of epithelial tissues undergoing cell rearrangement.

Focusing back on the embryonic tracheal system, the group next studied the effects of loss of *Src42A* function and found multiple defects involving a ramified structure known as the dorsal branch (a group of cells that gives rise to the trachea), including reductions in number of cells, and delays in branch extension and cell intercalation.

Knowing of the link between Src and E-cadherin in other contexts, Shindo et al. re-examined the function of E-cadherin in tracheal development using the cadherin gene, shotgun (*shg*). Loss-of-function *shg* mutants showed disruptions in cellular attachments in tracheal branches, while overexpression of the gene caused delays in dorsal branch elongation and cell rearrangements. Overactivation of *Src42A* caused reduced tracheal cell adhesion phenotype, which was partially suppressed by co-expression of E-cadherin, suggesting that E-cadherin is a rate-limiting component of the tracheal cell adhesion under control of Src.

Shindo M, et al. Dual function of Src in the maintenance of adherens junctions during tracheal epithelial morphogenesis. *Development* 135. 1355-64 (2008)

They next used a technology known as fluorescence recovery after photobleaching (FRAP), in which a fluorescent-tagged sample is zapped with high intensity light causing it to lose its fluorescence (known as "bleaching") and then watched to determine the rate of recovery, to study the effects of Src on adherens junctions, where cadherins normally accumulate. Looking at GFP-labeled α-catenin, an E-cadherin-binding molecule, as a measure of adherens junction dynamics, they made an interesting discovery. Entry of α-catenin-GFP into adherens junction was slowed down upon loss of Src function and increased upon gain of Src function. "We suspected that Src enhances turnover of α-catenin in adherens junctions," says Hayashi, who heads the lab in which the study was done.

One puzzling question remained. Although reduction of E-cadherin explains inhibition of cell adhesion by Src, it does not explain why activated Src permitted a larger amount of α-catenin to enter adherens junction. The hint was obtained when they investigated the expression of Armadillo (the *Drosophila* version of β-catenin, another binding partner of cadherin). They discovered that Src promoted Armadillo expression and its downstream target, E-cadherin. This led to the remarkable conclusion that, even as Src down-regulates the E-cadherin protein at the adherens junction, it upregulates E-cadherin transcription. This dual function explains the increased turnover rate of cell adhesion molecules upon elevation of Src, making adherens junctions more dynamic.

"The dual function of Src we've discovered provides an answer to the long-standing question of why embryonic epithelial tissues are plastic enough to be able to adopt a variety of morphologies," says Hayashi. "The mechanism also explains why Src-transformed cells not only detach from original tissues, but also re-settle frequently in a new position, an essential feature of malignant cancer metastasis."

Figure 1

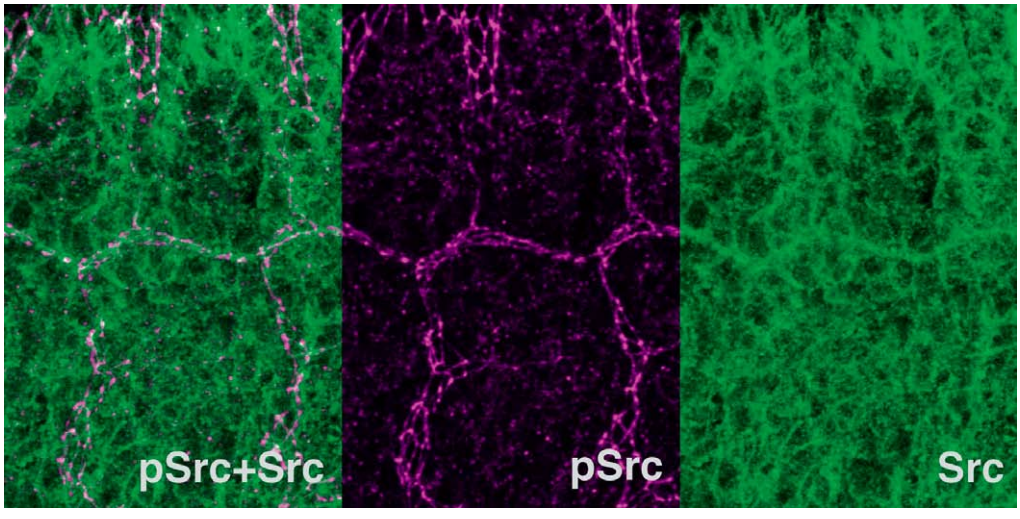


Figure 1
Activation of Src in adherens junctions in *Drosophila* embryos. Src42A is distributed broadly in cell membranes (right). A phosphorylated, active, Src42A (pSrc) was preferentially localized to adherens junctions of tracheal epithelium (center). Merged picture is shown at the left.

Figure 2

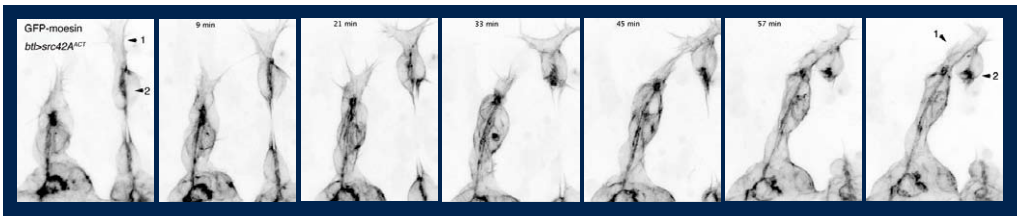


Figure 2(Movie)
Metastatic behavior of tracheal cells expressing activated Src42A. Two cells on one of tracheal branches detached from the stalk and settled on the adjacent branch.

Spring comes to the field

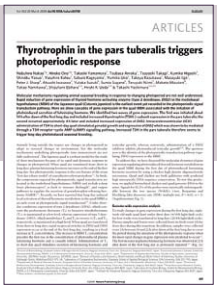
Takeya KASUKAWA

The circadian rhythm that runs on an approximately 24-hour cycle is perhaps the best-known biological clock, but many animals such as hibernating bears and migratory birds also follow a longer-term rhythm that regulates seasonal behaviors from feeding to reproduction. The molecular underpinnings of this "biological calendar," however, have largely remained a mystery.

A new collaborative study between Takeya Kasukawa of the CDB Functional Genomics Unit (Hiroki R. Ueda, Unit Leader) and Takashi Yoshimura's group at Nagoya University and others now reveals a "spring hormone" that works to trigger seasonal breeding in the Japanese quail, *Coturnix japonica*. In work published in *Nature,* the group found that the hormone thyrotrophin (TSH), which is secreted from the anterior lobe of the pituitary gland in response to light exposure mimicking that of a long day in spring, triggers seasonal breeding in this domestic fowl.

The work was enabled by the application of DNA microarray technology to the quail, which serves as a useful model for the study of seasonal breeding due to its rapid and dramatic responsiveness to changes in day length. As with many birds, the Japanese quail maintains gonads of small size in non-breeding periods, reducing its burden, developing them to functional size only at the start of mating season. Yoshimura's group had previously shown that thyrotrophin secretion was one of the earliest events in the long day-induced pathway.

The joint project began with a comprehensive analysis by DNA microarray of changes in gene expression when birds raised in short day length conditions were shifted to a longer light exposure environment, similar to the change in day length from winter to spring. Of the nearly 30,000 genes analyzed, roughly 300 showed changes in expression level associated with the day length shift, with TSH being the earliest responder. Further study revealed that this hormone is triggered in the pars tuberalis, a section of the anterior pituitary lobe, and binds to its receptor, TSHR,

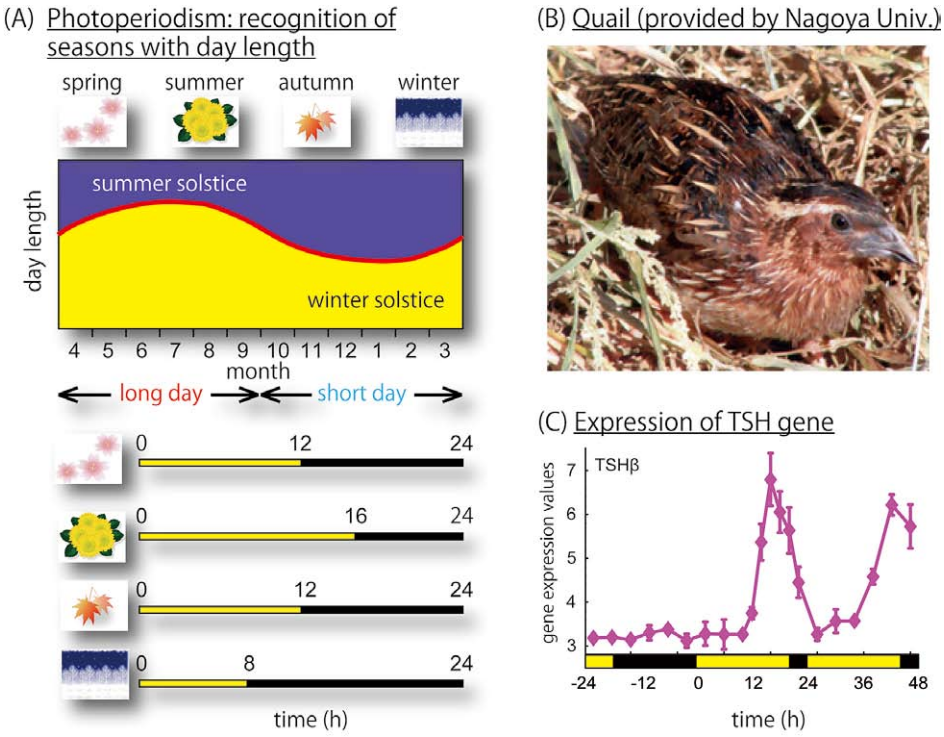

ARTICLES

Thyrotrophin in the pars tuberalis triggers photoperiodic response

Nakao N, et al. Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature* 452. 317-22 (2008)

in the overlying hypothalamus, enabling it to bypass the blood-brain barrier and enter the brain.The group next looked for a possible interaction between thyrotrophin and a second factor known as type-2 deiodinase, or DIO2, which Yoshimura's lab had previously shown to be important in gonad activation, and found that TSH indeed upregulates DIO2. They confirmed this in vivo by injecting TSH into cerebral ventricles, which stimulated both DIO2 expression and gonadal growth.

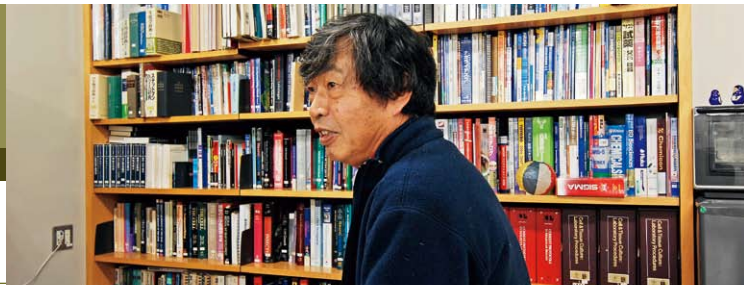
In addition to the identification of this molecular pathway involved in seasonal breeding, the discovery of the role of the pars tuberalis put paid to a longstanding question of the function of this hitherto mysterious structure. The new link between TSH, which is known for its roles in thermoregulation and metabolism, and reproduction also represents an important discovery, that may lead to new insights into seasonal biological cycles with implications in fields ranging from animal husbandry to seasonal affective disorders.



A) Many animals adjust their daily rhythms to the length of day in a given season. B) The quail used in this research is a common domestic fowl in Japan, and exhibits highly sensitive responses to seasonal change. C) On being shifted into a long-day light exposure regime, TSH levels are elevated by 14 H after midnight of the first day of exposure.

Vertebrate Body Plan

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



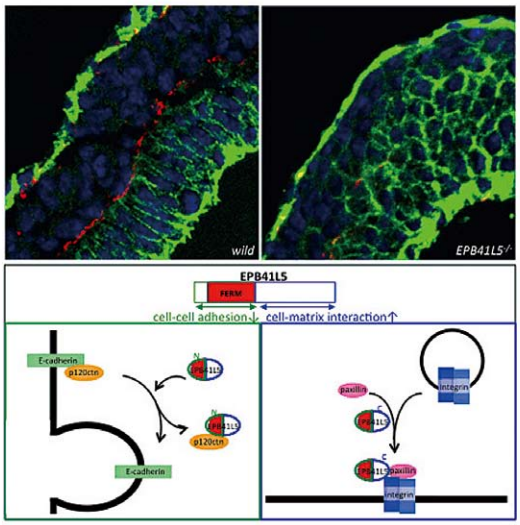
Shinichi AIZAWA Ph. D.

Shinichi Aizawa received his Ph. D. in biochemistry from the Tokyo Kyoiku University Department of Zoology in 1973. He spent the period from 1974 to 1979 as an investigator at the Tokyo Metropolitan Institute of Gerontology, then two years as a research fellow at the University of Washington. He returned to the Tokyo Metropolitan Institute of Gerontology in 1982, where he remained until 1986, when he moved to the RIKEN Tsukuba Life Science Center. He was appointed professor in the Kumamoto University School of Medicine in 1994, and served in that position until 2002. Since 2000 he has served as CDB deputy director and group director of the Vertebrate Body Plan lab, as well as head of the Animal Resources and Genetic Engineering Laboratory. In January 2007, he was appointed President of the Japanese Society of Developmental Biologists. He also serves as managing editor for the journal, *Mechanisms of Development*.

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. 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 however, remain unknown.

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



EPB41L5 regulates epithelial/mesenchyme transition post-transcriptionally; it binds to p120 catenin through its N-terminal FERM domain, thereby destabilizing E-cadherin toward endocytosis and to paxillin through its C-terminal, thereby activating integrin expression in cell surfaces or focal adhesion formation.

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Publications

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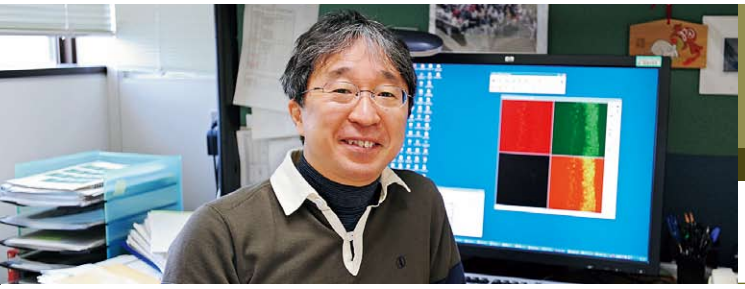


Neuronal Differentiation and Regeneration

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

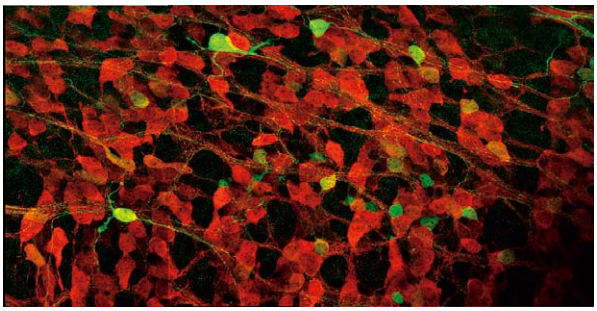
Hideki ENOMOTO M.D., Ph. D.

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 GDNFFamily of Ligands in neural development and function. He returned to Japan to take a position as a team leader at the CDB in 2002.

The architecture of the neural 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 (for 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).

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Publications

Uesaka T, et al. Diminished Ret expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice. *J Clin Invest* 118. 1890-8 (2008)

Gould T W, et al. The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are restricted to fusimotor subtypes. *J Neurosci* 28. 2131-46 (2008)

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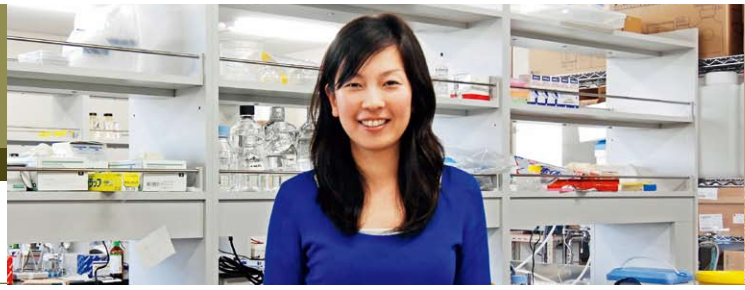
Neocortical Development

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

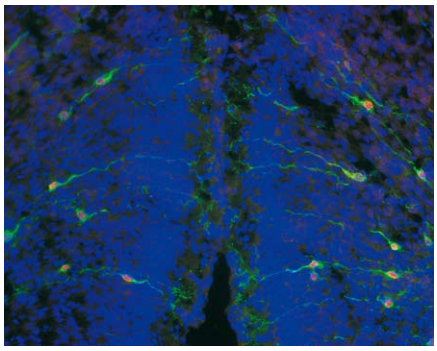


Carina HANASHIMA Ph. D.

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

The neocortex, 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 both 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 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 predetermined within the progenitor cells. We have found that specification of deep-layer projection neurons is dependent on transcriptional repressors that function cell-autonomously to prevent neurons from acquiring an earlier neuronal phenotype. These results imply cortical intrinsic programs may be co-opted in which neuron fate is established by temporal changes in gene expression. 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.

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Hanashima C, et al. The role of Foxg1 and dorsal midline signaling in the generation of Cajal-Retzius subtypes. *J Neurosci* 27. 11103-11 (2007)

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Morphogenetic Signaling

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

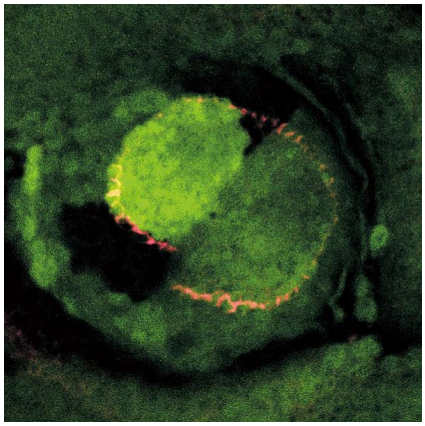
Shigeo HAYASHI Ph. D.

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

The main research interest in my lab focuses on the mechanisms by which cell-cell and tissue-to-tissue interactions are modulated during embryonic morphogenesis. Our strategy is to identify intercellular signaling systems and intracellular transducers that control cell-cell and tissue-tissue interactions. A range of vital developmental processes, including the ability to build complex structures through selective cell adhesion, to move through the body, and to form epithelial tissues, rely on interactions between cells, and between cells and the extracellular matrix. The means by which cells are able to communicate, congregate and work together to build a body is a central question in the study of morphogenesis.

The *Drosophila* tracheal system is a respiratory organ that delivers air directly to tissues through a network of finely branched epithelial tubes. Tracheal development begins by invagination of ectodermal epithelium that subsequently forms branches with specific types of cells migrating in stereotyped patterns. The branching patterns and cell fate are instructed by external cues, including FGF, WG and Dpp. We are studying the roles of these signaling molecules in the specification and migration of tracheal branches, as well as the mechanisms that coordinate cell movement and cell adhesion. We additionally use 4D confocal imaging of GFP-labeled embryos to study the dynamism of cell and organelle movement in living organisms. Using combinations of GFP markers and transcriptional enhancers of cell-specific expression, we have been able to capture movements of tracheal cells at resolutions sufficient to image cytoskeletal organization and cell adhesion structures in single cells.

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



Homophilic cell adhesion molecules Capricious and Tartan are required for proper placement of segmental boundaries in the leg. The boundary of pretarsas and tarsal 5 segment is marked by the red signal, which normally appears as a ring. When the boundary is flanked by a group of cells lacking Capricious and Tartan marked by a loss of green signal, the position of the boundary shifts to the interior and appears staggered.

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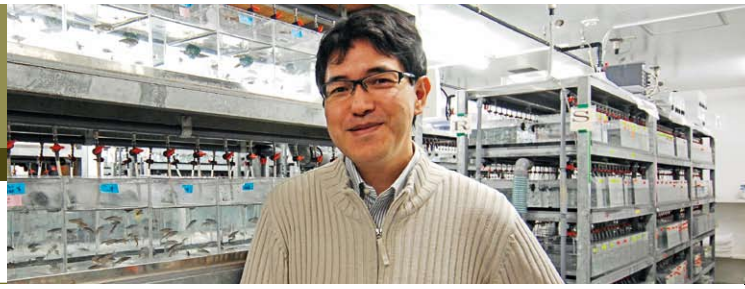
Vertebrate Axis Formation

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



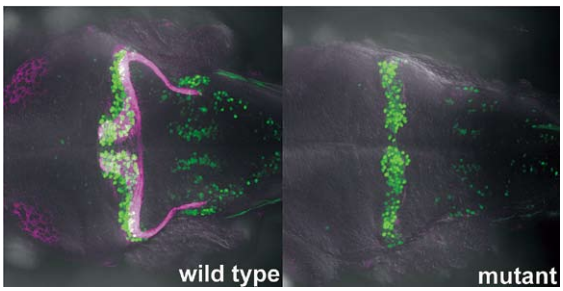
Masahiko HIBI M.D., Ph. D.

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

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

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

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



Development of cerebellar neurons in zebrafish larvae at 5 days post fertilization. Purkinje neurons (green) receive axons of granule cells (magenta) in wildtype but do not in one of the mutants isolated by our team.

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Hirata T, et al. Zinc-finger genes Fez and Fez-like function in the establishment of diencephalon subdivisions. *Development* 133. 3993-4004 (2006)

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Cell Lineage Modulation

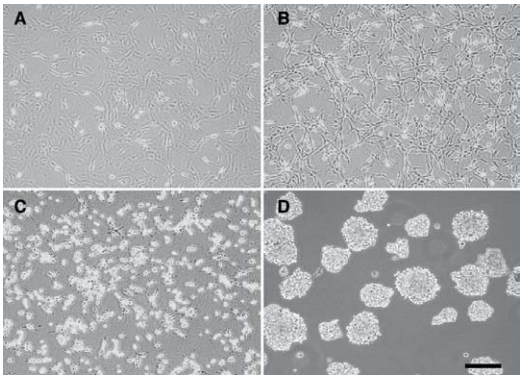
http://www.cdb.riken.jp/en/kondo

Toru KONDO Ph. D.

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

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

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



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

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Takanaga H, et al. Gli2 is a novel regulator of sox2 expression in telencephalic neuroepithelial cells. *Stem Cells* (In press)

Kondo T. Stem cell-like glioma cells. *Current cancer therapy Review* 4. 201-205 (2008)

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Evolutionary Morphology

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

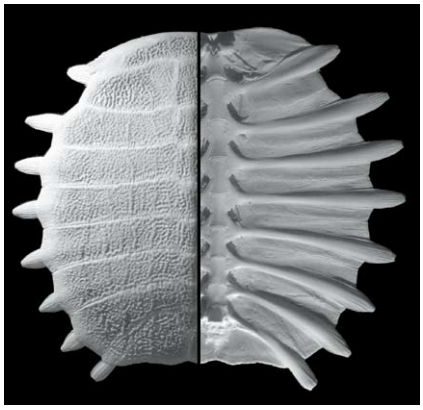
Shigeru KURATANI Ph. D.

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.

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

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

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



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

We have also recently started to study wing pattern evolution in moths in an effort to understand the relationship between phenotype and development. By employing morphometric strategies, we seek to identify relationships between developmental and functional modules in wing patterns, which we hope will lead to molecular developmental searches for genes favored by natural selection.

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Publications

Ota K G, et al. Hagfish embryology with reference to the evolution of the neural crest. *Nature* 446. 672-5 (2007)

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Sensory Development

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

Raj LADHER Ph. D.

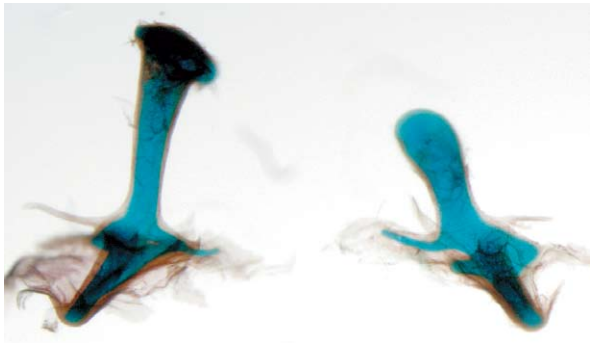
Raj Ladher received his B. Sc. in biochemistry from Imperial College, London in 1992 and his Ph. D. in developmental biology from the National Institute for Medical Research in 1996, for his thesis on the cloning of the *Xenopus* homeobox gene, *Xom*. He worked as a research associate at King's College,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

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

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

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

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



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

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Publications

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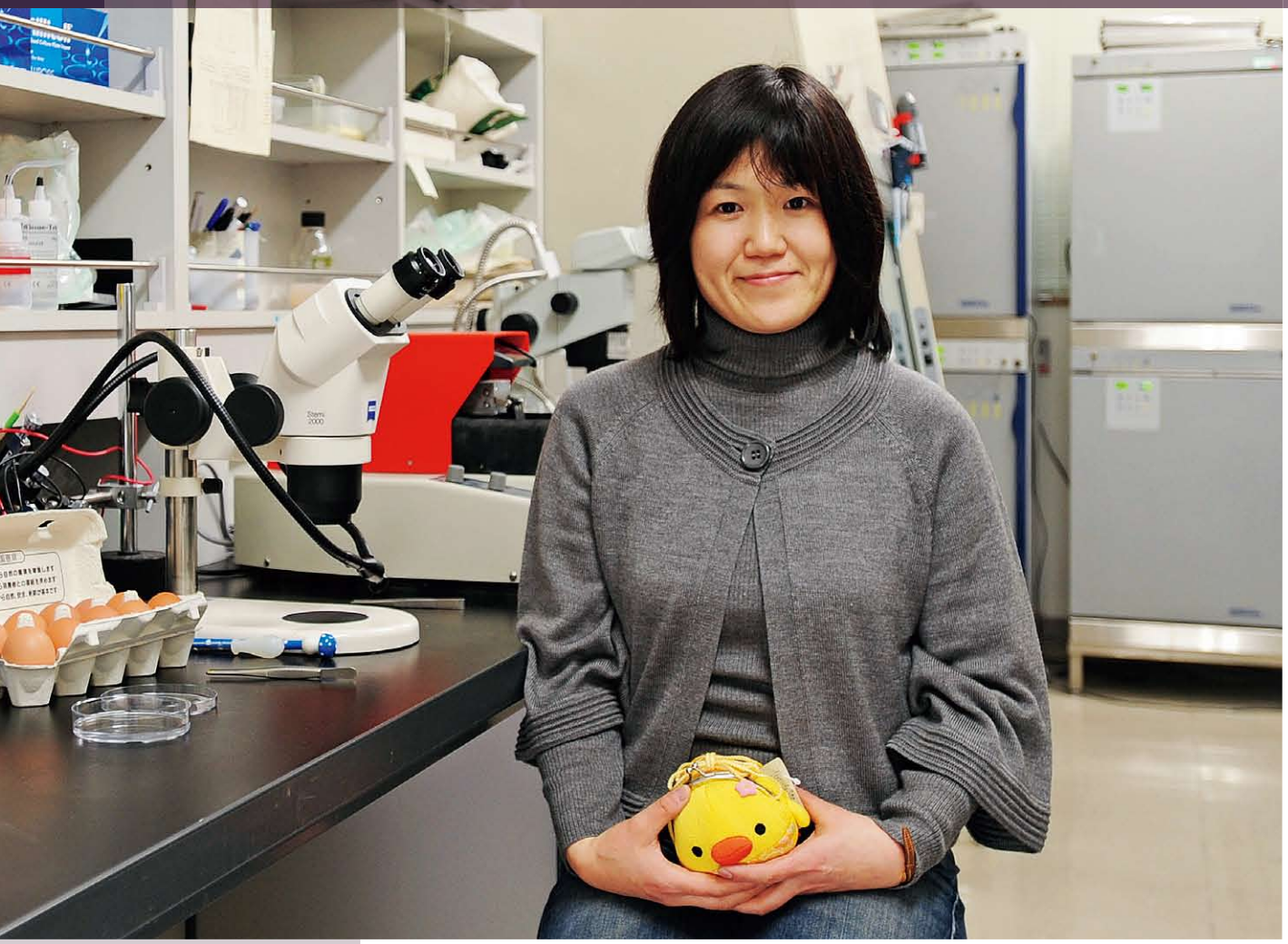
Shroom3 recruits ROCKs to the apical junction



Tamako NISHIMURA

Epithelial tissues are made up of highly ordered sheets of cells, that can stretch, squeeze, bend and roll into all manner of bulges, concavities and tubes. But for essentially two-dimensional sheets to take on these three-dimensional appearances requires changes in shape at the level of individual cells. This requires not only the regulation of morphological events within a given cell, but precise coordination across the many cells that make up the tissue, in order to assure that the process plays out true to the developmental plan. The invagination and closure of the neural tube, in which a sheet of cells destined to become the central nervous system forms a depression whose edges fold over to form a hollow cylinder, is one of the best-studied instances of such structural remodeling, but the molecular mechanisms that underlie this process remain incompletely understood.

A study this year conducted by Tamako Nishimura of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) identifies the role of one player in neural tube morphogenesis, the actin-binding protein Shroom3. In a report published in *Development,* Nishimura showed that this protein binds certain Rho kinases, recruiting them to the apical cell junction, a site at which uppermost lateral surfaces of epithelial cells connect, and which serves as a hinge and anchor point for much tissue remodeling activity.

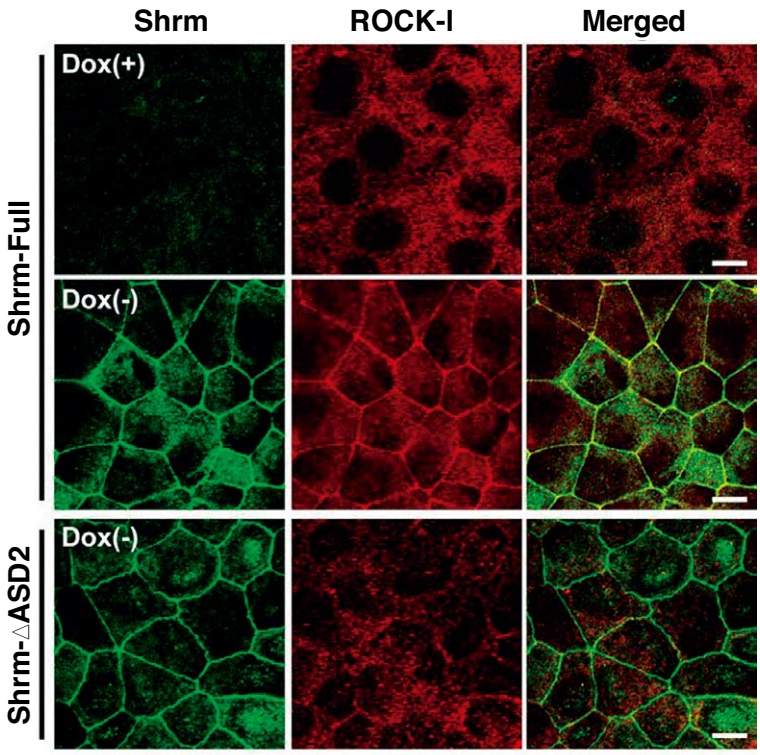
Shroom3 was originally identified as the culprit in a mouse mutant whose phenotype was characterized by incomplete neural tube closure (the name deriving from the mushroom-like appearance of the mutant embryo's head). Gene expression studies had shown that it is expressed strongly in the neural epithelium,

Nishimura T and Takeichi M. Shroom3-mediated recruitment of Rho kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. *Development* 135. 1493-502 (2008)

but its mechanism of action remained a mystery. To address this question, Nishimura first searched for binding partners, and found that a number of Rho kinases (known as ROCKs) associated with Shroom3 in immunoprecipitation assays. In vitro tests further revealed that Shroom3 and ROCKs co-localized at the apical cell junction, an effect that could be blocked by interfering with their binding activity.

Looking next in vivo, Nishimura studied the Shroom3-ROCK association in embryonic chicken, and confirmed that the two were concentrated in the apical junctions of neuroepithelial cells lining the neural tube. Blocking Shroom3 by RNAi resulted in the failure of ROCKs to localize to the apical junction, and consequent defects in neural tube closure. When she watched the interactions between cells in the closing neural tube, Nishimura observed assemblies of 5 or 6 cells into rosette patterns, suggesting coordinated contraction of the cell shapes, presumably dependent on actomyosin, which is known to be regulated by ROCKs. Such rosettes, again, were lost on ROCK inhibition.

The picture that emerges from Nishimura's findings suggests that Shroom3 recruits ROCKs to the apical junction, where they interact with myosin and actin cytoskeletal components to give shape to the forming neural tube. "There are still many unknowns in terms of the mechanisms behind changes in cell shape, such as those that occur in the neural tube," says Nishimura. "I think the Shroom/ROCK complex is particularly interesting in this context, for the way it localizes in a polarized way to only one aspect of the cells and thus contributes to the sculpting of the entire tissue."



Co-localization of Shroom3 and ROCK at apical junctions (middle row) and loss of ROCK localization in Shroom3 mutant cells (bottom row). Note the loss of tension in the mutant cell membranes.

Zeroing in on beta globins in the early embryo

Cantas ALEV

Our red blood cells, or erythrocytes, act as vehicle for conveying oxygen from the respiratory system to the cells of the body, a feat they achieve thanks to the ornate structure of hemoglobin, a molecule comprising alpha and beta globin subunits and an iron-based heme. There are multiple types of both alpha and beta globin, and different combinations of these types result in differing degrees of affinity for oxygen, which has implications in both embryonic development and adult physiology. In the chicken, the three subtypes of alpha globin (?, αD and αA) and the four beta globins (ρ, βH, βA and ε) show different spatiotemporal patterns of expression during the formation of the red blood system during embryogenesis, a process that plays out in two waves – an initial phase of primitive extraembryonic erythropoiesis, followed by a second wave that takes place within the embryo. This transition is characterized by a shift from the embryonic beta globins (ρ and ε) to βA, the major adult beta globin. But due to technical limitations, attempts to study the details of this sequence of events have yielded somewhat conflicting results.

In a study published in *Developmental Dynamics,* Cantas Alev and colleagues in the Laboratory for Early Embryogenesis (Guojun Sheng; Team Leader) and other CDB labs have shown through protein analyses that β A is expressed even in the early stage of primitive erythropoiesis in the chicken.

Previous reports using classical embryology and molecular biology had suggested that there was a clear separation between primitive (embryonic) and definitive (adult) beta globins, and that the transition between these two stages occurred as early as embryonic day 4 (E4) or as late as day 13. Analysis of mRNA transcripts, in particular, had indicated a switching on and rapid increase of βA expression in the period between days 5 and 7 of embryogenesis.

Alev et al., however, took a new approach to the question, first using mass spectrometry to sort out and quantify each of the globins from samples of embryonic blood. In contrast


BetaA, the Major Beta Globin in Definitive Red Blood Cells, Is Present From the Onset of Primitive Erythropoiesis in Chicken

Alev C, et al. BetaA, the major beta globin in definitive red blood cells, is present from the onset of primitive erythropoiesis in chicken. Dev Dyn 237. 1193-7 (2008)

to previous reports indicating that βA expression is first detectable at around E2 (stage HH13 in the Hamburger-Hamilton system for evaluating chicken development), the team found that it is present as early as two days earlier, coincident with the primitive wave of blood development. Looking even earlier, they found signs of βA expression as early as HH8, with significant transcription by HH9. Interestingly, even at this early stage of development, the level of the purportedly "adult" beta globin βA was seen concurrent with that of the "embryonic" ρ subtype (although at a much lower level). In situ hybridization further showed that, by HH10~11, βA beta globin is expressed in the extraembryonic mesoderm as well.

The findings from this multi-technique strategy are in general agreement with the scheme in which the transition from primitive (ρ and ε) to definitive (βA) beta globins occurs in the period of E5~7, but pushes back the start point for βA expression to the earliest stage of blood formation, suggesting that the distribution of the various subtypes may be a function of relative abundances rather than strict transcriptional control. "We still don't know what βA is doing in the primitive stage of hematopoiesis," admits Alev. "But it's interesting that the other adult beta globin, βH, is completely undetectable at that stage, so we'd like to try to work out how to account for these differences in the regulation of transcription."

Figure 1

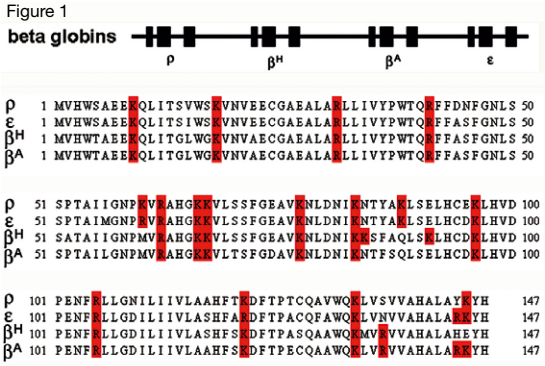


Figure 1
Each of the four beta globins has unique tryptic digestion pattern that can be used for mass-spectrometry based semi-quantitative assessment of its relative abundance.

Figure 2

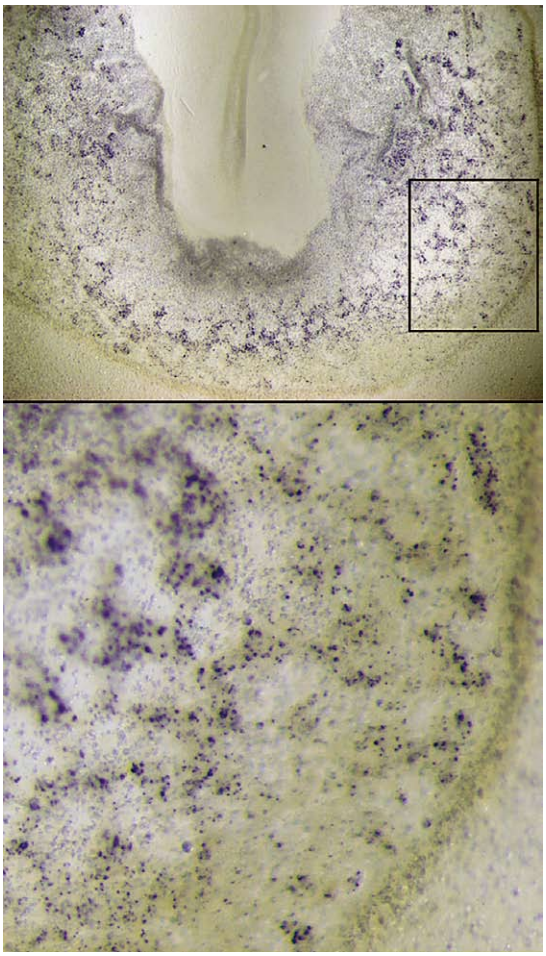


Figure 2
Expression of betaA in primitive erythrocytes revealed by intron-specific in situ hybridization.

Subtraction a plus for monoclonal antibody research

Chie WATANABE, Kazumasa TAKEDA

Antibodies have become an indispensable component of the experimental repertory in many life sciences laboratories, used in such techniques as immunohistochemistry, Western blotting and immunoprecipitation. It remains a challenge, however, to isolate antibodies of precisely the right affinity for antigens called for under a given experimental design. A method developed by Kazumasa Takeda and others in the Laboratory for Developmental Genomics (Asako Sugimoto; Team Leader) now looks set to lower the barrier to the creation of monoclonal antibodies to low-abundance antigens. In a proof-of-concept demonstration published in *Genes to Cells,* of this "antigen subtraction" approach, the team was able to isolate more than 30 monoclonal antibodies specific to structures in the *C. elegans* embryo, including P granules (specifically present in cells of the germline), muscle, pharynx and hypodermis, as well as previously unreported cellular structures. Importantly, this method can be applied to other species as well, which will allow researchers in other labs to develop new, highly specific antibody probes for cells and tissues at specific developmental stages.

The project traces its roots back more than 20 years, to when Takeda was using monoclonal antibodies as part of a virus research project at Kyoto University. On reading a review on apoptosis and roundworm germline development, he wondered whether he might be able to apply the techniques he'd been using in virology to develop antibodies for specific *C. elegans* structures as well, and quietly began to experiment. In the late 1980s, he generated and screened more than 1,300 hybridomas (cancer cell engineered to produce a single species of antibody). "I had to be a bit careful when I was working on these," admits Takeda, "as they weren't really part of the research program of the lab I was working in at the time."

Despite his intensive initial efforts, Takeda was frustrated to find that many of his antibodies were not specific to any single structure in immunostaining, and tended to

Takeda K, et al. Efficient production of monoclonal antibodies recognizing specific structures in Caenorhabditis elegans embryos using an antigen subtraction method. *Genes Cells* 13, 653-665 (2008)

stain the entire *C. elegans* embryo. Reasoning that the solution would lie in finding a way to sift out non-specific antibodies, he devised a clever two-stage system in which first antibodies against abundant and non-specific antigens are pooled and then used to subtract out the corresponding antigens from the embryonic extracts. The mixture that remained after this subtraction should then be enriched with less abundant proteins, which could then be used for a second round of immunization, hopefully yielding targets capable of marking specific cell or tissue types. Takeda found that in the first round of immunization, only about 3% of monoclonal antibodies showed a specific pattern in immunostaining tests; the rest generally stained the entire embryo. But after subtraction, almost 50% produced signals in specific structures or cell types.

Takeda had to discontinue this project for various reasons, but in 2003, after retiring from Kyoto University, he emailed Sugimoto to ask her if she would be interested in his collection of hybridomas and monoclonal antibodies, which led to an offer to join her lab and resume his work on an official basis. Takeda and Chie Watanabe, a technician in the lab, then developed a collection of 35 monoclonal antibodies for a range of structures including P granules, body wall muscle, hypodermal cells, and pharynx. This antibody library has already been used to produce images by immunostaining of previously unknown histological and cellular structures, which can be viewed on the Sugimoto lab homepage: http://cdb.riken.jp/dge. The lab has already received more than 30 requests for antibodies from labs around the world, and is pleased to make them available to the global *C. elegans* research community. Within the lab, the antibodies have opened up new direction for research and are now being used in analyses of the structure and assembly mechanism of P granules.

"The *C. elegans* research community has always been based on the open sharing of information and findings," says Sugimoto. "So we have made the entire database accessible to everyone in the field, in the hopes of distributing these antibodies as widely as possible."

Figure 1

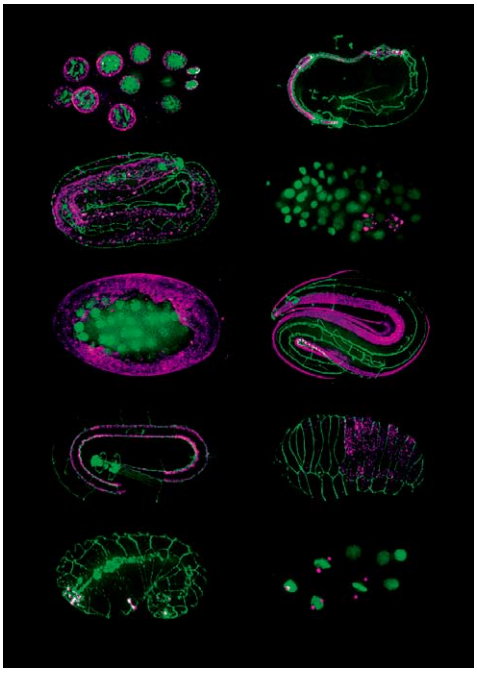


Figure 2

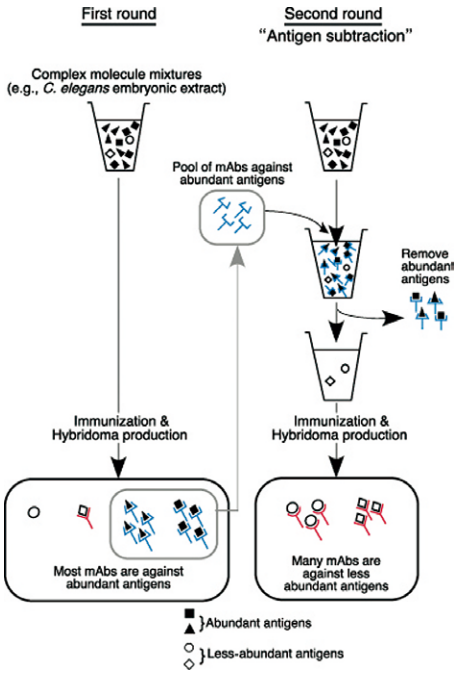


Figure 1
Images showing immunostaining using antibodies generated by antigen subtraction.

Figure 2
A schematic of the antigen subtraction method.

Novel ectodermal factor blocks mesodermal fate

Rieko YAKURA-NISHIZAWA, Noriaki SASAI

The development of the embryo is a tale of either/or gateways and roads not taken; decisions made at an early stage in development can put constraints on all those made subsequently. The same is often true of the induction of differentiation of pluripotent cells in culture, which requires step-by-step instructions to guide cells to adopt a terminally differentiated fate. For this reason, the identification of those early signals that steer undifferentiated cells toward a specific germ layer is of fundamental interest to scientists working both in vivo and in vitro. The African clawed frog, *Xenopus laevis,* provides an excellent model for studying questions of early vertebrate development through analyses of a pluripotent region of the nascent embryo known as the animal cap, which is the site of in vivo germ layer specification and is amenable to tissue culture as well. That notwithstanding, there have been no reports of a zygotic factor able to regulate one of the earliest lineage decisions during gastrulation, the choice between ectodermal and mesodermal fates.

That changed this year, when Noriaki Sasai and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) identified a novel ectodermal factor that prevents mesodermal differentiation in both *Xenopus* animal cap and mouse embryonic stem cells in a report published in *Cell*. This factor acts by an unsuspected molecular mechanism, the inhibition of p53, a gene best known for its role in genomic remodeling and cancer.

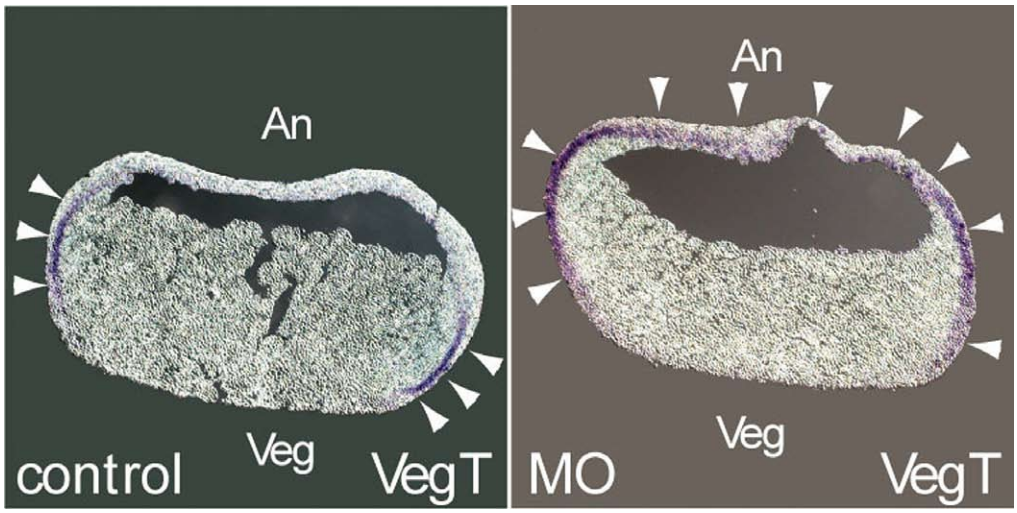
Having observed that mRNAs from stage 11.5 animal caps (about a half-day into development) suppressed mesodermal differentiation, the group screened a library of cDNAs to try to pinpoint this inhibitory function. “There were already a number of reports of maternal lineage determinants,” says Sasai, “but the fact that these were residual proteins that had been expressed in the egg, rather than newly in the embryo, made it unlikely that they could account for fate specification during gastrulation.” On completing the screen, five molecules showed mesoderm-inhibiting activity, and as four were already relatively well-characterized, they narrowed their focus to the fifth, a gene encoding zinc-finger protein known as XFDL156.


Ectodermal Factor Restricts Mesoderm Differentiation by Inhibiting p53

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

Sasai first tried overexpression experiments in animal caps that had been induced toward the mesodermal lineage, and found that XFDL suppressed mesodermal, but not neural or endodermal, markers. The converse experiment, in which XFDL function was blocked by antisense morpholinos, resulted in mesodermal expansion, suggesting that this factor suppresses mesodermal differentiation in the ectoderm. The question was, how?

As recent studies have shown a role for the transcription factor p53 in mesodermal differentiation, Sasai looked for a possible functional relationship between these two proteins in animal cap assays. After confirming by a double knockdown that its function was dependent on p53, he next tested the effect of XFDL on p53 (and its co-factor p300) and determined that it effectively inhibited p53's ability both to activate the mesoderm marker gene, *Mix*.2 and to promote apoptosis in the animal cap. Immunoprecipitation showed that XFDL and p53 co-precipitated, indicating a direct interaction between the two. Further analyses, by gel shift assay and chromatin immunoprecipitation, revealed that XFDL-bound p53 fails to bind to its target genes.

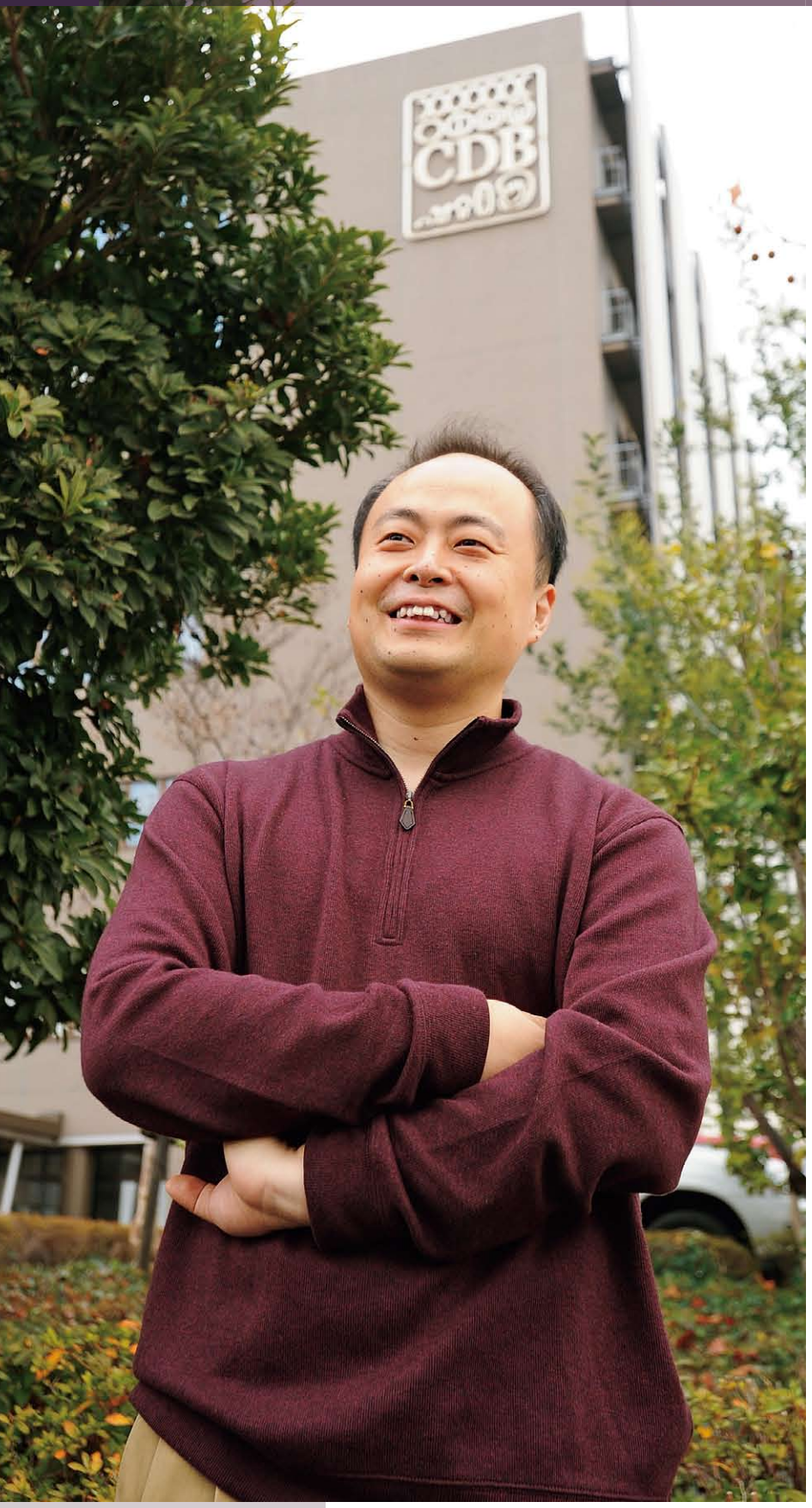


Expression of mesodermal marker (VegT, shown by arrowheads) limited to presumptive mesoderm in wildtype embryo (left) and expanded throughout presumptive ectoderm region on XFDL156 knockdown (right).

Investigating the structural underpinnings of this interaction, Sasai et al. next made a number of p53 constructs engineered to lack various protein domains, allowing them to identify a regulatory domain in the carboxyl-terminal region as the sequence necessary for XFDL binding. Interestingly, a search in other genomes for related genes turned up a pair of zinc-finger protein encoding genes in the mouse that shared the ability to inhibit mesodermal differentiation and to interfere with p53, suggesting that mammalian ES cells may use a mechanism for ectodermal differentiation similar to that in the frog.

"With these findings. I think we have managed to add a piece that has long been missing from the puzzle of early development from undifferentiated, pluripotent cells," says Yoshiki Sasai. "We're hopeful that this will take us one step closer to the day when we will be able to control every major step in the process of differentiation from ES cell to neuron."

Improved model yields new insights into Hirschsprung's disease



Toshihiro UESAKA

The long tract of the gut is innervated by a network of peripheral neurons that control, among other functions, the peristaltic action that moves food through the lower half of the alimentary canal. In Hirschsprung's disease, a congenital disorder, neurons fail to develop in the large intestine, which can lead to symptoms ranging in severity from chronic constipation to total blockage and enlargement of the colon, which can be life-threatening. The primary genetic cause of Hirschsprung's has been linked to mutations in the *Ret* gene. Recent evidence has also shown that the binding of the protein GDNF (glial cell line-derived neurotrophic factor) to its receptor GFRα1 activates RET, which is necessary for the survival of enteric neurons, leading to the question of precisely how Ret regulates neuronal survival and death in the developing colon, particularly with reference to Hirschsprung's disease.

Toshihiro Uesaka and colleagues in the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto; Team Leader) have developed a mouse model of Hirschsprung's in which Ret gene expression levels can be reduced conditionally, allowing them to study the gene's involvement in an animal model with greater precision than ever before. This work was published in *The Journal of Clinical Investigation.*

Uesaka had previously shown that loss of GFRα1 function at a specific stage late in mouse development caused a near-total loss of innervation in the large intestine. "In the conditional *GFRα1* mouse, we found an almost complete loss of the neuronal network in the lower intestine due to apoptotic cell death, a phenotype that closely resembles Hirschsprung's disease," says Uesaka. "As this disease is characterized by mutations in the *Ret* gene, we suspected that apoptosis might be involved there as well."

In mice in which *GDNF, GFRα1* or *Ret* are ablated unconditionally, the enteric nervous system fails to develop beyond the stomach. In the sense that it produces a loss of the enteric neural network, this phenotype could


Research article

Diminished *Ret* expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice

Uesaka T, et al. Diminished Ret expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice. *J Clin Invest* 118. 1890-8 (2008)

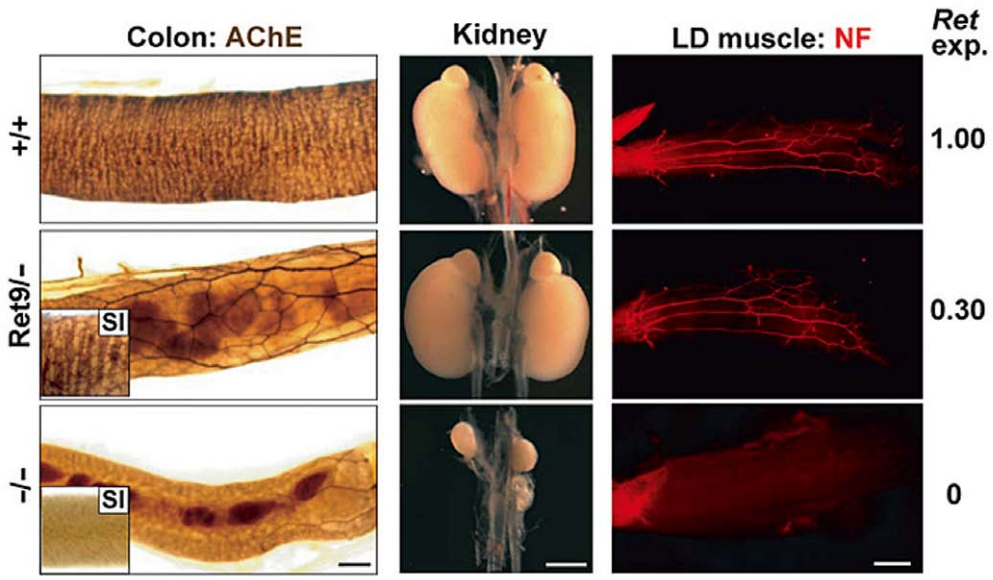
be said to resemble Hirschsprung's disease, but in fact such extreme cases are rare, and many patients show only a loss of neurons in the colon. Previous reports have also shown that *Ret*-mutant mice exhibit abnormal kidney and motor neuron development as well, which is not seen in Hirschsprung's, indicating the need for a more sophisticated experimental model.

"By designing a system for conditionally inactivating Ret at a specific developmental stage in the mouse fetus, we were able to confirm that, just as with GFRα1, the loss of Ret function at a specific developmental stage results in a reduction in the innervation of the colon," comments Uesaka. "We knew that in human Hirschsprung's patients there's a mutation in the *Ret* gene of a region that regulates its expression level, so we next looked at a series of mutant mice in which Ret expression levels were reduced to various extents."

Mice in which Ret expression was reduced to 50% of the wildtype level were asymptomatic, but in a different mutant in which levels were 30% of wildtype, colonization of the gut by enteric neural crest-derived cells initially appeared to play out as normal, but these cells subsequently disappeared. This phenotype occurred in about half of all the animals, in a gender distribution reminiscent of that in Hirschsprung's. The phenotype was limited to gut innervation without any of the other defects, such as kidney and motoneuron anomalies, that are seen in unconditional *Ret* knockouts. And, interestingly, the gender distribution showed a slight male bias, consistent with the tendency in the Hirschsprung's patient population.

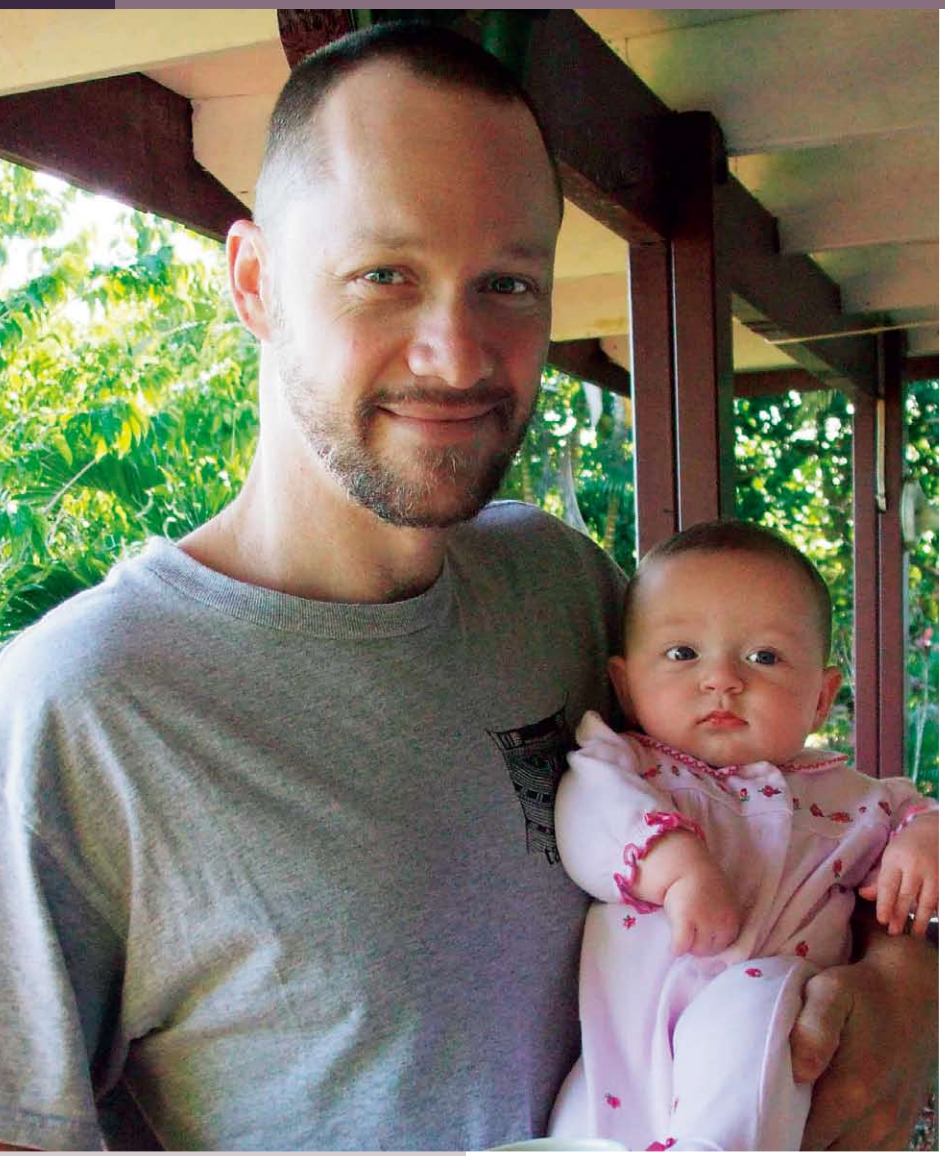
Uesaka next looked into the mechanism by which low levels of Ret expression might bring about such neuronal loss, and found that in the mutants neuronal migration into the colon is delayed. Reductions in Ret induced at later developmental stages also caused a loss of colonic innervation, suggesting that lower Ret levels may both interfere with the survival and maintenance of enteric neurons and trigger the programmed cell death that underlies Hirschsprung's pathogenesis.

"It had previously been thought that Hirschsprung's disease was caused by a defect in cell migration, not by cell death," says Uesaka. "But our findings suggest that insufficient supply of a survival signal is at fault. I am hoping to look more closely at the underlying mechanisms in the future to contribute to a better understanding of this disease."



Reduced expression of *Ret* causes aganglionosis in the distal colon in vivo.

Unsuspected neuronal specificity in GDNF function



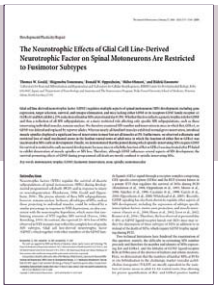
Thomas (and Margot) GOULD

Neurons are born with an appetite for molecular factors that regulate multiple aspects of their development and function, and in many cases are necessary for their very survival. One such neurotrophic factor, GDNF (glial cell-line derived neurotrophic factor), is known to play important roles in gene expression, targeting, survival, and synapse pruning in spinal motor neurons, which innervate skeletal muscles and convey efferent signals from the brain. Skeletal muscle comprises different kinds of muscle fibers – the extrafusal fibers that contract and extend to generate tension, and the intrafusal fibers, which are sheathed in sensory receptors that detect the speed and length of a muscle as it is stretched. Mice lacking GDNF or its receptors are known to suffer motor neuronal deficits, but it has never been determined whether this is a general effect, or specific to a given subpopulation.

Research by Thomas Gould of the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto; Team Leader) reveals that, somewhat surprisingly, loss of the function of GDNF or its receptors affects only the gamma motor neurons, which innervate intrafusal fibers. The study, published in *The Journal of Neuroscience,* further highlighted the exquisite specificity of GDNF signaling function by showing that the period in which GDNF is needed for motor neuron survival is limited to the first few days after birth.

In starting their work, Gould et al. were confronted by a number of technical challenges in teasing out the details of GDNF function, the thorniest of which is that mice entirely lacking GNDF function die at around the time of birth due to dysfunctions in kidney and gut. “The enormous repertoire of mice in the lab made this sort of thorough analysis possible,” says Gould. “”Our conditional mutant mice permit the deletion of GDNF receptors in specific tissue types such as motor neurons, which avoids the lethal phenotype caused by constitutive deletion and thus facilitates the investigation of the role of GDNF signaling in specific regions or cell types.”

The difficulty of visualizing and counting motor neurons presented a second hurdle to the team, which it met by developing a new labeling method which they used first to verify the normal number of lumbar motor neurons in wildtype and *GDNF*-knockout newborn mice. When they looked at different hindlimb muscles, they found that in most muscles innervation was normal in both wildtype and mutant. Only three muscle types – peroneal, gluteus maximus, and iliopsoas – showed deficits, consistent with previous findings showing a role for GDNF in peroneal axon guidance. Intriguingly, however, in the occasional GDNF ligand-receptor knockouts in which peroneal innervation remains normal, the mice still lacked greater numbers of neurons that could be accounted for by deficits in the other two groups alone.

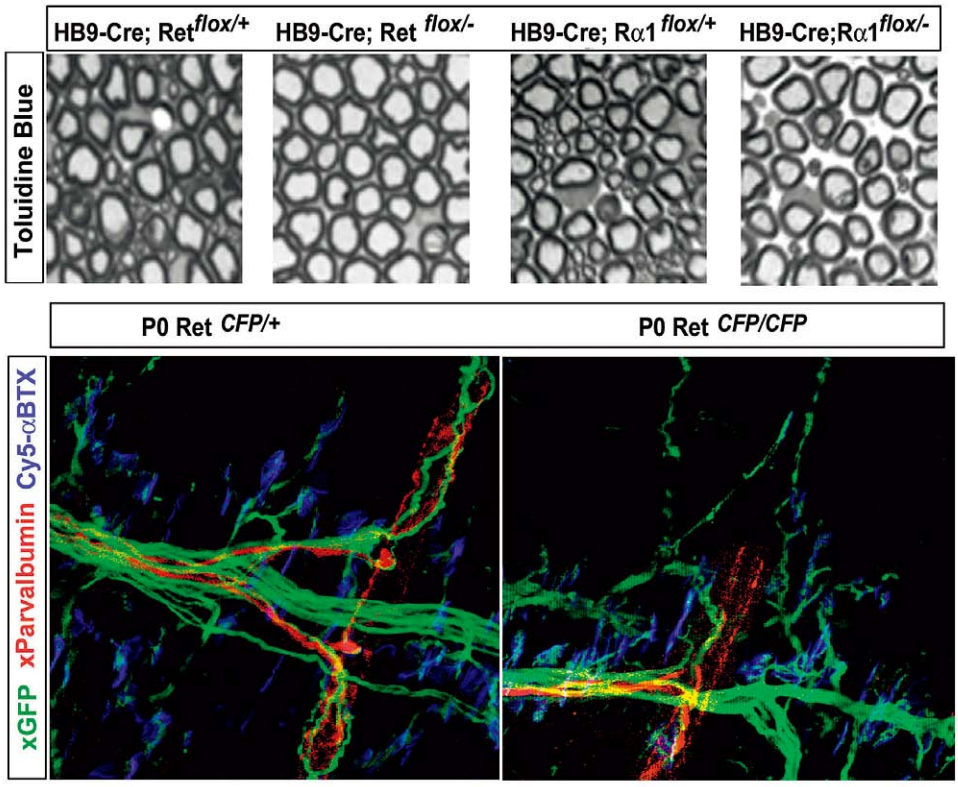


Gould T W, et al. The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are restricted to fusimotor subtypes. *J Neurosci* 28. 2131-46 (2008)

This prompted Gould to look at the possibility that rather than those innervating a specific muscle group, motor neurons projecting to a specific muscle fiber type might be affected by loss of GDNF signal. Indeed, while extrafusal skeletomotor neurons (also known as alpha motor neurons) were completely unaffected in knockouts of Ret (a vital component of the GDNF receptor complex), this same mutation caused near-total reduction in the number of fusimotor (or gamma) neurons. Similar fusimotor deficits were seen in knockouts of *GDNF* and *GFRα1* (the other half of the Ret receptor complex). Observation of myelinated axons in motor neuron-specific *Ret* and *GFRα1* conditional knockouts confirmed that this deficit was confined to small-diameter, gamma motor neurons. Finally, the analysis of mice expressing the fluorescent reporter CFP in one or both Ret loci (*Ret-CFP* knockin mice) revealed that the reductions in gamma motor neuron counts result at least partially from programmed cell death during initial innervation of intrafusal muscle fibers.

The team also crossed mice with conditional mutations in either *Ret* or *GFRα1* to those expressing a tamoxifen-inducible Cre to study the requirement for GDNF at different points in fetal and postnatal development, and found that gamma motor neurons depend on the GDNF pathway only during the late developmental period during which cell death occurs.

"In addition to the biology, what we found has some interesting implications for medicine," says Gould. "In ALS (amyotrophic lateral sclerosis), a fatal degenerative disease characterized by the selective loss of alpha motor neurons, for example, it had been thought that the loss of these neurons might be an instance of dysregulation of GDNF signaling. But our findings show that it is the gamma neurons, rather than the alpha neurons, that appear most sensitive to the trophic actions of GDNF, which raises questions about the clinical relevance of this pathway in the pathogenesis of ALS."



Top Row, Sections of the fourth lumbar ventral root stained with toluidine blue from P42 mice reveal a loss of small- but not large-diameter motor axons in motor neuron-specific Ret and GFRa1knockouts. Bottom Row, In contrast to those of WT mice (left panel), intrafusal muscle fibers detected by parvalbumin-immunoreactive sensory nerve terminals (red) fail to receive motor innervation from g MNs (GFP immunoreactivity, green) in P0 Ret knockout mice (right panel). In contrast, extrafusal muscle fibers (detected by the presence of nAChR clusters, blue) receive normal motor innervation from a MNs.

Untangling the roots of the germline

Kazuki KURIMOTO

Evolution has devised two ways for the germline to arise during development: through preformation, in which germ cells are determined by maternal factors segregated into a few cells from the very beginning of development, and epigenesis, in which signals delivered by the embryo itself instruct undifferentiated cells to adopt germline fate. Several popular model organisms, including *C. elegans* and *Drosophila,* take the preformation route, but most species, including mammals such as human and mouse, feature epigenetic germline development. So the question of how genetic signals induce the first few germ cells is one of fundamental importance and interest.

A genome-wide study of transcriptional dynamics conducted by Kazuki Kurimoto and others in the Laboratory for Mammalian Germ Cell Biology (Mitinori Saitou; Team Leader) has revealed how a complex network orchestrated by the regulatory factor Blimp1 guides the specification of primordial germ cells (PGCs) in the mouse. This work, which brought a sophisticated single-cell microarray and PCR analytical approach to bear on the question of what sets these cells apart from their somatic neighbors early in mammalian development. The article was published in the journal *Genes and Development*.

“The entire mouse germline arises from a small group of about 40 cells quite early in the embryo’s development,” says Kurimoto, “which meant we had to be able to analyze very small samples. The single-cell technology developed in our lab a few years ago was essential to making that possible.” The study, which looked at transcriptional dynamics and gene interactions across the entire genome in both wildtype embryos and those carrying mutations in the definitive PGC marker *Blimp1* (also known as *Prdm1*), represents the first such comprehensive study in any mammalian cell lineage.

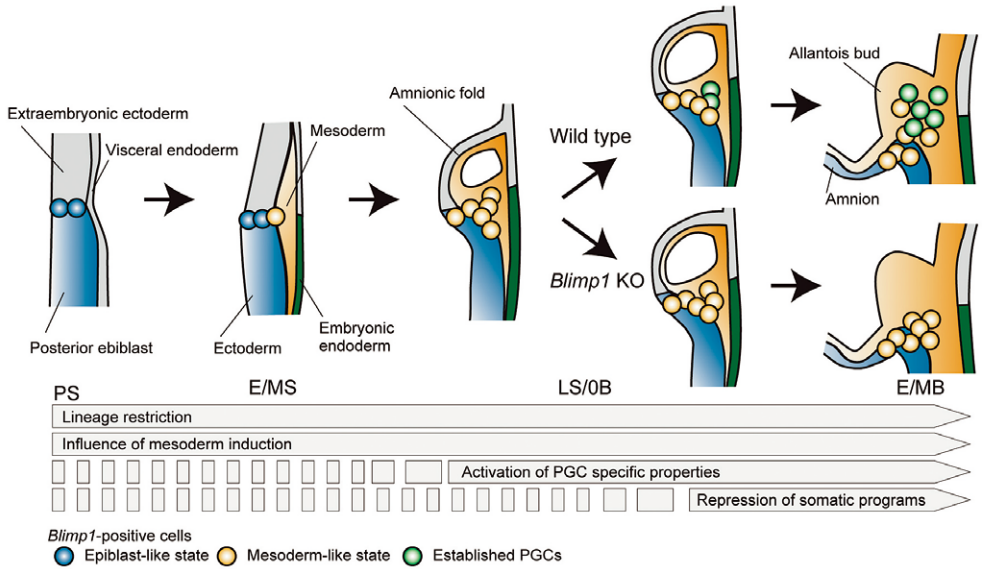
The team began by comparing gene expression patterns in cells taken from embryos at 6 different stages of development between day E6.25, when the germline is first specified, and 8.25, when the somites begin to appear and germline commitment is complete. One mainstream theory of germline specification has it that Blimp1 acts to repress “somatic” genetic programs, such as those enacted by Hox genes (represented

Kurimoto K, et al. Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. *Genes Dev* 22. 1617-35 (2008)

here by *Hoxb1*). The picture that emerged from this close analysis, however, was a bit more complex: undifferentiated cells (marked by the gene *Sox2*) that become specified by Blimp1 for germline fate first transiently express *Hoxb1* at levels similar to those in the surrounding soma before ultimately repressing this and other somatic genes.

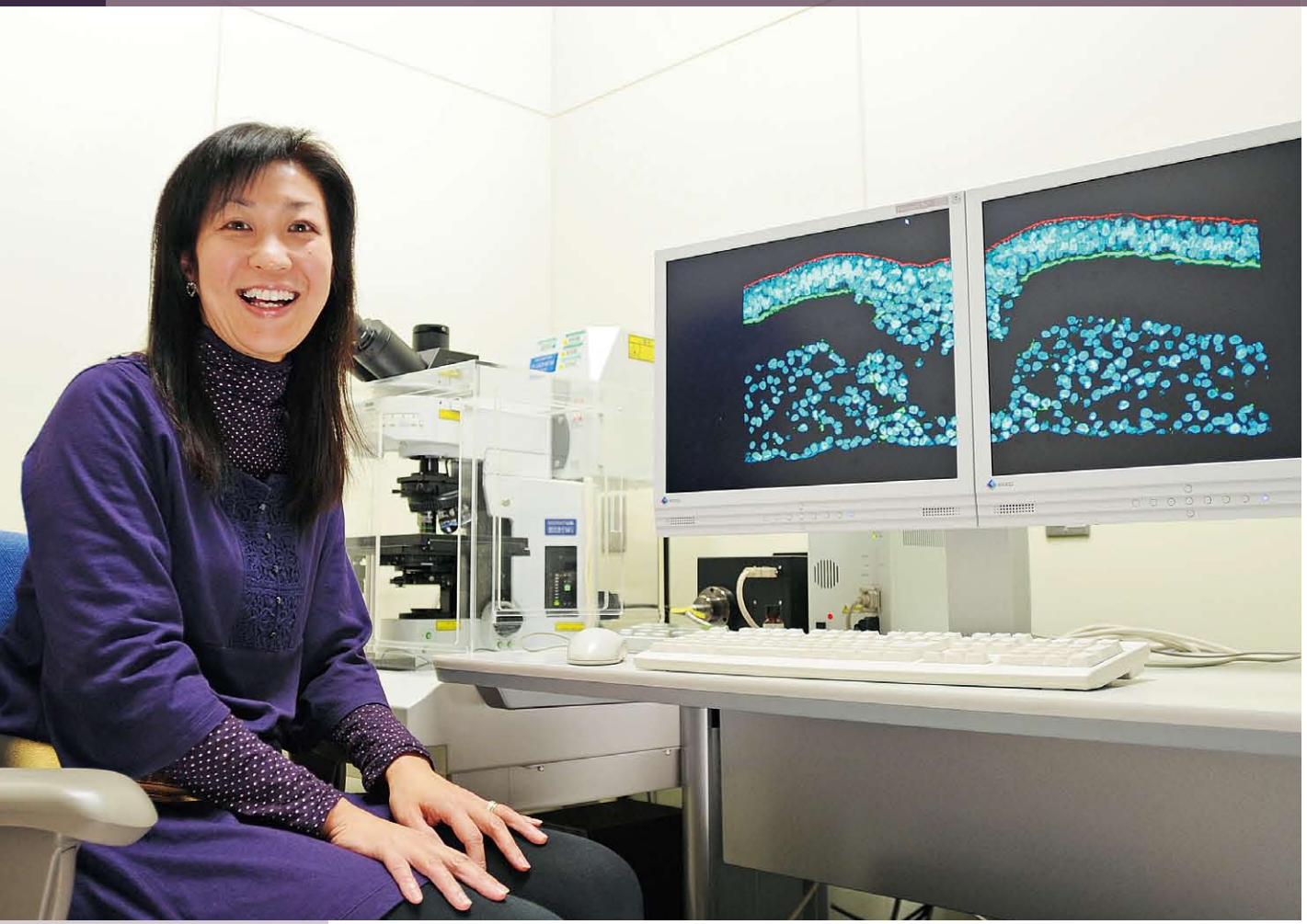
Comparison of genome-wide expression patterns in wildtype and *Blimp1*-mutant embryos also shed light on the dynamics and functions of specification and somatic genes in the germline anlage. Among the highlights of the spectrum of new insights were the findings that nearly all genes relating to the establishment or maintenance of DNA methylation are repressed in PGCs, and that germline specification occurs without a requirement for epithelial-mesenchymal transitions. This comprehensive trove of gene expression profiles of cells undergoing PGC specification now allows a more detailed understanding of what distinguishes the nascent germ lineage from its somatic neighbors at the genome-wide scale.

Kurimoto's analysis of loss-of-Blimp1-function embryos yielded even more intriguing gold, with the revelation that while this gene certainly plays a master role, it apparently does not work alone. While *Blimp1* was essential for the repression of almost all of the genes normally downregulated in PGCs, the transcription of many genes that had been presumed to be under the sole control of *Blimp1* was unaffected in the mutants, suggesting that other factors must also play a part. "What we saw was that some genes specific to the germline would switch on to some extent even if *Blimp1* wasn't working," says Kurimoto. "That said, in the mutant, the individual cells still couldn't coordinate all the many genes required. It seems that Blimp1 functions something like the conductor of an enormous orchestra, making sure that everything plays according to the score."



Differentiation of PGCs at different stages of early development.

Breaking away: A new role for RhoA in EMT



Yukiko NAKAYA

The cells of the embryo can be classed roughly by their structures into epithelial and mesenchymal types; epithelium is characterized by its more rigid borders, clear apical-basal polarity, and well-defined physical junctions with other cells and the extracellular basement membrane, while mesenchymal cells show none of these interactions and have more free-form morphologies. These cell types, however distinct, are not permanent, and switches from epithelium to mesenchyme or vice-versa occur in many developmental contexts, a phenomenon known generally as epithelial-mesenchymal transition (EMT) or mesenchymal-epithelial transition (MET). Given the frequency and importance of such events, they have been widely studied in cultured cells, but due to the technical challenges it presents, the regulation of EMT in vivo has yet to be adequately investigated.

Working with chicken embryos, Yukiko Nakaya and others in the Laboratory for Early Embryogenesis (Guojun Sheng; Team Leader) have shown that EMT during gastrulation plays out as a serial, stepwise process, beginning with the breakdown of the basement membrane. In an article published in *Nature Cell Biology,* Nakaya reports that this initial stage in the epithelial-mesenchymal transition is controlled by the downregulation of the factor RhoA, a small Rho GTPase known to regulate cytoskeletal rearrangements. Gastrulation is a process that takes place during early development in which, through a series of tissue movements, the early mammalian embryo transforms itself from an almost featureless cylinder to a rudimentary but recognizable body composed of three germ layers: ectoderm, mesoderm and endoderm. In the chicken, epithelial cells from a structure known as the epiblast ingress to form mesoderm (the germ layer that gives rise to tissues including heart, blood, bone and muscle), requiring them to undergo EMT in which they dissolve their attachments to neighboring cells and the underlying extracellular substrate.

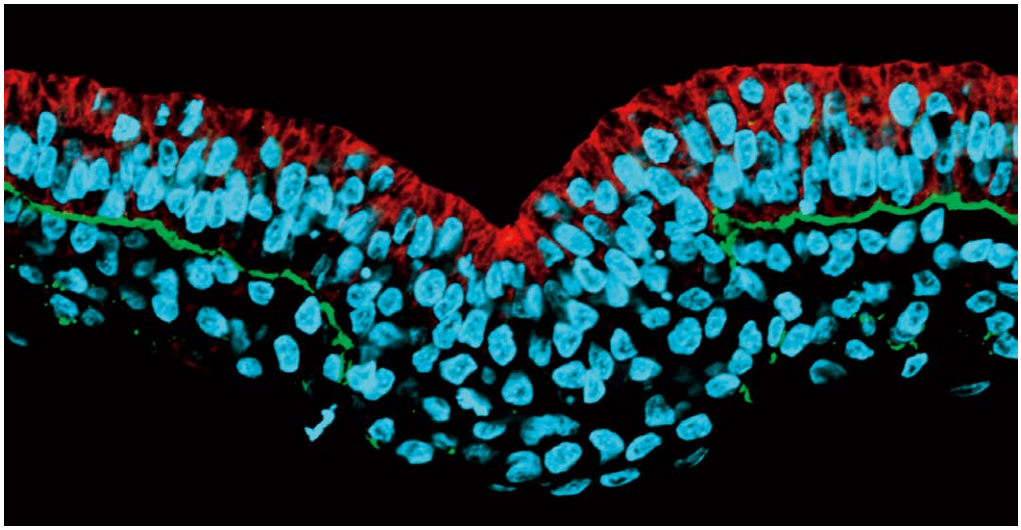

ARTICLES

RhoA and microtubule dynamics control cell–basement membrane interaction in EMT during gastrulation

Nakaya Y, et al. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nat Cell Biol* (2008)

"The epithelial-mesenchymal transition involves a series of distinct events that fortunately can be observed clearly and directly in the chicken embryo," says Nakaya. "What we wanted to do here was look at RhoA in this context, as it was known to play a part in regulating cytoskeletal changes in cultured cells, but was not very well studied in the living embryo."

The team first used electroporation to inject the RhoA gene into the chicken epiblast, and found that its misexpression caused a failure in the normal detachment of the epiblast from the basement membrane, a component of the extracellular matrix, preventing the occurrence of EMT. Looking more closely at the mechanics, Nakaya found that overexpression of RhoA caused a stabilization of basal microtubules, a cytoskeletal component responsible for maintaining cellular structure, which served to mediate the cell-basement membrane interaction. Inhibition of microtubules by a chemical agent caused the opposite effect, the breakdown of the basement membrane expanded beyond its usual region, a phenotype that could be induced by loss of RhoA function as well.



Cross –section of a gastrulation-stage chicken embryo with cell nuclei stained blue, RhoA in red and the basement membrane (marked by laminin) in green. Loss of RhoA coincides with breakdown of the basement membrane.

The findings from this study point to a scenario in which the interaction between epithelial cells and the basement membrane is mediated by the stabilization of microtubules by *RhoA*. The downregulation of RhoA in the epithelial epiblast allows for cells anchored by this cell-substrate interaction to break free and adopt the mesenchymal state, a critical first step in the EMT process. "Much of the work that has been done in EMT to date has focused on experiments done in vitro, so there have been a lot of calls to look at it in vivo as well," says Nakaya. "What we've been able to do in this study is identify an important new player in an EMT event taking place in a living embryo. And because EMT is very important in the invasion and metastasis of epithelial cancers as well, I hope more people will come to recognize the value of the early chicken embryo as a model for studying how it works."

How cells go pear-shaped

XiaoRei SAI

The organs of the body are born from simple sheets of cells that later take on their elaborate and complex forms during development, a process known as morphogenesis. It is known that such developmental processes involve changes at the level of individual cells, but what influences groups of cells to collectively change shape in a coordinated fashion remains largely a mystery.

The inner ear, which originates as a flat patch of epithelium that subsequently invaginates and pinches off to form a hollow cyst, provides an excellent system for the study of such organogenesis. In a study published in *Current Biology,* XiaoRei Sai and Raj Ladher of the Laboratory for Sensory Development (Raj Ladher; Team Leader) have revealed the role of extrinsic signaling in the morphogenesis of the inner ear in the chicken embryo. Through a series of experiments involving chemical inhibition of signaling pathway components, they showed that, following a preliminary induction phase, FGF signaling from the underlying mesoderm instructs the cells of the ectoderm that are destined to become the inner ear to expand basally, contributing to the transformation of columnar epithelial cells with parallel lateral sides into more trapezoidal forms, like voussoirs in an arch. This allows the cells to invaginate and form a concavity that ultimately pinches off to form the otocyst.

“We knew that morphogenesis was one of the first events after inner ear induction, which is controlled by a pair of FGF molecules, so we were curious as to whether FGFs played a role in this as well,” says Ladher.

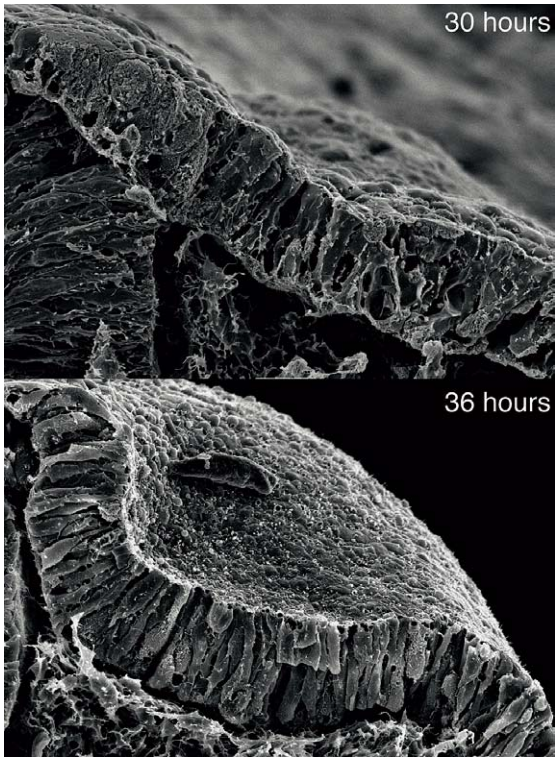
Sai and Ladher first confirmed that FGF was required for the morphogenetic stage of inner ear development by removing ectoderm that had been induced to become inner ear from embryos at different developmental timepoints. They found that even after induction, the tissue needed to remain in contact with its normal surroundings in order for morphogenesis to occur, suggesting that one or more external signals is required. Using microscopic beads soaked in the FGF factors

Sai X and Ladher R K. FGF signaling regulates cytoskeletal remodeling during epithelial morphogenesis. *Curr Biol* 18. 976-81 (2008)

known to induce the inner ear, they determined that FGF signaling to the basal aspect of the prospective inner ear was necessary to its subsequent morphogenesis.

Knowing that changes in cell shape are often the result of cytoskeletal rearrangement, the team next looked at microtubules and actin during the morphogenetic phase, and noted that while microtubules appeared not to change, actin filaments gradually became depleted from the basal aspect of the ectoderm in a manner suggesting that a local change in actin polymerization (rather than its localization) was responsible.

In looking to connect FGF signaling with the change in actin dynamics, they homed in on one of two known FGF signaling pathways, involving the phosphorylation of phospholipase Cγ (PLCγ), which they had linked to basal FGF signaling through inhibition of PLCγ and another downstream factor, protein kinase C (PKC). Suspecting that the mechanism responsible for the basal expansion of ectodermal cells was actin depolymerization, they first surveyed for well-known actin depolymerization factors, but were unable to detect them in the basal otic ectoderm. They turned next to myosin II, a factor that typically mediates the contraction of actin filaments, but which had recently been shown to play a part in actin clearance and turnover as well.



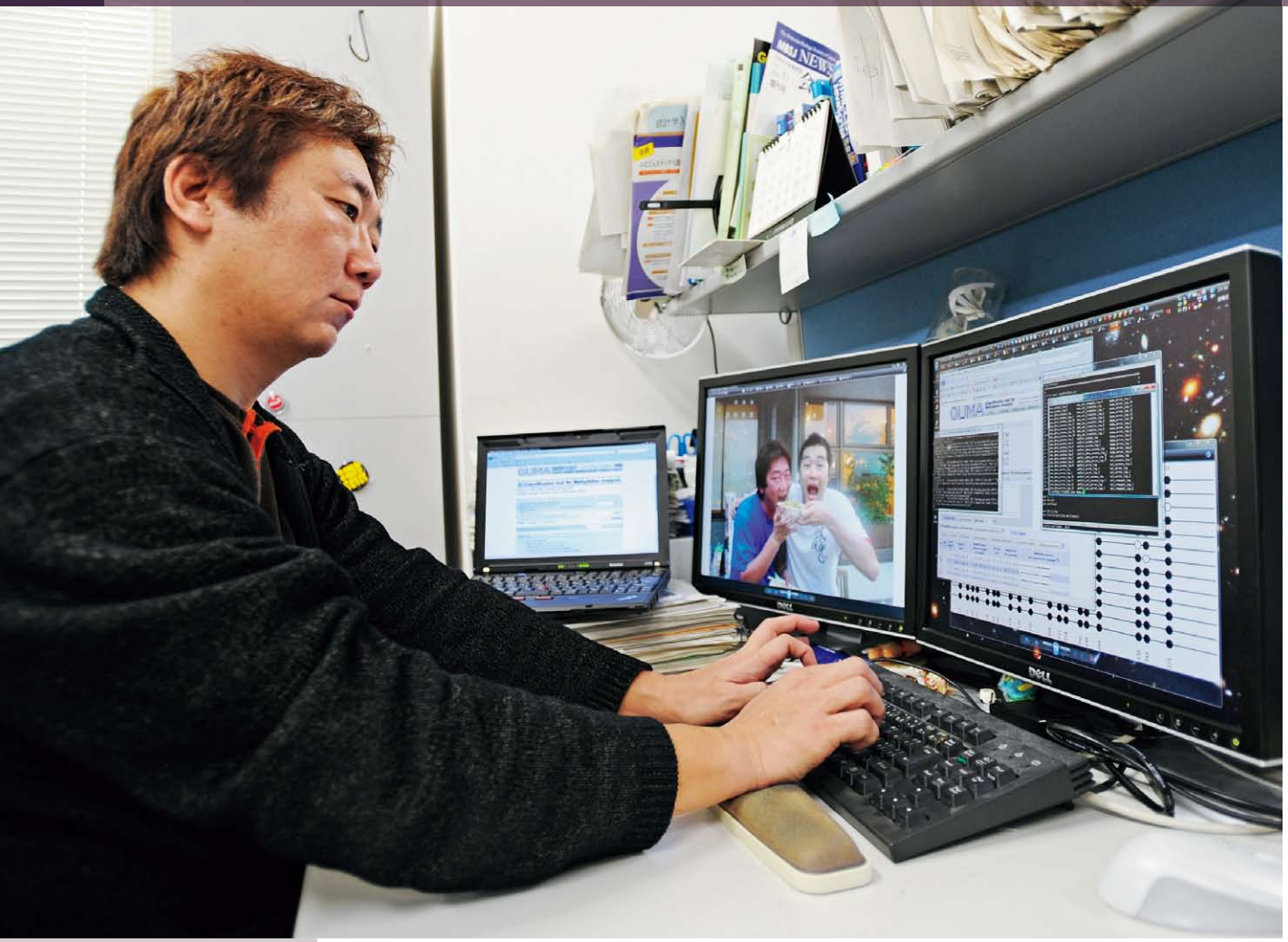
Scanning electron micrographs of the chick inner ear at 30 and 36 hours of development. The inner ear is a flat epithelial sheet at 30 hours, by 36 hours the inner ear has invaginated. In this report, the Ladher team shows that FGF controls the cytoskeletal remodelling events that lead to this morphological change.

Activation of non-muscle myosin II relies on the phosphorylation of a regulatory region known as the myosin light chain (MLC), and when the team used an antibody specific to phosphorylated light chain, they found it localized to the basal side of the otic tissue. Inhibition of myosin II activity also prevented invagination, suggesting that its activity is needed for morphogenesis of the inner ear.

With strong evidence that the FGF–PLCγ cascade and activated myosin light chain are both involved in the basal expansion of otic ectoderm during its morphogenesis, they sought to link them by studying the effects of inhibition of upstream factors on MLC phosphorylation, and found that it could be blocked by the inhibition of either FGF or PLCγ. Using a general inhibitor of protein synthesis, they further showed that this activation of myosin II was a direct effect of FGF signaling, as even in the absence of translation, the activation of the FGF-PLCγ-myosin II pathway caused the depolymerization of F-actin in the basal aspect of otic ectodermal cells, necessary for the apical constriction and basal expansion that gives them their wedge-shaped morphology.

“This is the first time to our knowledge that the shaping of cells involves something more than cell-intrinsic mechanisms,” says Ladher. “And as we see very similar processes in other aspects of development, like the formation of the neural tube from an originally flat plate of cells, it will be interesting to see how general the use of extrinsic signaling is in other such morphogenetic events.”

QUMA tool for bisulfite sequencing analysis



Yuichi KUMAKI

Bisulfite sequencing is a technology for determining the methylation status of stretches of genomic DNA, one of the most widely studied forms of epigenetic marking. The technique relies on the interesting fact that when DNA is treated with the ion $HSO3^-$, known as bisulfite, it causes all cytosine residues in the sequence to convert into the related nucleobase, uracil. This occurs in all cases but for one important exception: methylated cytosines remain unchanged. This makes it possible to determine the methylation status of specific sites in the treated sequence with high confidence. One of the main limiting factors in the application of bisulfite sequencing to basic and medical research, however, remains the analysis of the data generated by the DNA sequencer, which requires multiple steps of processing and refinement, including subjective quality checks that may introduce researcher bias.

That all may change with the release of QUMA (quantification tool for methylation analysis), a new software tool for bisulfite sequencing analysis developed by Yuichi Kumaki and colleagues in the Laboratory for Mammalian Epigenetic Studies (Masaki Okano; Team Leader), which addresses some of the shortcomings of previous such applications. The software, which is described in an article in *Nucleic Acids Research,* is available for download under a Creative Commons License from: http://quma.cdb.riken.jp/

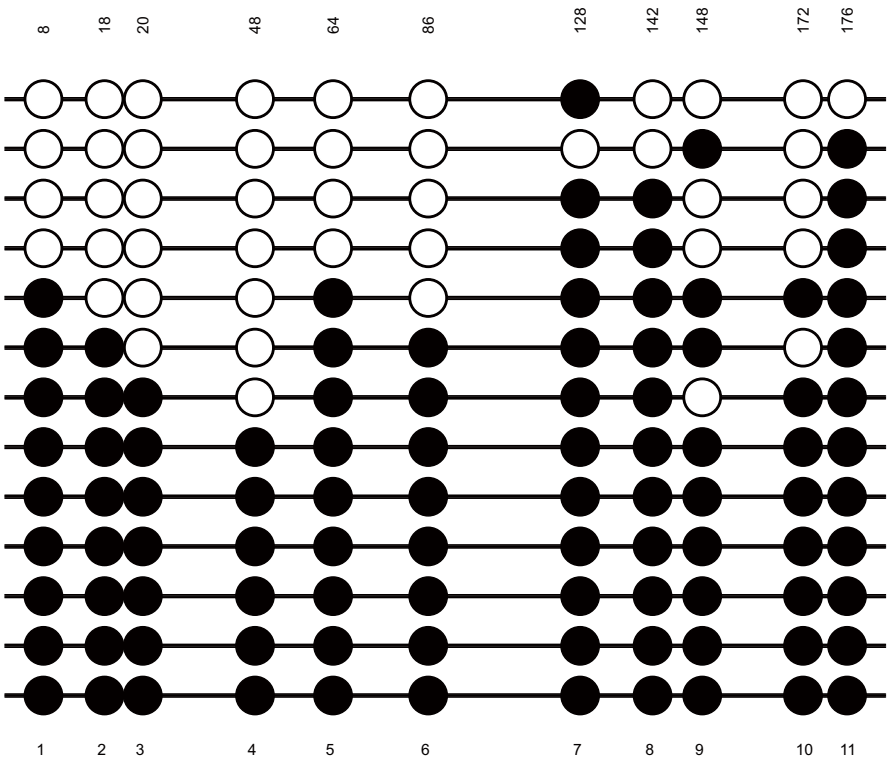
“I started developing QUMA because I had seen and experienced myself how much time was required for tasks like aligning sequences by hand and generating output for figures, ” says Kumaki. “I think that people studying DNA methylation will find that this tool will reduce the amount of time they spend on these routine tasks.”


QUMA: quantification tool for methylation analysis

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

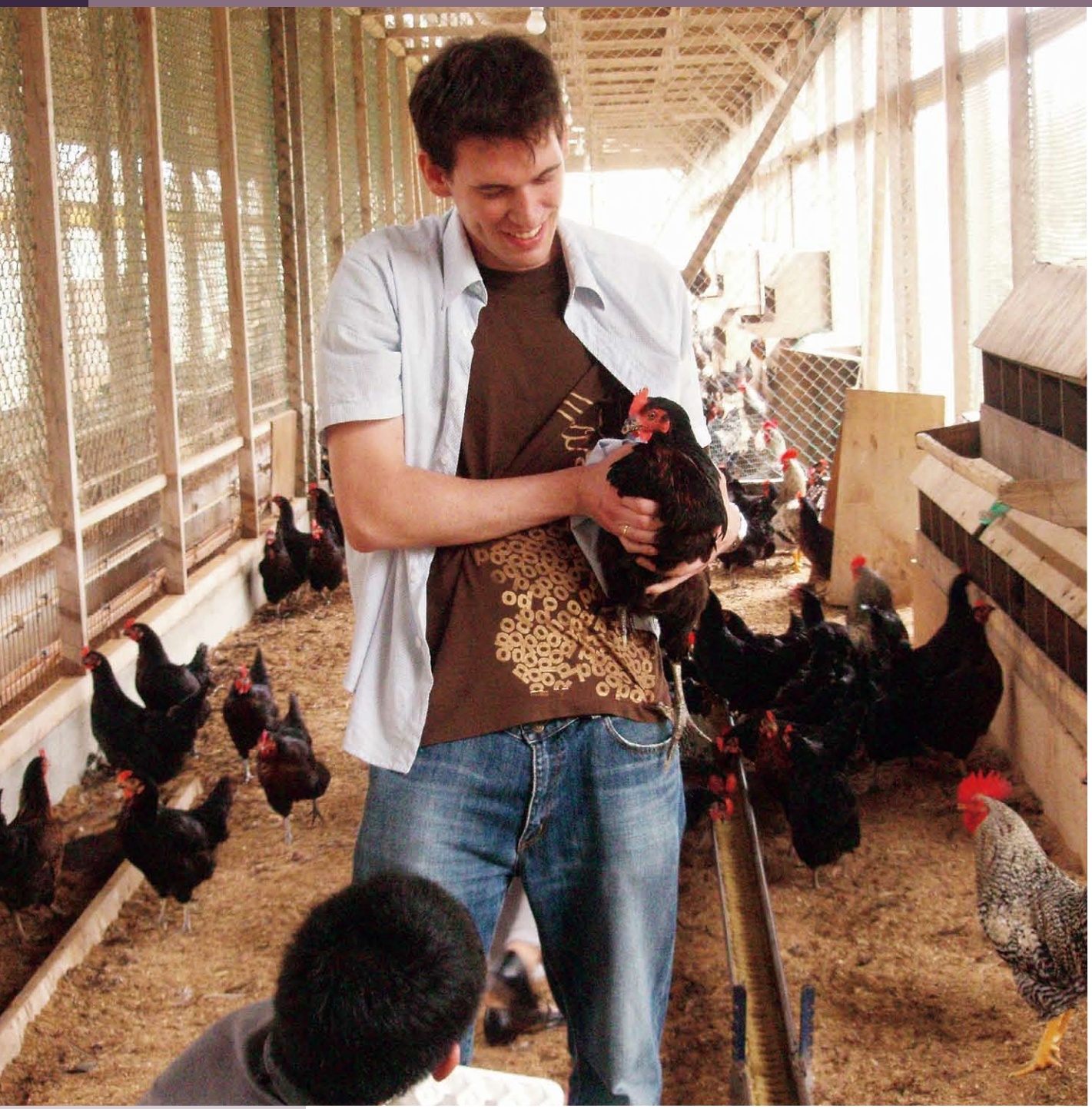
QUMA (pronounced koo-ma), boasts a number of features geared toward making bisulfite sequencing studies simpler. The user interface is easy to use and modify, and nearly all of the output data can be downloaded and configured to user specs. It provides a one-stop service for virtually all of the data-processing functions needed to analysis the methylation of sequences, such as sequence alignment, trimming, quality checks, visualization and statistical analysis. This represents a significant improvement over previous software that required, for example, manual alignment and trimming of sequences prior to analysis. QUMA puts quality control in the hands of the user with adjustable cutoff parameters, which can be shared with analysis data, increasingly their reproducibility. And, for users who wish to work with sensitive data, the entire software package can be run over a local network from a CD-ROM.

“We’re looking forward to receiving feedback from the DNA methylation community on ways to improve the program, and suggestions for new features, such as the ability to analyze methylation status of individual alleles,” says Kumaki. “The code is entirely open source, and distributed free via our website, so we hope that this finds a broad user base in the basic and medical research communities.”



Sample output automatically generated by QUMA’s user-configurable image generator.

Blood cell type diversity in the early embryo



Brendan McINTYRE

In chickens, as in other vertebrates, the emergence of the blood system takes place in two waves, a first that originates in extraembryonic tissue, and a second within the developing embryo itself. The nature of this early hematopoiesis is of great interest to scientists and clinicians alike, but the small size of the embryo in most popular model organisms makes it difficult to obtain enough material to conduct comprehensive studies. The comparatively large size of the chicken embryo at the transition from primitive (extraembryonic) to definitive hematopoiesis in the embryo proper makes it a good platform for investigating the changes that take place during this critical stage in the development of the blood system.

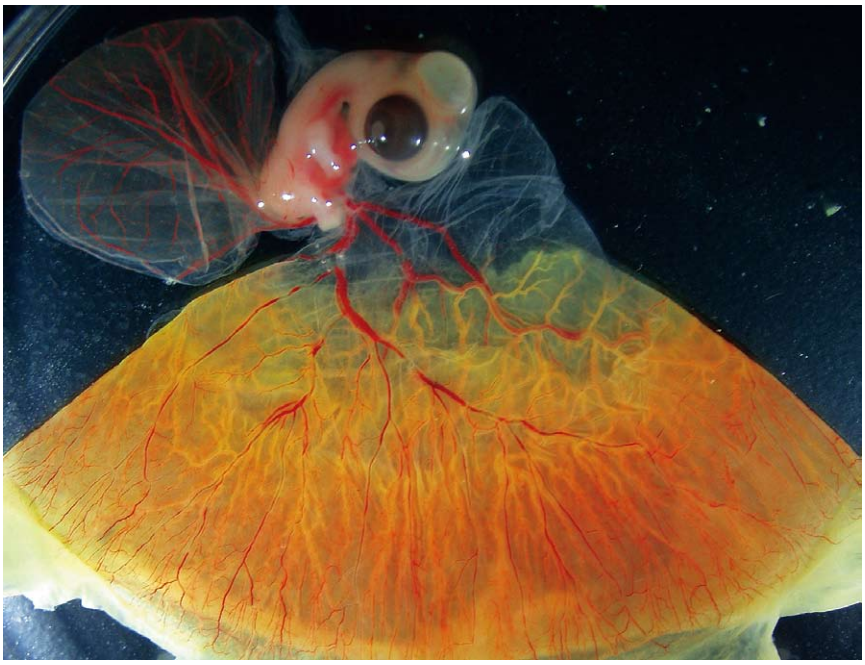

BMC Developmental Biology

Expression profiling of circulating non-red blood cells in embryonic blood

McIntyre B A, et al. Expression profiling of circulating non-red blood cells in embryonic blood. *BMC Dev Biol* 8. 21 (2008)

Brendan McIntyre and others in the Laboratory for Early Embryogenesis (Guojun Sheng; Team Leader) took advantage of this plentiful supply to examine gene expression profiles of blood during the period between embryonic days 4 and 6, when the specification of definitive hematopoietic lineages occurs. Through microarray data analysis, he found that circulating non-red blood cells include a full complement of blood progenitors and stem cells, meaning that this population may serve as a useful alternative to embryonic stem cells and perinatal blood for scientists interested in embryonic hematopoiesis.

The team began by taking samples from chicken embryos at days E4 and E6, and centrifuging them to sort the heavier red cells from the other lighter components of the blood system. Additional cell sorting by FACS and classical staining techniques enabled them to isolate a population of cells from which all red blood had been excluded. RT-PCR, a technique for amplifying gene transcripts, showed that the non-red blood population expressed the hematopoietic stem cell (HSC) marker CD34, while the red blood fraction did not, indicating that the non-red sample represented the sole source of blood stem cells in the embryo. McIntyre et al. next used Affymetrix gene chips to compare levels of gene expression, and identified a number of candidates whose expression was higher in the non-red blood cells (but, significantly, was not elevated in the heart). Their analysis revealed that numerous genes associated with hematopoietic stem cells, including those used in cell signaling, cell-matrix adhesion and communication between cells at gap junctions. In addition to these HSC genes, the team found markers of the myeloid and lymphoid lineages, providing the first evidence in chick of lymphoid differentiation at this early stage of development. The search also yielded six functionally uncharacterized genes, which it is hoped will provide new insights into HSC function and differentiation on further study.



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.

Science communications activities

The CDB takes engagement, outreach and public understanding of science very seriously, and organizes various events and programs every year to build relationships of mutual understanding and trust with the community at large.



2008 Open House

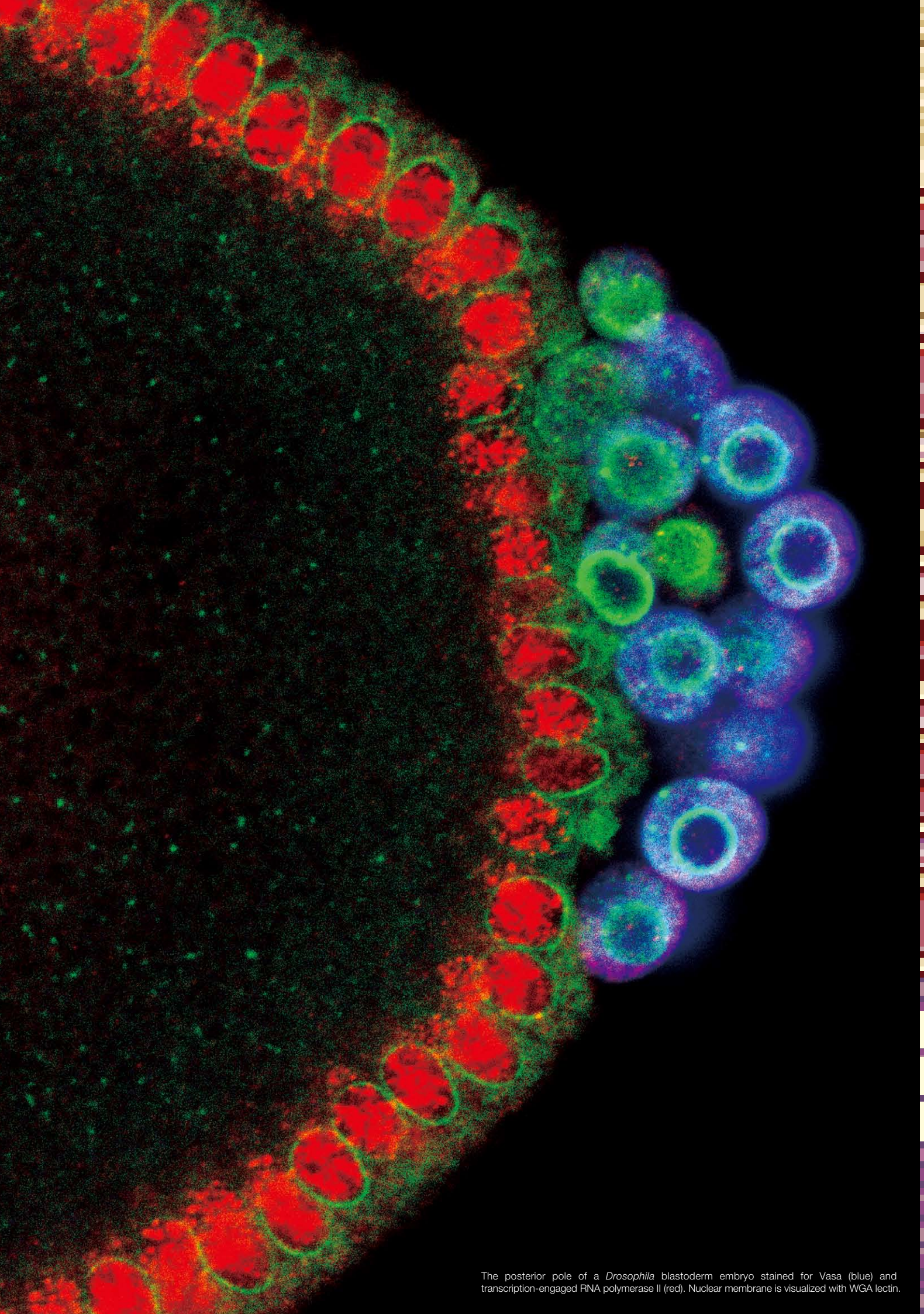
The CDB held its fifth annual Open House in May, welcoming more than 1,000 visitors who came to learn about the Center's research ongoing projects and recent achievements through a variety of exhibitions and activities. In the morning, CDB Group Director Shigeo Hayashi (Laboratory for Morphogenetic Signaling) gave a talk on "Learning about development and regeneration from insects." Two themed exhibits on "Development and Disease" and "Head and Brain Patterning" gave guests the opportunity to speak with researchers about their work and how CDB research connects to "big picture" academic and healthcare issues. This year's Open House was held in conjunction with the RIKEN Molecular Imaging Research Program and the city of Kobe's Medical Industry Research Project, and drew people from around the Kansai region of western Japan.




NIHITI ©Takagi Masakatsu Photo:Kazuhiro Tanda

An Evening of Science and Music

Over 800 people filled the Kyoto Kaikan Hall on August 22, 2008 to take in "An Evening of Science and Music", sponsored by the Japan Society for Science and Technology (JST) and co-organized by RIKEN. This event was part of an initiative by JST to combine art and science with the goal of conveying the wonders of science to a wider audience. This collaborative event featured a talk by Masatoshi Takeichi (Director of the RIKEN Center for Developmental Biology) followed by a performance by Masakatsu Takagi (a popular video artist and musician). One of the highlights of the evening was the world premiere of Takagi's newest piece "NIHITI", the concept of which originated from his visit to the CDB earlier this year to learn more about cells and biological phenomena. A dialogue between Takeichi and Takagi followed the lecture, where they exchanged their views on common ground between art and science. Asked about what inspired him in his work, Takeichi said, "Regardless of whether you are looking at flowers or insects, all life phenomena are beautiful. I carry out research with the hope that by attempting to reveal the essence of life, we will stumble upon an ultimately beautiful phenomenon. Art and science are alike in that they both seek ultimate beauty."



The posterior pole of a *Drosophila* blastoderm embryo stained for Vasa (blue) and transcription-engaged RNA polymerase II (red). Nuclear membrane is visualized with WGA lectin.

Cell Asymmetry

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

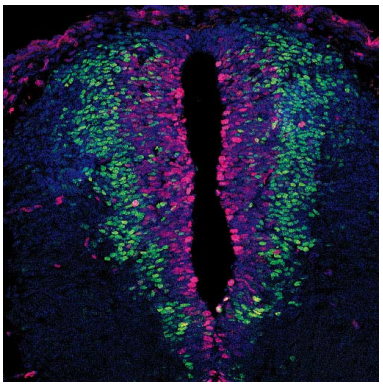
Fumio MATSUZAKI Ph. D.

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

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

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

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



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

Staff

Group Director
Fumio MATSUZAKI

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Publications

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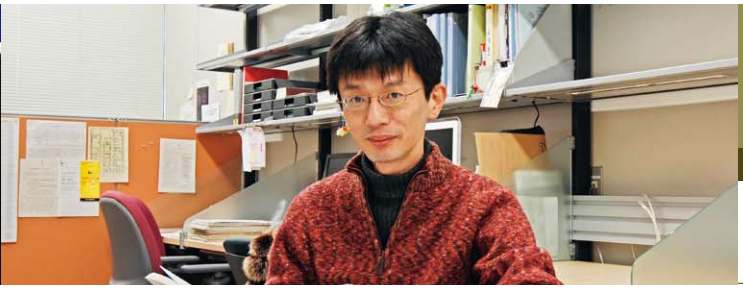


Germline Development

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

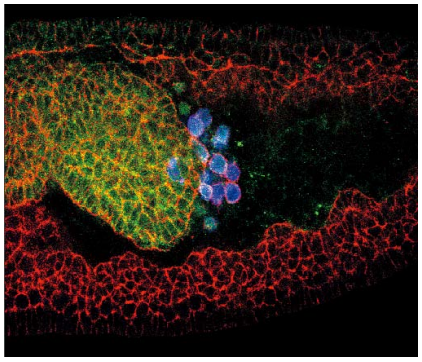
Akira NAKAMURA Ph. D.

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.

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

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

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



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

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Publications

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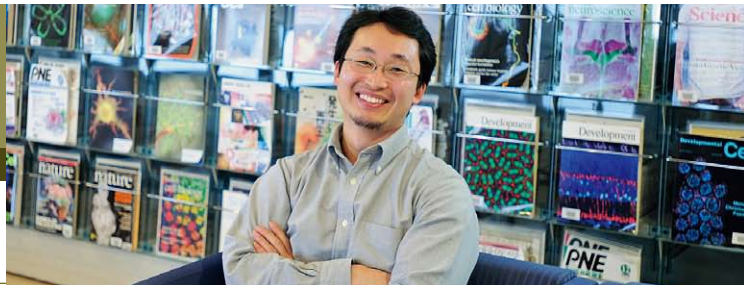
Chromatin Dynamics

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



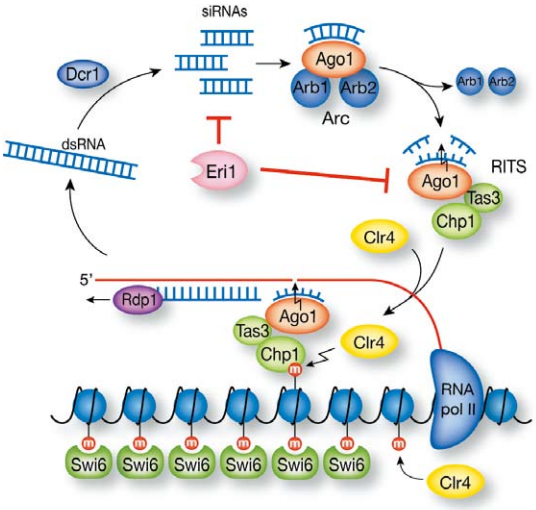
Jun-ichi NAKAYAMA Ph. D.

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.

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 post-transcriptional gene silencing by double-stranded RNA molecules are some of the best known epigenetic phenomena. Recent studies have begun to show that these different mechanisms are closely inter-related, but a detailed understanding of these systems has yet to be developed.

Our team investigates how modifications to the structure and configuration of chromatin (complexes of nuclear DNA and proteins that provide the structural basis of chromosomes) contribute to epigenetic gene regulation and how such modifications are transmitted over generations of cellular division by studying events at the molecular scale in the 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 construct and maintain higher-order chromatin structures. We also seek to clarify the picture of how dynamic rearrangements of chromatin structure are triggered by examining the structure and function of protein complexes that bind to and modify histones. Through these approaches we aim to understand the molecular mechanisms that underlie complex epigenetic phenomena in developmental processes.



Model for the RNAi-mediated heterochromatin assembly in fission yeast.

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Publications

Sadaie M, et al. Balance between distinct HP1 proteins controls heterochromatin assembly in fission yeast. *Mol Cell Biol* 28, 6973-88 (2008)

Iida, T, et al. siRNA-mediated heterochromatin establishment requires HP1 and Is associated with antisense transcription. *Mol Cell* 31, 178-89 (2008)

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Stem Cell Biology

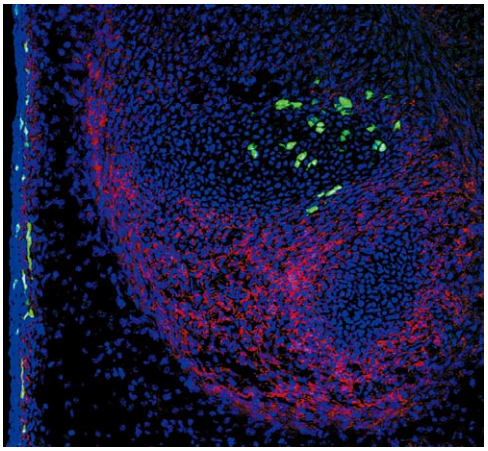
http://www.cdb.riken.jp/en/nishikawa

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

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 Medical School in 1987 to take a professorship in the Department of Immunology, and returned to Kyoto in 1993, as professor in the Department of Molecular Genetics at the Kyoto Graduate School of Medicine. He was appointed CDB group director in 2000.

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. Our current interest in this project is the development of hematopoietic stem cells, endothelial cells and pigment cells. These three ongoing research projects within our lab allow us to explore the problem of cell renewal in self-maintenance from multiple angles, an approach that we hope will provide new insights into this fundamental process.



PDGFRα+ mesenchymal stem cells derived from neuroepithelial cells in 14.5 embryo.

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Publications

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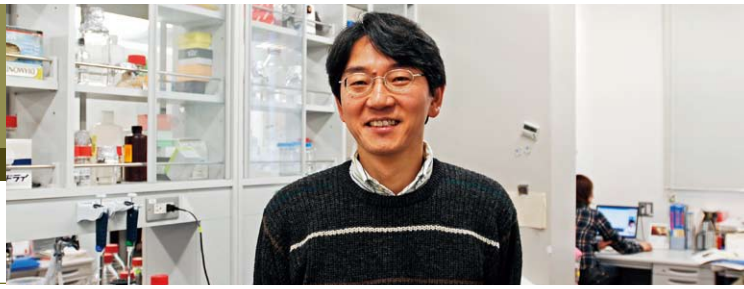
Cell Migration

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

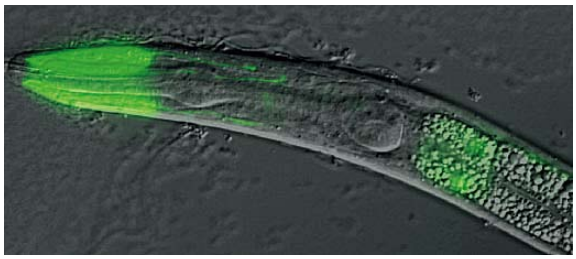


Kiyoji NISHIWAKI Ph. D.

Kiyoji Nishiwaki received his B. Sc. and M. Sc. from Osaka City University and was awarded a Ph. D. by the same institution for work on the molecular biology of *C.elegans* in 1994. He joined NEC Corporation in 1986 as a researcher in the Fundamental Research Laboratory, studying the molecular genetics of *C. elegans*. He left NEC in 1992 to work as a visiting researcher at Johns Hopkins University, then returned to the company in 1993 to continue his work on nematode molecular genetics. He remained at NEC until receiving an appointment as team leader at the RIKEN CDB.

In many forms of organogenesis, we can observe the coordinated movement of epithelial cells in sheets. For example, in the development of the lung, tubular epithelial sheets repeatedly extend and subdivide in branching patterns and thereby give rise to the minutely ramified and intricate structure of the airway. Epithelium is characterized by the presence of a basement membrane, an extracellular substrate in the form of a protein matrix, which plays important roles in regulating the direction and distance of epithelial cell migration. The main component of the basement membrane is collagen, but it comprises hundreds of other proteins as well, and abnormalities in the function of these proteins can lead to a range of developmental anomalies and adult pathologies.

We study the function of basement membranes in migration of epithelial sheets using a model organism called *C. elegans,* which is a tiny roundworm species about 1 mm in length. In this worm, the gonads develop following a stereotyped path in which cells at the leading end of the migrating gonad (known as distal tip cells, or DTCs) travel along a U-shaped route in the larval body, thereby giving rise to an organ of that shape. The proper migration of the developing gonad relies on surface interactions mediated by the basement membranes of the gonad and the body wall. We study various mutant worms in which the direction of gonadal cell migration is abnormal to search for clues to the genetic and molecular bases of DTC guidance. One of the genes we have been focusing on encodes a metalloprotease named MIG-17, which localizes in the gonadal cell basement membrane and plays an important part in the determination of the DTC's migratory route by breaking down or modifying other membrane proteins. We have also discovered that FBL-1, a member of the fibulin family of secreted proteins, is localized to the basement membrane in response to MIG-17 activity and also plays a role in directing cell migration. It is our hope that research such as this will provide insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions in which cell migration is aberrant.



Expression of *fbl-1::Venus* in the head region.

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Publications

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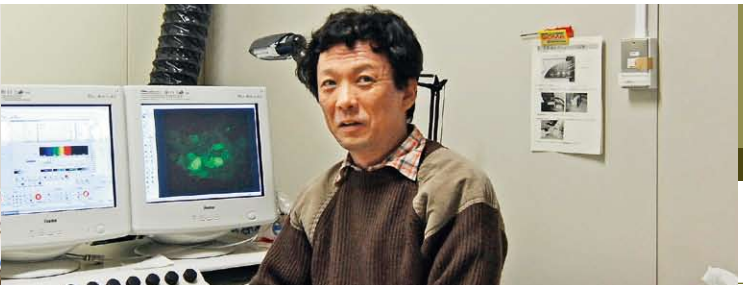


Pluripotent Cell Studies

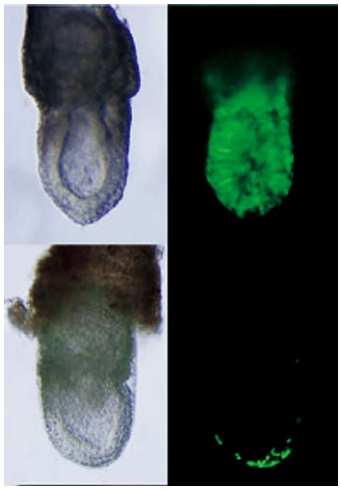
http://www.cdb.riken.jp/en/niwa

Hitoshi NIWA M.D., Ph. D.

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

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.



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 when treated with dexamethasone (lower panels).

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Toyooka Y, et al. Identification and characterization of subpopulations in undifferentiated ES cell culture. *Development* 135. 909-18 (2008)

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Mammalian Epigenetic Studies

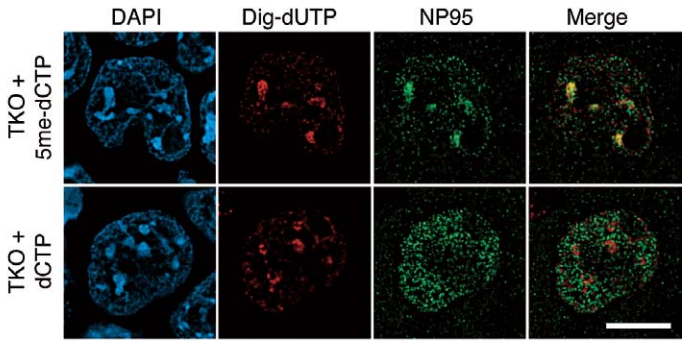
http://www.cdb.riken.jp/en/okano

Masaki OKANO Ph. D.

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.

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.



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

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Mammalian Molecular Embryology

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

Tony PERRY Ph. D.

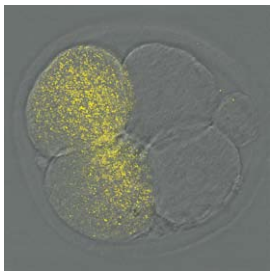
Tony Perry received his B.Sc. in Microbiology from the Department of Bacteriology at the University of Bristol, England and his doctorate from the University of Liverpool four years later. From 1989 he became a postdoctoral fellow and temporary lecturer working on epididymal sperm maturation at the University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

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

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

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

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



Twelve hours earlier, this 4-cell mouse embryo had just two cells. One of these had been injected with oocyte cytoplasm (containing fluorescent mitochondria so that it's easier to see). In the frog, this classic experiment causes the injected cell to stop dividing due to a long-sought cytostatic factor (CSF). The result: a 3-cell embryo. But the embryo above has 4-cells - the experiment doesn't work very well in the mouse, requiring a novel approach to identifying mouse CSF.

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Mammalian Germ Cell Biology

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

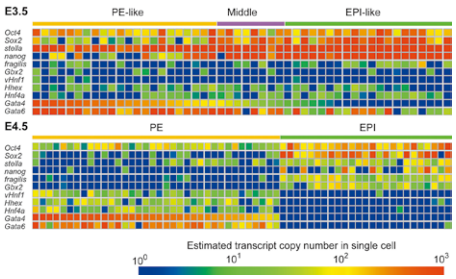
Mitinori SAITOU M.D., Ph. D.

Mitinori Saitou received his M.D. from the Kyoto University Faculty of Medicine in 1995, and received his Ph. D. in 1999 for his study of the structure and function of tight junctions under Shoichiro Tsukita in the Kyoto University Graduate School of Medicine. He then moved to the Wellcome Trust/Cancer Research Campaign Institute (present name: Wellcome Trust/Cancer Research UK Gurdon Institute), where worked as a postdoctoral research associate in Azim Surani's laboratory, focusing on the origin of the germ line in the mouse. He was appointed team leader at the CDB in 2003, and received a three-year grant from the Japan Science and Technology Corporation (JST) PRESTO program that same year, and was affiliated to the Kyoto University Graduate School of Biostudies as associate professor in 2004. He will assume a professorship at the Kyoto University Graduate School of Medicine in 2009.

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

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

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



Heat map representation of the copy numbers of the indicated genes in single ICM cells at E3.5 (upper) and E4.5 (lower). PE, primitive endoderm. EPI, epiblast.

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Organogenesis and Neurogenesis

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

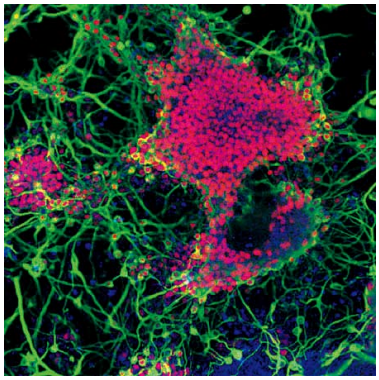
Yoshiki SASAI M.D. , Ph. D.

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*.

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 disease. Using a system developed in our lab, we have succeeded in inducing mouse and primate embryonic stem (ES) cells to differentiate into a range of specialized neuronal types. The application of similar techniques to human ES cells represents a field of study that, although still at an early stage, shows immense clinical promise.



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

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

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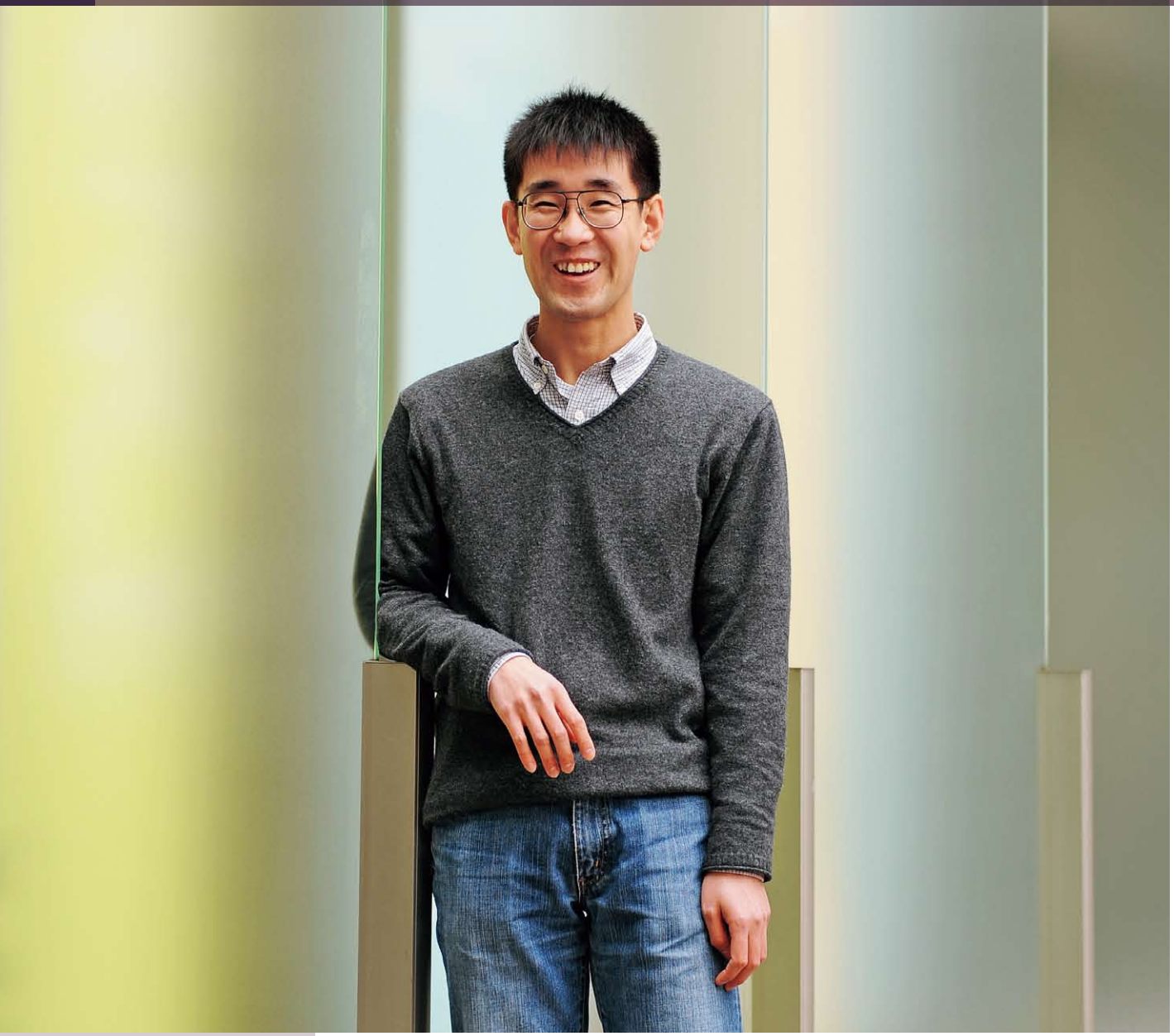
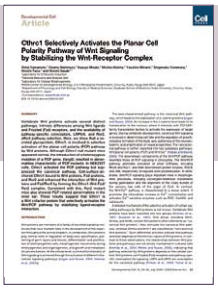
Cthrc1 ties Wnt PCP factors together



Shinji YAMAMOTO

Wnt activate pathways that play central roles in a wide range of developmental processes, from body axis specification to stem cell proliferation to morphogenetic cell movements. Two of the main programs are the canonical pathway, in which Wnt stabilizes β-catenin allowing it to interact with various transcription factors in the nucleus, and the non-canonical, planar cell polarity (PCP) pathway, which features the activation of numerous small GTPases and downstream factors important in cell morphology and motility. Different Wnt ligands are associated with each of these pathways (Wnt 1, 3a and 8 are known as canonical, while Wnt5a and 11 are non-canonical), but the means by which pathways are selectively activated have remained unclear.

Work by Shinji Yamamoto and others in the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) shows for the first time how an extracellular cofactor enables the selective activation of a Wnt pathway. The report, published in *Developmental Cell,* reveals how a secreted glycoprotein, Cthrc1, helps switch on the PCP molecular cascade by stabilizing the interaction between Wnt ligands and their receptors.



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

In the PCP pathway (as in canonical Wnt signaling) the primary receptor is the surface protein Frizzled, but importantly, the co-receptors in the two pathways differ; canonical signaling uses LRP5 and LRP6, while PCP uses a molecule named Ror2.

Yamamoto's first inkling of a role for *Cthrc1* came from a screen for genes expressed in the notochord (a transient axial structure found in all chordate embryos). Drawn by the fact that it codes for a secreted protein expressed in both the notochord and inner ear, suggesting the possible involvement of the Wnt/PCP pathway, he first created a null mutant, but found no phenotype when *Cthrc1* alone was ablated. When he studied its loss of function in mice carrying a heterozygous mutation in another PCP pathway element (*Vangl2*), however, he found defects in several developmental processes, including neural tube closure and orientation of the cochlear hair cells in the inner ear.

Using co-culture immunoprecipitation, a method newly developed by the Sasaki lab to detect interactions between proteins, Yamamoto et al. next asked whether Cthrc1 binds with the co-receptor Ror2. Intriguingly, they found that it not only binds this PCP-specific co-receptor, but to Wnt ligands and the Frizzled receptor as well, opening up the possibility that Cthrc1 strengthens the interaction between components, some of which are general to Wnt signaling and others that are specific to the PCP pathway. Conversely, Cthrc1 appears to suppress canonical Wnt signaling by some unknown mechanism.

"The fact that the *Cthrc1* solo knockout had no phenotype suggests that there are other genes able to compensate for its loss of function," says Sasaki, "so one of the next challenges will be to identify other genes that work in the selective activation of either the canonical or non-canonical Wnt pathways."

Figure 1

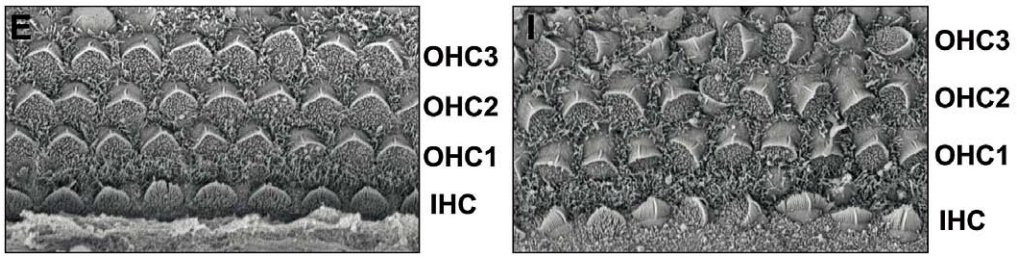


Figure 1
Misorientation of inner ear hair cells in a Cthrc1 homozygous / *Vangl2* heterozygous mutant (right), a phenotype typical of defects in the planar cell polarity (PCP) pathway.

Figure 2

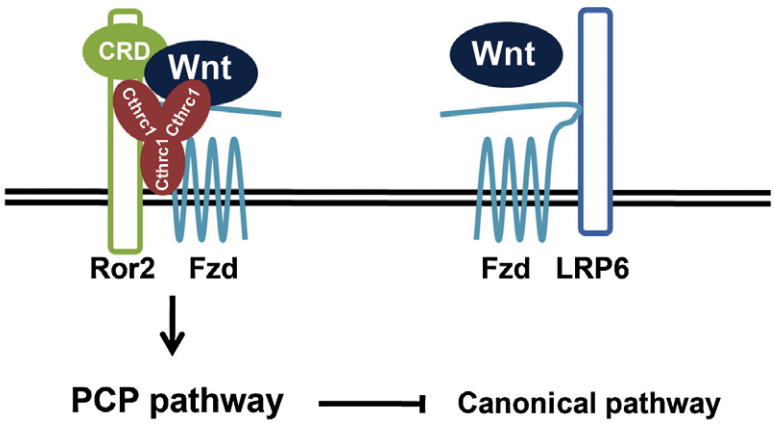


Figure 2
Model of the proposed function of Cthrc1 in stabilizing the interaction between Wnt/PCP pathway components (left). The canonical Wnt ligand-receptor combination is shown at right.

New germline specification gene found

Yoshiyuki SEKI, Masashi YAMAJI, Kazuki KURIMOTO

Germ cells diverge from their somatic counterparts fairly early during mammalian development, undergoing at least three processes: the repression of somatic genes, the reacquisition of the potential for pluripotency, and subsequent epigenetic reprogramming to a committed germline fate. The genetic factors involved in germline specification have been traced as far back as day 6.25 of embryonic development, when the gene *Prdm1* (also known as *Blimp1*) is switched on in a handful of cells in the epiblast, in what is believed to be the first critical step in the pathway to determining germline fate. A recent genome-wide study of transcriptional dynamics in early germline progenitors by the Laboratory for Mammalian Germ Cell Biology (Mitinori Saitou; Team Leader) has revealed, however, that the network is more diverse than previously expected, with *Prdm1* acting as a sort of conductor keeping this genetic orchestra in harmony.

Masashi Yamaji and others from the Saitou lab discovered that a gene identified in their previous analysis, *Prdm14*, plays a critical role in the establishment of the germ cell lineage. In a study published in *Nature Genetics,* they reported that this gene, which encodes a transcription factor expressed only in the germline, is necessary for two of the three hallmark events in the acquisition of germ cell fate.

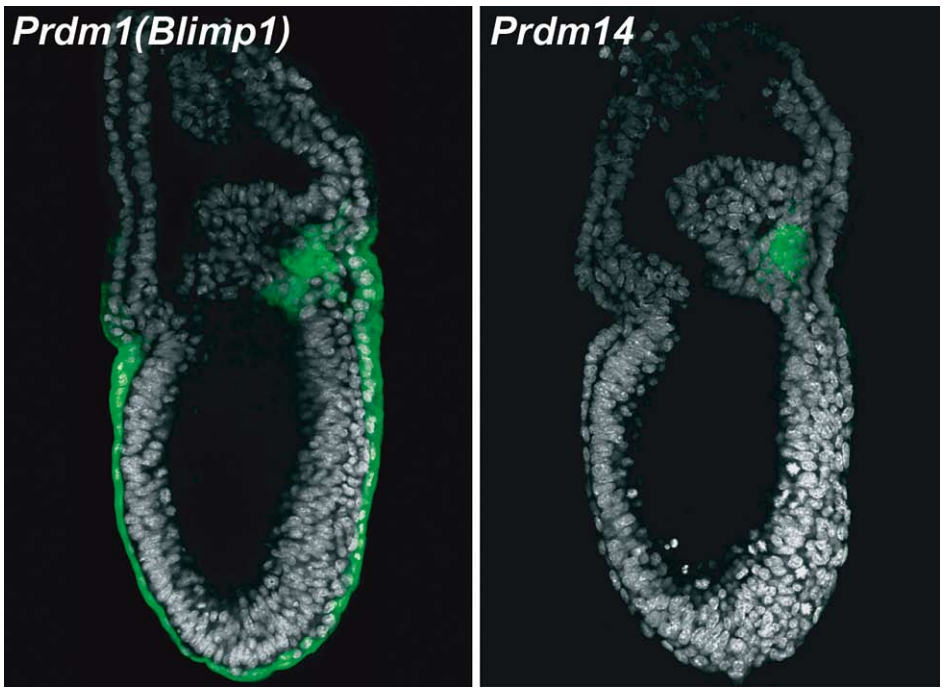
Prdm14 is first expressed transiently in the inner cell mass, a cluster of cells in the interior of the 3.5 day-old embryo that give rise to the embryo proper, but fades out by day 5.5, before reappearing at around E6.5 at a stage called the early streak, in which the germline master gene *Prdm1* is also just beginning to be expressed. This early germline-specific expression prompted Yamaji et al. to look more closely for a role in the specification of primordial germ cells (PGCs). To do so, they established mutant mouse lines in which Prdm14 was deleted. The homozygous *Prdm14*$^{-/-}$ mutants were of normal appearance, but both the males and females were sterile, their gonads being entirely devoid of germ cells.

Yamaji M, et al. Critical function of Prdm14 for the establishment of the germ cell lineage in mice. *Nat Genet* (2008)

The team examined germline development in the knockout embryos to determine the stage at which Prdm14 functions, and found that while somatic genetic programs (as represented by *Hoxb1*) were repressed, the cells failed to regain pluripotency (indicated by expression of *Sox2*) or to undergo the widespread epigenetic reprogramming indicated by the erasure of histone H3 lysine(K)9 di-methylation and upregulation of H3K27tri-metylation). Further in vitro experiments using primordial germ cells from the *Prdm14* mutants showed that, as suggested by their inability to reinitiate *Sox2* expression, they were also unable to de-differentiate to pluripotent embryonic germ cell-like cultures, which is another feature of wildtype PGCs.

Interestingly, when Yamaji and colleagues analyzed the position of *Prdm14* in the genetic network associated with PGC specification they found that although its initial activation does not require *Prdm1*, its subsequent maintenance and/or upregulation do. The trigger for *Prdm14's* activation appears rather to be dependent on the Bmp4-Smad pathway, a separate signaling routine at work in the early mammalian embryo.

"We now know that the specification of the germ cell lineage is orchestrated by the two transcriptional regulators, Prdm1 and Prdm14," says Saitou. "Our next challenges will be to clarify the biochemical mechanism by which these two key factors function and to reconstruct germ cell specification in vitro. We hope that this work will provide a useful foundation for reproductive and regenerative medicine."



Expression of *Prdm1(Blimp1)* and *Prdm14* in the emerging PGCs visualized by *Prdm1*- and *Prdm14*-mVenus, respectively.

Hypothalamic neurons from ES cells

Takafumi WATAYA

The hypothalamus is a small ventral region of the brain that sits atop the pituitary gland and serves as a critical interface between the nervous and endocrine systems. Although it only accounts for about 1/300 of the total brain mass, the hypothalamus plays important roles in the regulation of the autonomic nervous system and a set of behaviors that include feeding, sexuality, and the fight-or-flight response. Despite its importance, however, the development of the hypothalamus remains imperfectly understood, due in part to a lack of effective means for studying its differentiation in vitro.

A series of experiments by Takafumi Wataya and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) demonstrating a technique for inducing hypothalamic differentiation from mouse embryonic stem (ES) cells in culture stands to add significantly to our understanding of the development of this tiny but complex brain region. In an article published in the *Proceedings of the National Academy of Sciences USA,* Wataya showed that, by modifying a technique used for inducing neuronal differentiation in pluripotent cells, it is possible to generate populations of cells expressing specific hypothalamic markers at high efficiency.

The study began when scientists in the Sasai lab noticed that mouse ES cells cultured using a method known as SFEB (for serum-free culture of embryoid body-like aggregates) very rarely expressed hypothalamic marker genes, although this technique had been used with great success in giving rise to other


Minimization of exogenous signals in ES cell culture induces rostral hypothalamic differentiation

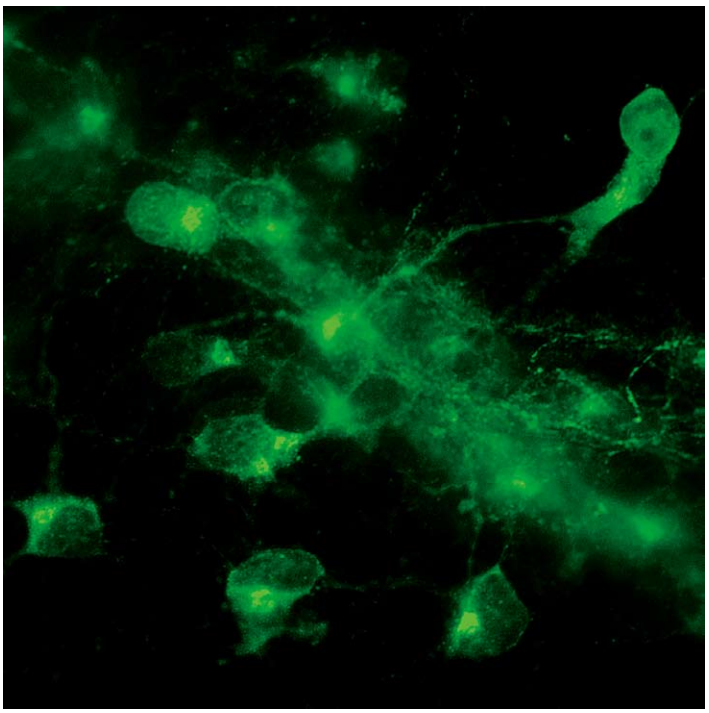
Wataya T, et al. Minimization of exogenous signals in ES cell culture induces rostral hypothalamic differentiation. *Proc Natl Acad Sci U S A.* 105. 11797-801 (2008)

specific neuronal populations, including telencephalic (forebrain) precursors. “We knew that the medium typically used in SFEB culture contained a number of bioactive factors, such as insulin,” says Wataya, “so we tested whether these might somehow be interfering with hypothalamic differentiation.”

Assays in which ES cells were SFEB-cultured in the presence or absence of insulin at different stages revealed that this hormone had a strong inhibitory effect on the differentiation of precursor cells expressing the hypothalamic marker gene, *Rax*. Interestingly, SFEB culture in the absence of insulin and other exogenous growth factors yielded populations of cells in which 60~70% expressed *Rax* and other genes indicative of hypothalamic neuronal fate. When the *Rax*-positive precursors were treated with the growth factor Sonic hedgehog, they found that cells went on to differentiate into neurons specific to different hypothalamic nuclei involved in hunger and satiety, which are of great potential value for studies of the cellular bases of metabolic regulation.

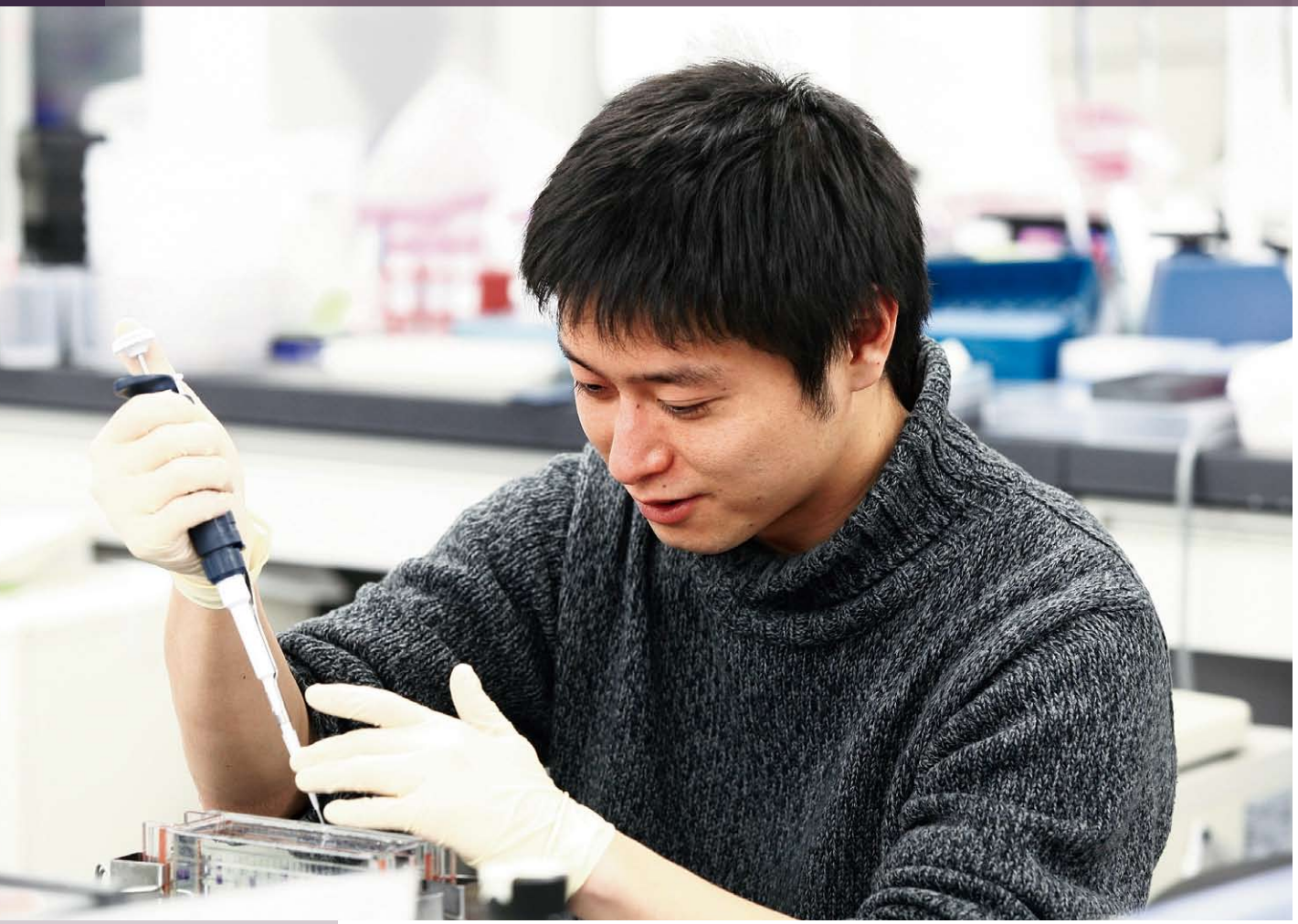
“Hypothalamus function is implicated in a wide range of medical conditions, including appetite and metabolic problems such as anorexia and overeating, but its small size has made it difficult to study in vivo,” says Wataya. “So we’re hopeful that the ability to grow large populations of homogeneous hypothalamic neurons through ES cell culture will make a contribution to the study of pathogenesis as well as the screening of new drug candidates.”

The discovery that exogenous factors work to inhibit hypothalamic differentiation also intriguingly suggests that this rostral-ventral region may be thought of as a kind of zero-point in the Cartesian positional patterning of the early neuroectoderm. Taken together with the enormous biological importance and high degree of evolutionary conservation of this region, Wataya’s findings raise the interesting possibility that the hypothalamic precursor fate stands as a default outcome in brain development, opening new avenues of understanding of the evolution of nervous systems as well.



Hypothalamic vasopressinergic neurons derived from mouse ES cell.

Pairing up and settling down



Shinsuke NAKAO

Individual cells are capable of moving over surfaces by using a variety of mechanisms, such as the formation of ruffling regions of cell membrane known as lamellipodia that allow the cell to creep across a substrate. But when two cells encounter each other, the interaction causes them to settle down, this loss of movement being one aspect of a phenomenon known as contact inhibition. A number of signaling and adhesion factors, including members of the classic cadherin family of molecules, have been implicated in this contact-mediated inhibition of cell movement, but a detailed understanding of the process has remained elusive. Shinsuke Nakao and others in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) have added another piece to this puzzle with the revelation that another cadherin known as OL-protocadherin is involved in recruiting cytoskeletal regulators to cell-cell contact sites, a previously unknown means of modulating cell migration.

OL-protocadherin (OL-pc) shows a similar like-binds-like mode of interaction to that seen in other cadherins, although its homotypic bonds are comparatively weaker. Previous work from the Takeichi lab had shown that rather than acting as a straightforward adhesion molecule, OL-pc might instead function in other cellular processes – neurons in mice lacking OL-pc, for example, showed defects in axon extension.

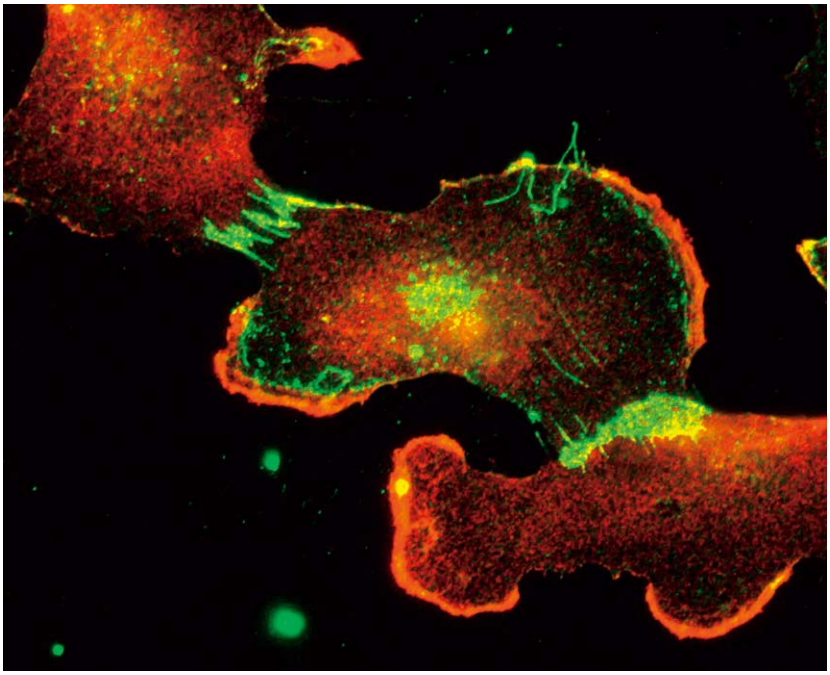
This novel role prompted the group to look for interactions with other molecules, using a technique known as a pull-down assay, in which the OL-pc protein was used as “bait” to survey for other proteins that bind with it. Their search for binding partners turned up the protein named Nap1 that is known to associate with a second factor called WAVE1, which functions in the assembly of the actin cytoskeleton protein into forms necessary for cell migration.

Nakao S, et al. Contact-dependent promotion of cell migration by the OL-protocadherin-Nap1 interaction. *J Cell Biol* 182. 395-410 (2008)

After identifying the specific molecular region at which Nap1 binds to OL-pc, Nakao et al. next investigated its cell biological function by transfecting OL-pc into a line of cells that shows high motility when cultured at low densities. Typical of other cadherins, OL-pc tends to accumulate in regions of contact between cells, while Nap1 and WAVE1 are concentrated in lamellipodia. In cells transfected with exogenous OL-pc, both Nap1 and WAVE1 became detectable at cell-cell contacts as well, phenotypes that were not seen when OL-pc lacking the Nap1-binding region was used.

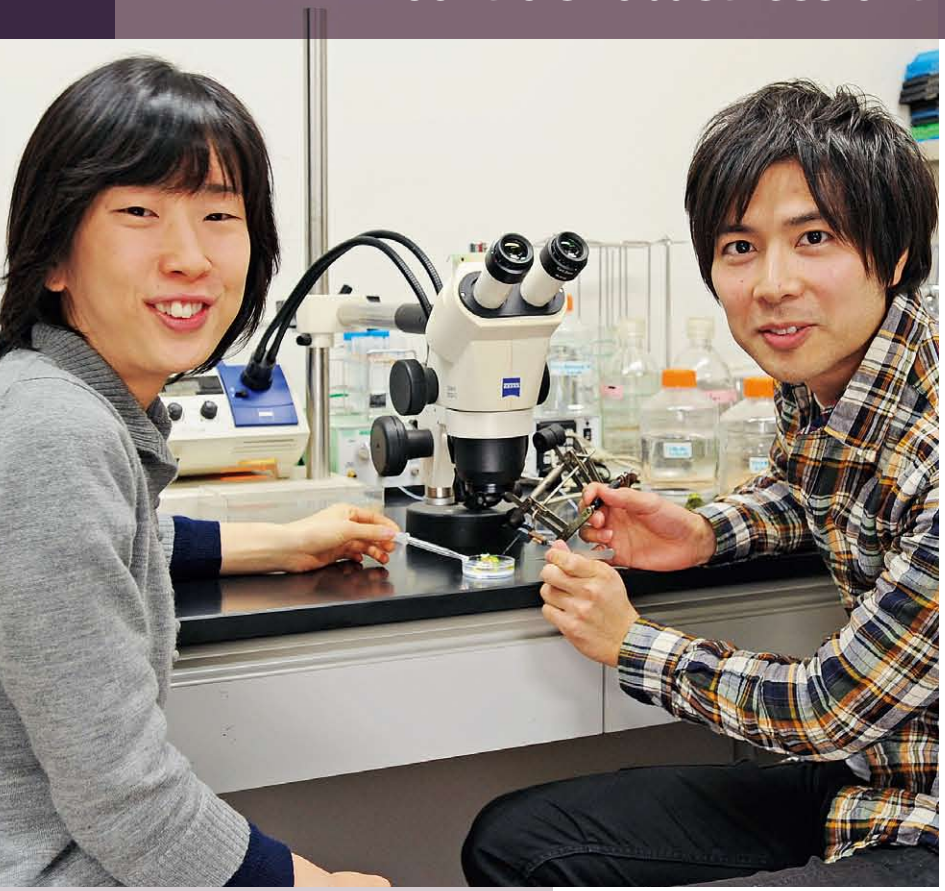
As Nap1 and WAVE1 are known to regulate cell movement, Nakao was curious about a possible role for OL-pc in this process as well. In cells moving freely at low culture densities, OL-pc misexpression had no apparent effect when observed using time-lapse microscopy. But when the cells were grown in more crowded quarters, enabling the formation of stabler cell-cell contacts, OL-pc-expressing cultures showed higher motility than their untransfected counterparts, with the cells moving in a jumpy, uncoordinated fashion. Tests in which Nap1 and WAVE1 were knocked down using RNAi revealed that OL-pc's function relied at least in part on the function of these actin regulators.

Interestingly, cells lacking N-cadherin, a classic cadherin expressed in neurons, mimicked the OL-pc misexpression phenotype, which, combined with observations of altered organization of adherens junctions in transfected cells, suggests that the OL-pc-Nap1-WAVE complex acts by remodeling actin assembly at cell-cell contact sites, causing changes in the structure of the cell-cell junction. The group speculates that this may be the basis for the increase in cell motility in contact inhibition mediated by cadherin adhesion is weakened due to the alteration of cell-cell junction structure.



Co-localization of OL-pc (green) and Nap1 (red) at cell-cell contacts in cells transfected with OL-pc.

Knowing when to stop: New stability factor controls robustness of the dorsal-ventral axis



Tomoko HARAGUCHI, Hidehiko INOMATA

One of the key questions in development is how tissues know when to stop growing. Early experiments on the head organizer by Hans Spemann and colleagues famously showed that this signaling region can prompt the growth of a second dorsal axis when transplanted into the ventral region of an amphibian embryo. Interestingly, the features, such as heads, that develop in the embryo with the organizer largely removed maintain sizes similar to those found in wildtype embryos, suggesting that dorsal-ventral developmental routines are robust against massive perturbations in their regulatory environment. But while the interplay of factors that assigns back and belly fates is well characterized, it has never clearly been shown how this robustness, in the face of change, is maintained.

A study by Hidehiko Inomata and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) identified a new factor responsible for stabilizing the dorsal-ventral axial pattern in *Xenopus laevis,* a popular system for the study of signaling in early development. This work, published in the journal *Cell,* shows that a secreted scaffold protein, ONT1, and the neural inducer Chordin (a BMP antagonist) in contact with a degradatory enzyme, allowing it to fine-tune BMP signaling in the axial tissue.

The study emerged in part from a theoretical conundrum surrounding the network of dorsal-ventral regulatory factors: the mutually inhibitory relationship between dorsalizing (such as Chordin) and ventralizing proteins (BMP4) appeared to be vulnerable to instabilities capable of triggering catastrophic changes in the patterning of the embryo. The observation that this rarely occurs under either natural or experimental conditions opened up the possibility that some unknown factor might be at work restoring the axial balance when things begin to go astray. One such factor, ADMP, was shown in a study by a different lab to work in this manner, but its loss-of-function phenotype suggested that it alone could not account for the sturdy robustness of this system.

Inomata's work began with the identification of the ONT1 protein in chicken by others in the Sasai lab. A member of the olfactomedin family and related to other neurodevelopmental regulatory proteins such as Noelin and Tiarin, ONT1 was interesting also for the expression of its homolog in the dorsal side of the embryo, from which the nervous system arises in frog. Using morpholinos to study its knockdown phenotype, the group found that loss of ONT1 function resulted in dorsalization as evidenced both by morphology and gene expression.

Wanting to know more about how it might relate to other components of the BMP regulatory network that controls dorsal-ventral patterning, they used tagged ONT1 in immunoprecipitation assays against a range of BMPs, their receptors and modulators, as well as other candidates such as Wnt and Nodal. Strong affinity was shown for the BMP antagonist Chordin, but also intriguingly with a group of proteinases (collectively referred to as B1TP proteins) known to degrade Chordin. Analysis of deletion mutations showed that the interactions with Chordin and the B1TP enzymes depend on different ONT1 domains.


Robust Stability of the Embryonic Axial Pattern Requires a Secreted Scaffold for Chordin Degradation

Inomata H, et al. Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. *Cell* 134. 854-65 (2008)

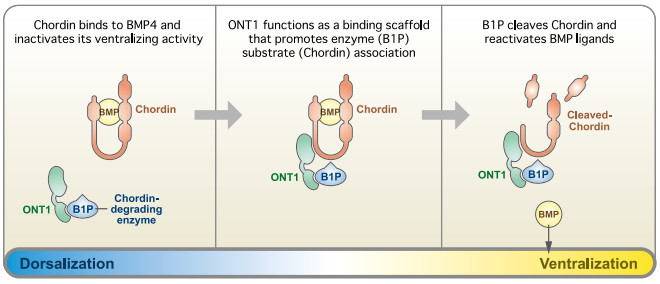
This dual role of ONT1 yoking Chordin to enzymes brought up the possibility that this protein works to titrate Chordin levels in dorsal-ventral patterning. Despite the apparently primary role of Chordin as a BMP antagonist, this inhibitory system is quite robust in response to changes in Chordin availability. Knowing that even a significant gain of Chordin function usually has only moderate dorsalization, Inomata tested whether the combination of Chordin protein injection and ONT1 deletion would disrupt the system's stability, and found that it did result in a hyperdorsalized phenotype as measured by gene expression and morphology.

In vivo tests of ONT1 at different concentrations showed that its role as a scaffold linking Chordin and B1TP enzymes is dose-dependent; normal levels of ONT1 reduce Chordin by promoting its degradation but, seemingly paradoxically, its overexpression actually leaves Chordin protected by binding to individual proteins and preventing the enzymes from coming into contact with and digesting their target. This scaffold model was further supported (at the molecular level by) immunoprecipitation assays that revealed increased Chordin-B1TP association in the presence of ONT1.

Interestingly, the group found that ONT1 is negatively regulated by ventralizing BMP signals, just as is Chordin. Given that its mode of action as a facilitator of Chordin degradation differs from ADMP, a previously reported pro-BMP (or to be more accurate, anti-anti-BMP) factor also shown to have only a moderate loss-of-function phenotype, they performed a double knockdown to see if the two work in concert. The ONT1-ADMP morphant indeed showed much stronger dorsalization, suggesting the factors play complementary roles, a kind of double safeguard that underlies the dorsal-ventral system's robustness.

"Past studies have developed a pretty clear picture of deterministic events in development," says Inomata, "but we have a poorer of understanding of how developmental programs recover when confronted with potentially catastrophic perturbations. This work shows that there is a kind of emergency manual for dealing with crisis situations, and maintaining this system's robustness."

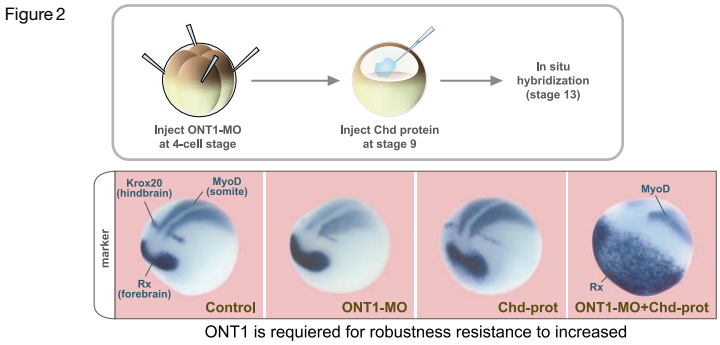
Figure 1



ONT1 binds to B1P and Chd as a scaffold protein

Figure 1
ONT1 binds to B1P and Chd as a scaffold protein.

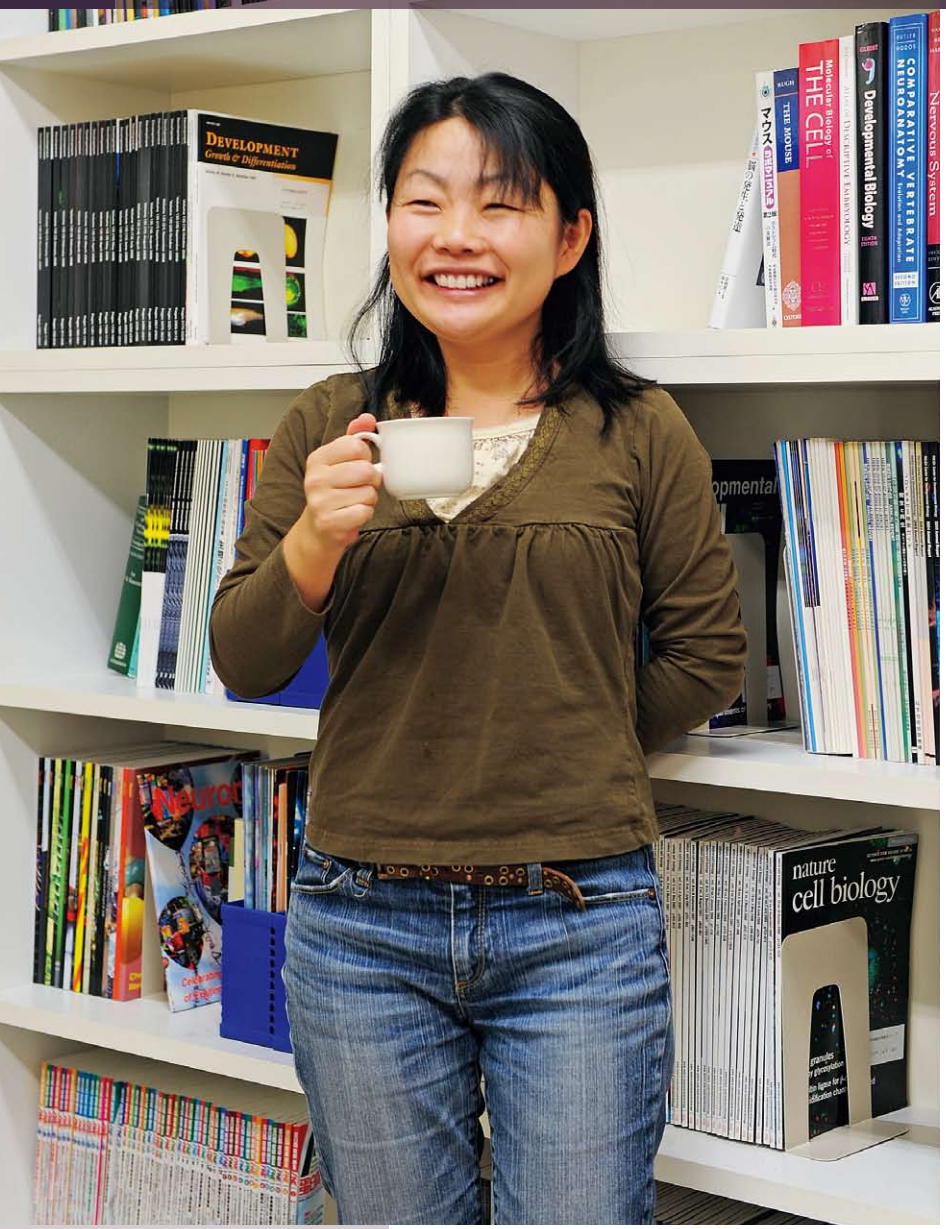
Figure 2



ONT1 is requiered for robustness resistance to increased Chordin in DV patterning of the early Xenopus embryo

Figure 2
ONT1 is required for robustness resistance to increased Chordin in DV patterning of the early Xenopus embryo.

Molecular basis of neural progenitor diversity



Ayano KAWAGUCHI

Mammalian brains feature dozens of neuronal subtypes generated principally by the division and differentiation of progenitor cells during embryonic development. In the neocortex, for example, elongated progenitor cells straddle and shuttle across the ventricular zone along this apical-basal axis, undergoing mitosis at the apical surface to give rise to the multiple strata of cortical neurons. But while molecular markers have been identified that distinguish apical progenitors from their basal counterparts, the mechanism that underlies their differentiation has remained largely a mystery.

A thoroughgoing statistical analysis of gene expression profiles of individual neocortical progenitor cells by Ayano Kawaguchi and colleagues in the Laboratory for Cell Asymmetry (Fumio Matsuzaki; Group Director), as well as the Laboratories for Mammalian Germ Cell Biology (Mitinori Saitou; Team Leader) and Systems Biology (Hiroki R. Ueda; Team Leader), yielded new insights into the molecular basis of their heterogeneity. Using a combination of single-cell cDNA amplification, cluster analysis, and in situ hybridization, the group identified a set of genes that distinguishes the stem cell-like apical progenitors from their more differentiated basal counterparts, as well as a possible role for Notch signaling in maintaining these two progenitor populations. Their findings were published in the journal *Development*.

Kawaguchi began by manually selecting a random assortment of individual cells (including several mature neurons used as controls) from the ventricular/subventricular zone of embryonic mouse forebrains, and used a high-efficiency single-cell PCR technique to analyze their expression of a set of 20 marker genes known to be associated with various populations of progenitors and neurons. This profiling work enabled them to class the cells into five groups: undifferentiated and neuronally-biased apical progenitors from the ventricular zone, subventricular (basal) progenitors, young neurons, and mature neurons.

To validate these initial findings, the group next performed hierarchical cluster analyses of the cells using microarray data. In both supervised and unsupervised analyses, the gene expression profiles of the individual cells allowed them to be grouped into four clusters closely corresponding to the results of the cDNA analysis. Cluster I cells included the full set of undifferentiated progenitors, and Cluster IV cells all the differentiated neurons. Clusters II and III showed highly similar gene expression and were most closely matched with the neuronally-biased ventricular zone progenitors and the basal progenitors, respectively. Clustering revealed that these two groups occupy the same branch of the hierarchical tree as the neurons, setting them apart from the undifferentiated progenitors in Cluster I.

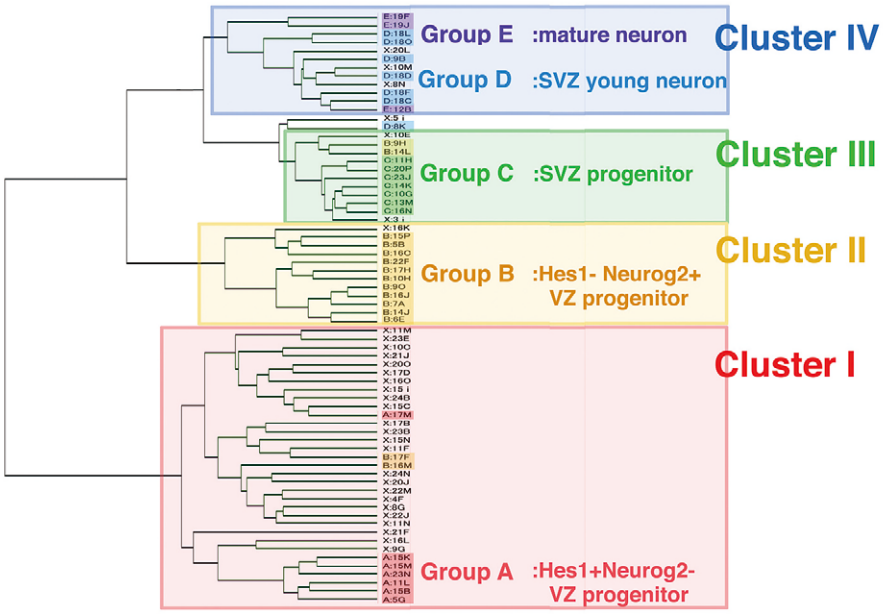
Kawaguchi A, et al. Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis. *Development* 135. 3113-24 (2008)

Equipped with a clear in vitro picture of neuroprogenitor taxonomy, Kawaguchi next looked in vivo, to see if gene expression patterns would match the predictions of the cluster analysis. In situ hybridization revealed distinct patterns of expression along the apical-basal axis, consistent with the expectation that the Cluster II cells represented progenitors that have begun to differentiate, and Cluster III basal progenitors in the subventricular zone. The high degree of similarity in gene expression between Clusters II and III raised the possibility that the Cluster II cells were young progenitors in the ventricular zone en route to more basal destinations in the subventricular zone, where they would mature and divide to produce a pair of terminally differentiated neurons.

Cluster I cells, in contrast, appeared more likely to be undifferentiated apical progenitors, whose nuclei undergo elevator-like shuttling in vivo. Looking at levels of molecules associated with neuroprogenitor fate determination, Kawaguchi et al. found a remarkable heterogeneity in factors in the Notch signaling pathway, although it was unclear whether this was due to transient contact or loss of contact by individual cells with sources of Notch ligands in their microenvironments, or an inherent diversity within the Cluster I population.

In any case, Notch signaling provided the strongest distinction between the Cluster I and Cluster II/III groups. Expression of the Notch ligand Delta-like 1 was seen most strongly in the Cluster II cells, suggesting that these young basal progenitors are an important Notch signaling source. Inhibition of this pathway in cultures including Cluster I (apical progenitor) cells resulted in dramatic increases in the number of cells that converted from an apical to a basal progenitor fate. The picture that emerges is one in which young basal progenitors express a ligand capable of inhibiting the differentiation of their neighbors as they make their way to the subventricular zone, thereby maintaining the balance between undifferentiated and committed progenitors.

“This is the first work to provide a genome-wide basis for the molecular properties and variety of neural progenitors in mammalian developing brains,” says Matsuzaki. “This allows us to clearly delineate one dynamic feedback system controlling the balance between self-renewal and differentiation among progenitors.”



Clustering of neural progenitor cells from a mid embryonic stage based on single cell gene profiling.

New-found control principles for biological clocks

Maki UKAI-TADENUMA, Yuichi KUMAKI

The advent of high-throughput genomics about a decade ago has yielded an extraordinary wealth of information on genome sequences, cDNAs, transcriptional start sites and binding sites for a growing spectrum of species. This burgeoning trove has simultaneously created the demand for new analytical tools, using computer algorithms to sift through the data, sometimes across genomes, in search of patterns of interest, identify new genes and regulatory factors and potentially synthesize new ones. As with any attempt to make sense of dizzyingly complex systems, such tools tend to work best when set to work on problems with at least some well-defined parameters. But once this combination of high-quality data sets and appropriate tools is in place, they represent a powerful new means of interrogating the molecular codes that underlie the machineries of life.

Using the mammalian circadian clock as a model system, Yuichi Kumaki and Maki Ukai-Tadenuma of the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader) have done just that. In a study conducted in collaboration with scientists at the University of Pennsylvania, Kinki University and INTEC Systems Institute, the Ueda team constructed a database of DNA regulatory regions in mammalian genomes, surveyed it with statistical methods to predict new targets, and finally validated their model with synthetic regulatory elements. Their work, published in the *Proceedings of the National Academy of Sciences USA,* demonstrates the power of the systems biology approach in uncovering new biological principles.

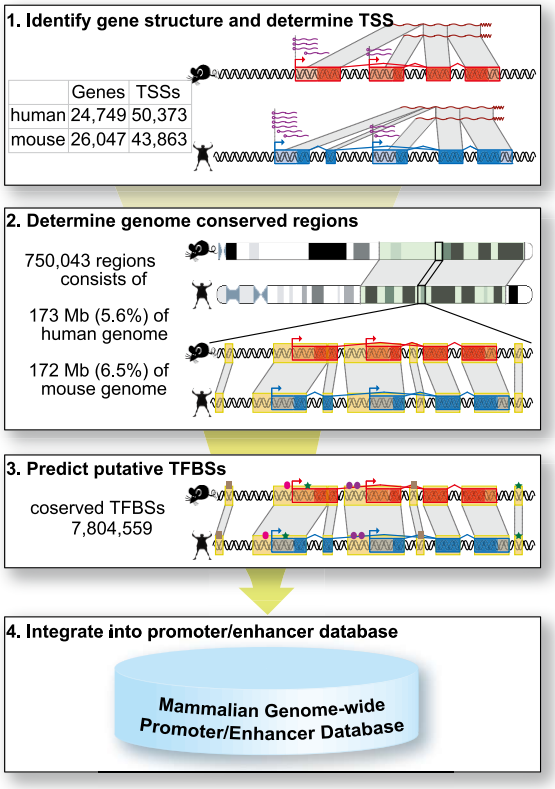
The oscillatory nature of circadian gene expression in mammals is maintained by the cyclical interaction of transcription factors, which produce periodic transcriptional read-out through three sets of elements whose activity peaks in morning (E-box), daytime (D-box) and night (RRE). While dozens of such clock-control elements are known, as an estimated 5-10% of mammalian genes show circadian expression, much remains to be learned. To address this question, the Ueda team assembled a database of human and mouse enhancers and promoters (two types of DNA regulatory regions), and customized a statistical method known as a Hidden Markov Model (HMM) to compare data and search probabilistically for new clock-controlled elements.


Analysis and synthesis of high-amplitude Cis-elements in the mammalian circadian clock

Kumaki Y, et al. Analysis and synthesis of high-amplitude Cis-elements in the mammalian circadian clock. *Proc Natl Acad Sci U S A* 105. 14946-51 (2008)

They found that the Hidden Markov Model performed best in non-coding regions conserved between mouse and human, and that while D-box and RRE elements showed no bias in their distribution, E-box sequences tended to be grouped around transcriptional start sites. Their search turned up more than 6,500 predicted E-box, D-box and RRE elements. After winnowing out false positives, tests for clock-like temporal expression patterns using the top 100 putative clock-controlled elements for each of the three categories showed highly consistent peak expression times in approximate phase with other circadian elements.

The team next validated their candidates in vitro, picking the ten highest-confidence E-box, D-box, and RRE elements, and transfecting them fused to a luminescent reporter into cells to observe their transcription. After stimulation to synchronize the cells' circadian rhythms, 4 of the transfected E-box candidates, 7 of the D-box, and 6 RREs showed strong daily oscillations. They next checked the expression of these 17 genes in vivo, examining endogenous transcripts from seven different mouse tissues and found circadian patterns of expression for 13.



Cartoon of experimental design of the PNAS study.

As systems biology seeks not only to find what is in a system, but also conceptually what might be, the Ueda team looked back to the HMM. The Hidden Markov Model is essentially a means of inferring probabilities about unknown states in a system by looking at known properties of the same system, such as probabilities of the state generating a given outcome ("emission") and of transition from state to state. Kumaki and Ukai-Tadenuma emitted sequences from the E-box, D-box and RRE models, and filtered out the naturally occurring ones, leaving only those that appeared not to be present in the genome (but which nonetheless are predicted to have similar activity to their natural counterparts). Taking one "high-scoring" and one "low-scoring" element for each of the models, both of which contained the same consensus sequence but different flanking sequences, they tested them in a synthetic reporter system and found that all of the high-scoring elements showed high-amplitude circadian transcriptional activity. This synthetic reporter assay clearly provided the evidence that the flanking sequences around the consensus sequence can function to titer the amplitude and oscillating rhythm of their transcriptional output.

Thinking that such flanking sequences might play a role in changing the binding affinity (and thereby alter the amplitude) of clock gene regulators, the team analyzed the affinities of activators and repressors in competitive binding assays. Interestingly, among the E-box elements (but no D-box or RRE), those which had previously been classed as low-scoring had higher affinity for activators and approximately normal affinity for repressors, while the affinities for activators and repressors in high-scoring E-box elements was closer, suggesting that a balance between direct activators and repressors is important for generating high-amplitude E-box output.

Principles proven: Transcriptional logic of the mammalian biological clock

Maki UKAI-TADENUMA, Takeya KASUKAWA

Much like their mechanical counterparts, mammalian biological clocks rely on complex inner workings. In mouse, the circadian clock is established by a system of interlinked genetic regulatory loops consisting of at least three sets of clock-controlled DNA elements that mediate transcriptional output at different times of day: morning, daytime and night. With three such elements already identified, the genetic regulation of the biological clock already rivals the most sophisticated clockworks in its complexity, but the dynamic principles that govern this system have never been clearly defined.

A study by Maki Ukai-Tadenuma of the Laboratory for Systems Biology and Takeya Kasukawa of the Functional Genomics Unit (both of which are led by Hiroki R. Ueda; Team Leader/Unit Leader) provided new insights into the transcriptional logic of the mammalian circadian clock. In an article published in *Nature Cell Biology,* Ukai-Tadenuma and Kasukawa describe how they simulated this circuitry *in cellulo* using synthetic factors to manipulate and observe the system dynamics of the biological clock.

The work began with the development of a clock-cell culture system in which the expression of a synthetic transcriptional activator and repressor were put under the control of morning (E'-box), daytime (D-box) or night (RRE) clock-control DNA elements. By transiently transfecting the host cells with the activator and repressor, along with a reporter construct used to monitor output, the team was able to watch their transcriptional activity in real-time over the course of several days per transfection.

Testing a hypothesis that a morning activator combined with a nighttime repressor would be sufficient to produce daytime transcriptional output (which was based on experimental results from previous work in the Ueda lab), they put their synthetic activator and repressor under the control of E'-box and RRE elements, and observed the resulting output oscillations as indicated by the luminescent reporter. The transcriptional output tracked very closely (within 1 hour) of the rhythm seen in the output of the natural daytime phase controlled by D-box elements.

A related principle for nighttime output, determined by a daytime activator and morning repressor, yielded similar confirmation of theory; the transcriptional output of the synthetic circuitry oscillated in near-lockstep with the natural daytime clock, providing an important proof-by-synthesis validation of the logics underlying the regulation of the mammalian biological clock in vivo.

Confident that their synthetic system could faithfully mimic at least two natural daily rhythms, Ukai-Tadenuma and Kasukawa next attempted to generate other circadian phases. The pairing of a night activator and a daytime repressor, for example, generated high-amplitude transcriptional output in the pre-dawn period, while other combinations of morning, daytime and night activators and repressors likewise resulted in oscilla-

Ukai-Tadenuma M, et al. Proof-by-synthesis of the transcriptional logic of mammalian circadian clocks. *Nat Cell Biol* 10. 1154-63 (2008)

tions with regular circadian phases. Given these results, they reasoned that in principle, it should be possible to generate transcriptional output that would peak at times corresponding to (subjective) midday, dawn, dusk or late at night.

A previous report from the Ueda lab had proposed an even more general model for circadian oscillations under genetic control. In this model, the expression of a repressor in advance of an activator element ("repressor-precedes-activator") results in the delay of transcriptional output, while in the opposite case ("activator-precedes-repressor"), the phase is advanced. A corollary to this is that larger discrepancies between the repressor and activator phases ("repressor antiphasic to activator") create higher amplitude oscillations. Using this as a starting point, they searched for theoretical sets of parameters likely to generate specific outputs, and created an input-output plot against which they could test the theory using their synthetic activator and repressor. As with the morning, day and night rhythms, their experimental data matched nicely with the predictions of the general activator-repressor theory, showing that, in this system at least, the virtual can provide a window into the real, just as manmade clocks keep us informed of the natural passage of time.

"Physical simulations allow us to mirror and manipulate actual biological systems with increasing precision and ease, making it possible to perform all kinds of analyses," says Ueda. "This approach should be equally valid in the study of dynamic, complex biological systems other than biological clocks as well, so we are hopeful that it will find a broad range of applications."

Figure 1

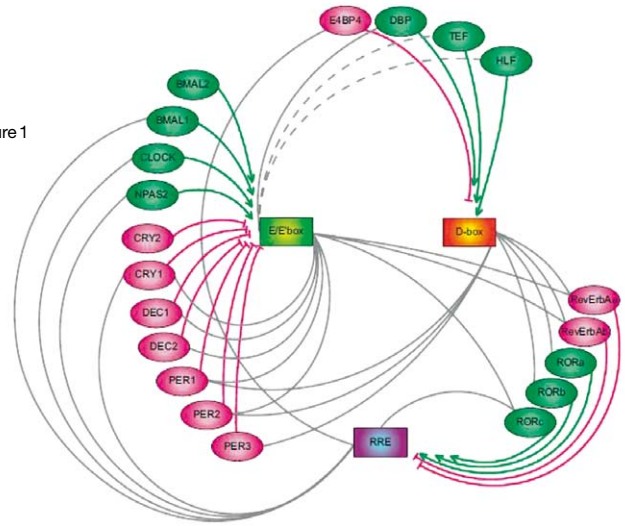


Figure 1
Scheme of complex circadian transcriptional network identified previously by Ueda lab.

Figure 2

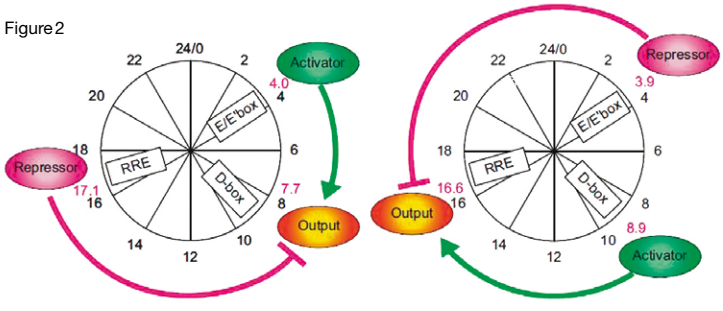


Figure 2
Cartoon of daytime transcriptional output generated by morning activator and nighttime repressor (left), and nighttime output generated by daytime activator and morning repressor (right).

Societies and Networks

The CDB strives to contribute to the advancement of developmental biology and related fields in Asia and around the world by supporting the work of numerous scientific societies and networks.

International Society of Developmental Biologists (ISDB)

http://www.developmental-biology.org/

The ISDB serves the global developmental biology community through its support for international meetings, the establishment of regional networks, its website listing developmental biology societies and meetings around the world, and its official journal, *Mechanisms of Development*. In 2006, CDB Director Masatoshi Takeichi was elected president of the ISDB.

Asia-Pacific Developmental Biology Network (APDBN)

http://www.apdbn.org/

The APDBN was established in 2004 to promote and support the study of development and related fields in the Asia-Pacific region. The APDBN provides multiple travel awards every year to subsidize regional travel by students and young investigators, hosts meetings and satellite sessions, and administers a mutant mouse collaborative program in conjunction with the CDB Laboratories for Animal Resources and Genetic Engineering.

Asia Reproductive Biotechnology Society (ARBS)

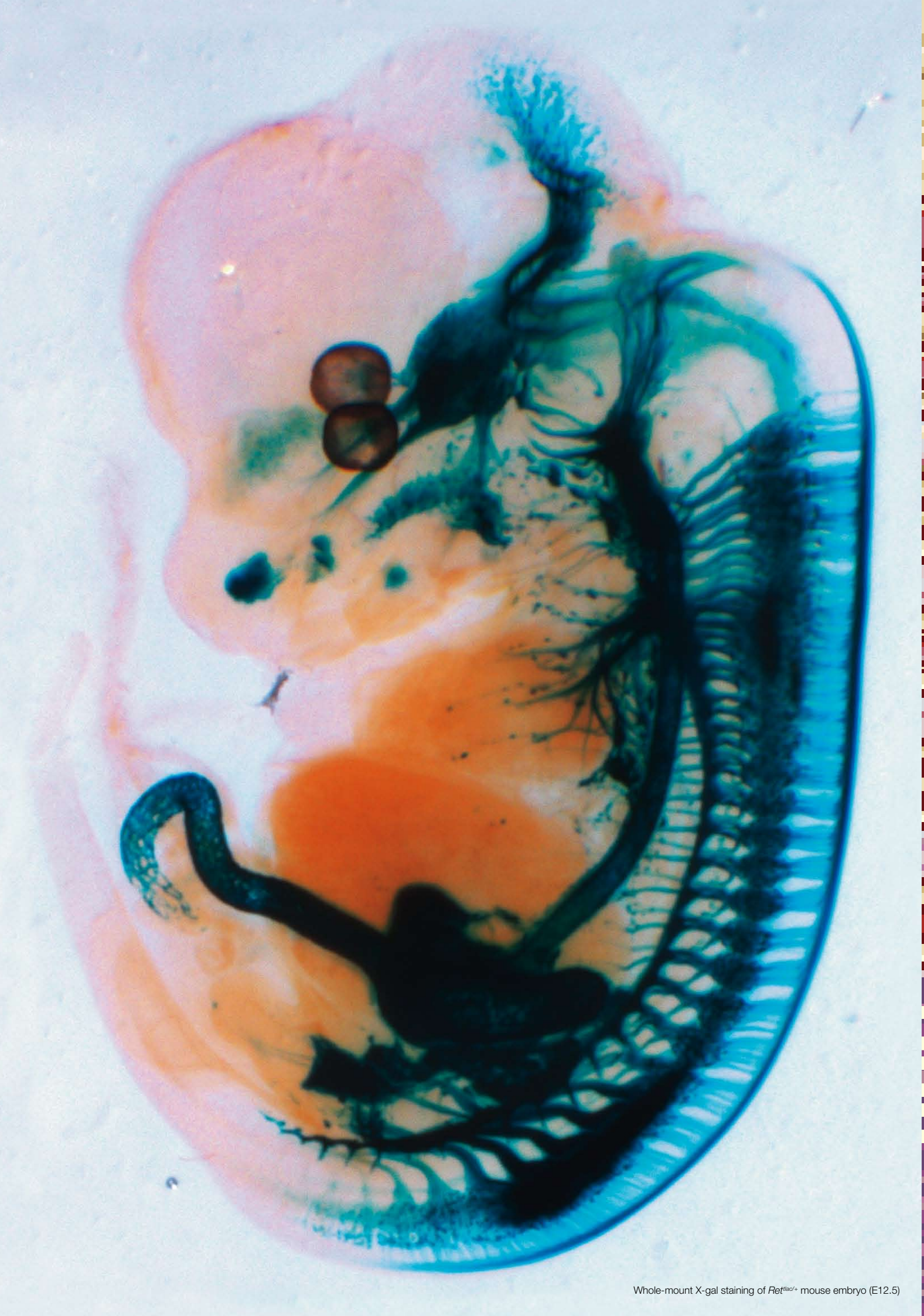
http://www.cdb.riken.jp/arb/

Established in 2004, the ARBS has held major annual meetings throughout Southeast Asia, as a forum for discussing the latest advances in reproductive biology with applications in veterinary and conservation biology, and with additional implications in the understanding of stem cells and regenerative medicine. The 2009 annual meeting of the ARBS will be held in Cambodia. The CDB hosts the ARBS website, and has served as co-host for the first four ARBS meetings.

Stem Cell Network, Asia-Pacific (SNAP)

http://www.asiapacificstemcells.org

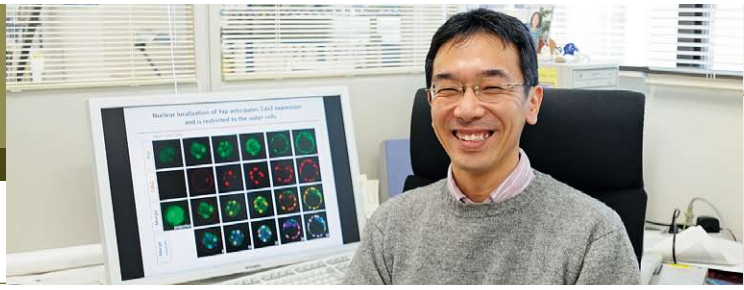
This network was launched in 2007 to help develop the field of stem cell research in the Asia-Pacific region. Scientific and government representatives from Australia, China, India, Japan, Korea, Singapore, Taiwan and Thailand participated in the launch meeting in Bangkok, and the organization held its first scientific meetings in Beijing and Kyoto, and launched its website in 2008. CDB Deputy Director Shin-Ichi Nishikawa serves as chair of the SNP steering committee.



Whole-mount X-gal staining of *Ret*$^{tlacZ/+}$ mouse embryo (E12.5)

Embryonic Induction

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

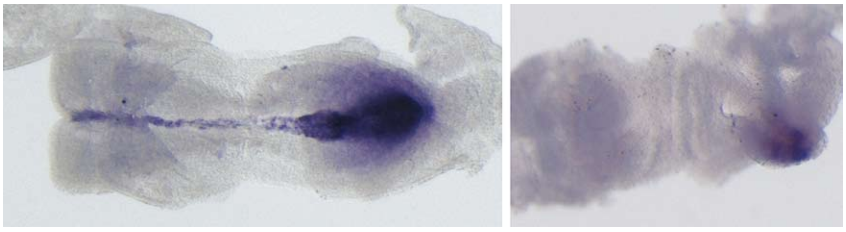


Hiroshi SASAKI Ph. D.

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.

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.

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Publications

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

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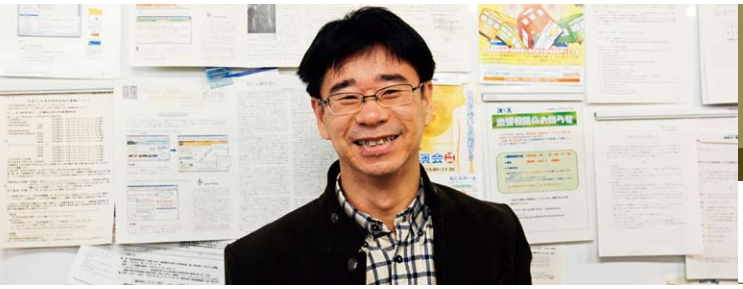


Cell Fate Decision

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

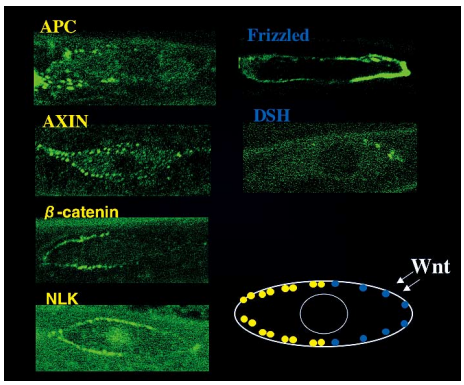
Hitoshi SAWA Ph. D.

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.

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.

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Publications

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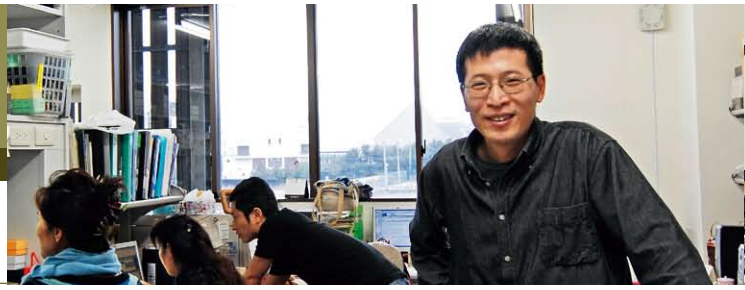
Early Embryogenesis

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



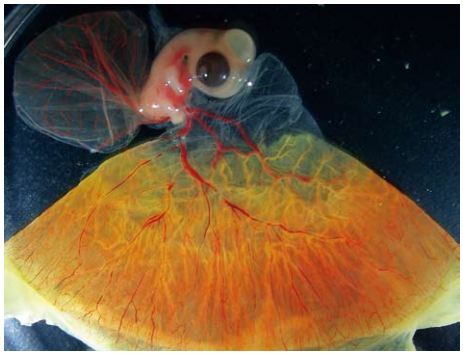
Guojun SHENG Ph. D.

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

The 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 plateand 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.

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Publications

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Developmental Genomics

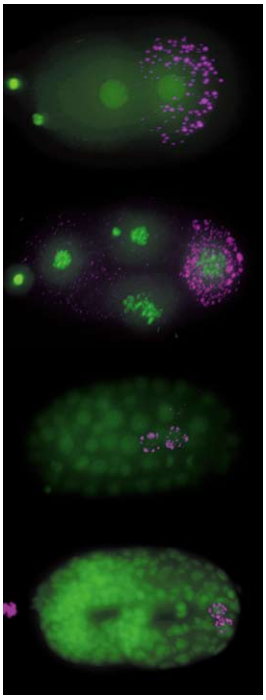
http://www.cdb.riken.jp/en/sugimoto

Asako SUGIMOTO Ph. D.

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.

Fertilized eggs give rise to complex animal structures through strict coordination of cell divisions, cell fate determinationand 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 spindles 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.

P granules (magenta) at 2-cell, 4-cell, ~100-cell and pretzel-stage *C. elegans* embryos. P granules are segregated only in the germ lineage. P granules were visualized by the KT2 monoclonal antibody. DNA (green) are stained by DAPI.

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

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Retinal Regeneration

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

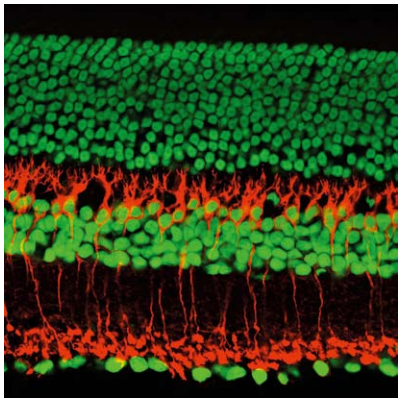
Masayo TAKAHASHI M.D., Ph. D.

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.

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

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



Immunostaining of bipolar cells in the adult mouse retina.

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Publications

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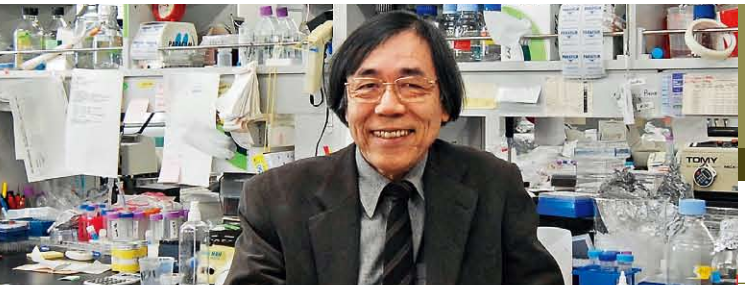


Cell Adhesion and Tissue Patterning

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

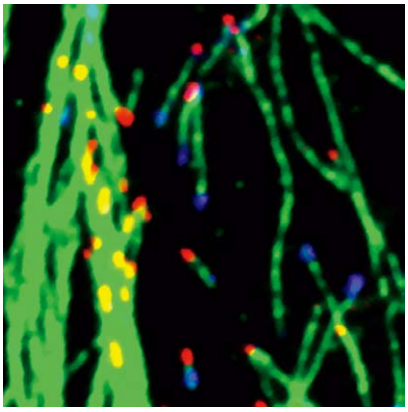
Masatoshi TAKEICHI Ph. D.

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.

The general research focus of our lab is to study the cellular and molecular basis of the mechanisms by which animal cells are organized into precisely ordered multicellular structures, such as tissues and neural networks. We are particularly interested in the roles played by cell-cell adhesion and recognition molecules in these processes. The cadherin family of adhesion molecules and associated signaling systems are essential for the construction of tissues; this adhesion machinery is the main subject of our research.

Mechanisms of cell-cell adhesion and its remodeling: Cell-cell adhesion is remodeled during morphogenesis or cancer metastasis, and this nature of cell-cell adhesion is important for regulating many types of morphogenetic cell behavior. Cadherin activity is modulated by the interactions with actin filaments or microtubules, whose processes are mediated by catenins, a group of cadherin-associated proteins. We are studying the mechanisms underlying the crosstalk between cadherins and such cytoskeletal systems, with the goal of gaining novel insights into the mechanisms of cell-cell adhesion remodeling.

Cell rearrangement and tissue patterning: A second area of interest to our lab is the mechanisms by which animal tissues are organized through the processes of dynamic cell rearrangement, such as cell migration and relocation. At cellular levels, we are exploring the roles of cadherin molecules in cell contact-dependent regulation of cell migration. At tissue levels, using the embryonic brains as model systems, our team is attempting to determine how cadherin and associated molecules control the morphogenesis of neuroepithelial layers, as well as the migration of neural precursor cells. We are also investigating the roles of protocadherins and Fat cadherins, members of the cadherin superfamily, in the regulation of cell-cell contacts and tissue organization.



Synapse formation: We are further interested in the mechanisms underlying the formation of neural networks, particularly interneuronal recognition during synapse formation. The cadherin-catenin complex is localized in synaptic contacts, and different cadherin subtypes are expressed by different neurons of the brain. We are investigating ways to determine how synaptic contacts are regulated by this adhesion system, as well as by other adhesion molecules, such as Ig-superfamily members.

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

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Publications

Meng W, et al. Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. *Cell* 135. 948-59 (2008)

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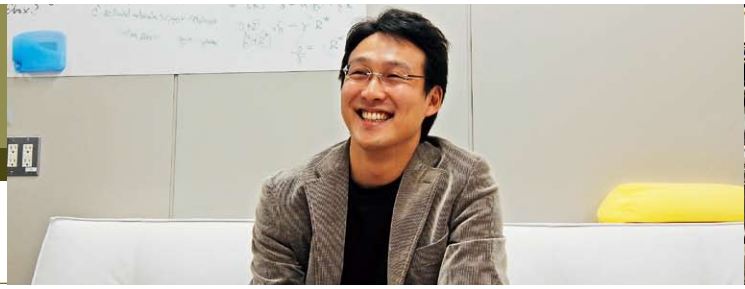
Systems Biology

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



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

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.

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 gene(s)." 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 control existing systems toward the desired state and design new ones based on an understanding of structure and underlying principles.

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

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

Clock sculpture outside Gare St Lazare train station in Paris. The cellular clocks inside us can be also become desynchronized on exposure to critical light pulse(s).

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Ukai-Tadenuma M, et al. Proof-by-synthesis of the transcriptional logic of mammalian circadian clocks. *Nat Cell Biol* 10. 1154-63 (2008)

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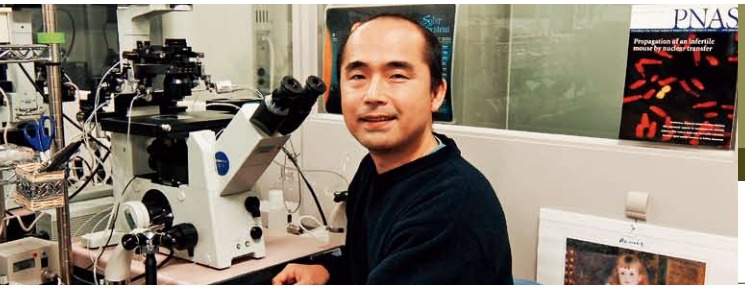

Genomic Reprogramming

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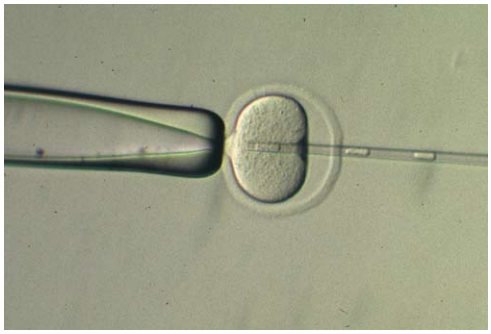
Teruhiko WAKAYAMA Ph. D.

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.

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 re-programming, 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 bio-technology of great promise, given its potential applications in the field of regenerative medicine, such as the generation of em-bryonic 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

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Publications

Bui H T, et al. The cytoplasm of mouse germinal vesicle stage oocytes can enhance somatic cell nuclear repro-gramming. *Development* 135. 3935-45 (2008)

Mizutani E, et al. Propagation of senes-cent mice using nuclear transfer embry-onic stem cell lines. *Genesis* 46. 478-83 (2008)

Ohta H, et al. The birth of mice from testicular spermatozoa retrieved from frozen testicular sections. *Biol Reprod* 78. 807-11 (2008)

Ono T, et al. Nuclear Transfer Preserves the Nuclear Genome of Freeze-Dried Mouse Cells. *J Reprod Dev* (2008)

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

Wakayama S, et al. Production of healthy cloned mice from bodies frozen at -20 degrees C for 16 years. *Proc Natl Acad Sci U S A* 105. 17318-22 (2008)

Animal Resources and Genetic Engineering Laboratory

Shinichi AIZAWA Ph. D.

Genetic Engineering Unit
Shinichi AIZAWA Ph. D.

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

Animal Resource Unit
Kazuki NAKAO

The Animal Resource Unit maintains and cares for CDB's laboratory mouse and rat resources in a specific pathogen free (SPF) environment. We also handle the shipping and receiving of mutant mice both within the CDB and with other institutions in Japan and overseas. In addition, we provide pregnant females, mouse embryos, and services for colony expansion and cryopreservation of fertilized eggs of strains prepared by CDB for in vitro fertilization (IVF) breeding. We also develop technologies for the study of reproductive biology.

Staff

Unit Leaders
Shinichi AIZAWA
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Mari KANEKO
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Megumi WATASE
Assistant
Mayumi MIZUSHIRO

Genomics Laboratory

Fumio MATSUZAKI Ph. D.

Genome Resource and Analysis Unit
Hiroshi TARUI Ph. D.

The Genome Resource and Analysis Unit aims to support a wide gamut of gene analysis research, ranging from DNA sequencing and gene expression analysis to storage and distribution of gene resources. Our facility can perform gene expression analysis and high-throughput gene screening using a DNA sequencing system that 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

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Osamu NISHIMURA
Part-Time Staff
Minako MOTOISHI
Assistant
Chiharu TANEGASHIMA

Functional Genomics Unit
Hiroki R. UEDA M.D., Ph. D.

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

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

Staff

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Technical Staff
Junko NISHIO
Kenichiro UNO
Assistant
Ikuko TADA

Electron Microscope Laboratory

Shigenobu YONEMURA Ph. D.

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, as well as support for live imaging using optical microscopy.

Staff

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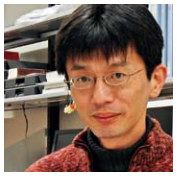
Proteomics Laboratory

Shigeo HAYASHI Ph. D.



Mass Spectrometry Analysis Unit
Akira NAKAMURA Ph. D.

The identification of the individual components of molecular complexes and elucidation of their structures can provide fundamental insights into the intricate mechanisms of development and regeneration. The Mass Spectrometry Analysis subunit uses LC-MASS spectrometry, a technology that enables the high-speed analysis of protein components from even minute biological samples. The subunit undertakes the analysis of protein modifications as well.

Staff

Lab Head
Shigeo HAYASHI
Unit Leader
Akira NAKAMURA
Technical Staff
Kaori SHINMYOZU

Division of Human Stem Cell Technology

Yoshiki SASAI M.D., Ph. D.

Despite their great promise and fundamental interest, stem cells – both embryonic and somatic – can be challenging to study and manipulate in vitro. The Division of Human Stem Cell Technology (DHSCT) 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 DHSCT provides technological expertise, training and support in hESC culture, maintenance, distribution and management, meeting the needs of both experienced and first-time users in the life sciences research community. Working with labs in academia and industry, the DHSCT seeks to contribute to achieving the goals of translational research, in line with the CDB mission to establish a solid scientific foundation for regenerative medicine. In parallel to hESCs, technical development for the use of human iPS cells is being planned.

Staff

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Yoshiki SASAI
Deputy Chief
Hitoshi NIWA
Research Specialist
Hiroyuki KITAJIMA
Research Scientist
Masatoshi OHGUSHI
Technical Staff
Michiru MATSUMURA-IMOTO
Company-Sponsored Research Trainee
Satoshi ANDO

On again and off again: Fgf signaling in inner ear development

Sabine FRETER

The past decade has seen dramatic progress in our understanding of the molecular factors that direct the early development of the ear. It is known that, in both chicken and mouse, FGF signaling from the mesoderm initiates the induction of the inner ear, and that Wnt signals from the overlying neural plate also play a role. But the order of events in this stepwise process, and the specific functions of these two signaling pathways in inner ear induction have proven elusive.

New work by Sabine Freter and colleagues in the Laboratory for Sensory Organogenesis (Raj Ladher; Team Leader) now provides a higher resolution view into this problem. In an article published in *Development,* the team revealed how FGF and Wnt work in setting up and then progressively restricting the fate of the embryonic region that ultimately gives rise to the inner ear.

Findings from previous studies, in which it was found that explants from the same pre-otic region isolated from embryos at closely spaced developmental stages (defined by number of somites) showed differing expression of certain genes. To see if this would hold true for genes used in inner ear induction, Freter removed presumptive explants at the 5-somite stage and tested for the otic marker *Soho1,* finding only minimal expression. When she took explants from the same region at the 7~8 somite stage, however, they showed high levels of *Soho1* transcription.

Returning to previous findings from the Ladher team which showed that FGF signaling is needed to trigger the first steps toward otic differentiation, but that FGF expression subsequently fades in the region, Freter decided to test whether the downregulation of FGF was merely incidental, or in fact a prerequisite for subsequent stages in the process. Using a genetic trick to leave FGF permanently switched on, and she found that, while it worked to expand the field of tissue from which the inner ear ultimately should emerge (which


Progressive restriction of otic fate: the role of FGF and Wnt in resolving inner ear potential

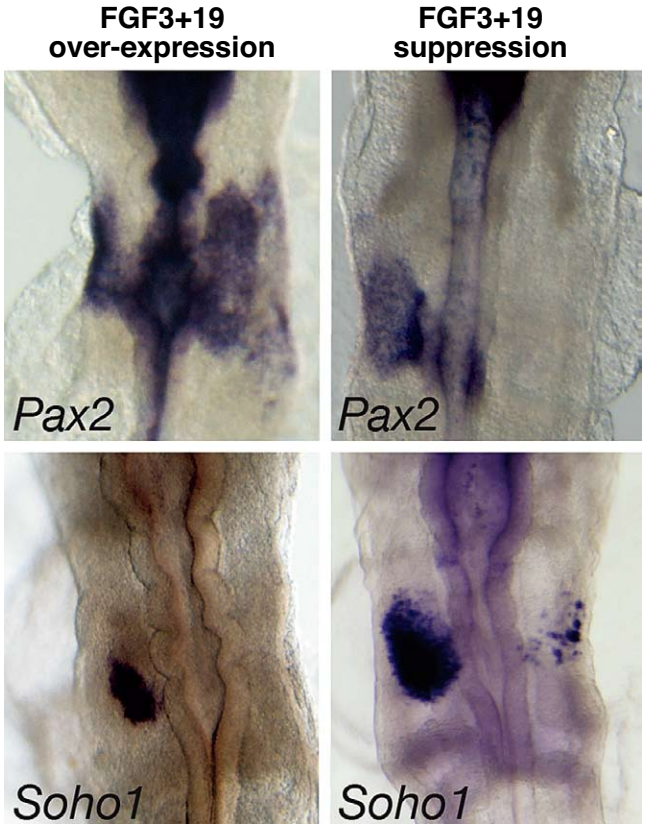
Freter S, et al. Progressive restriction of otic fate: the role of FGF and Wnt in resolving inner ear potential. Development 135. 3415-24 (2008)

they call the otic-epibranchial progenitor domain, or OEPD), genes needed to induce differentiation into the inner ear proper failed to switch on. This came as something of a surprise, as the link between FGF expression and otic development is well established. Indeed, when the team tried suppressing FGF signaling, they found again that the development of the inner ear failed. (Interestingly, the expression of non-otic genes was unaffected by constitutive FGF.)

In chick, a second signaling pathway known as Wnt has also been implicated in otic development. Using a mutant downstream of Wnt that results in the pathway being constantly activated, Freter looked for effects in the otic and non-otic areas of the OEPD. In a near mirror image of the case for FGF in the second stage of otic development, she found that Wnt was required for otic development, but not so for the epibranchial regions of the OEPD.

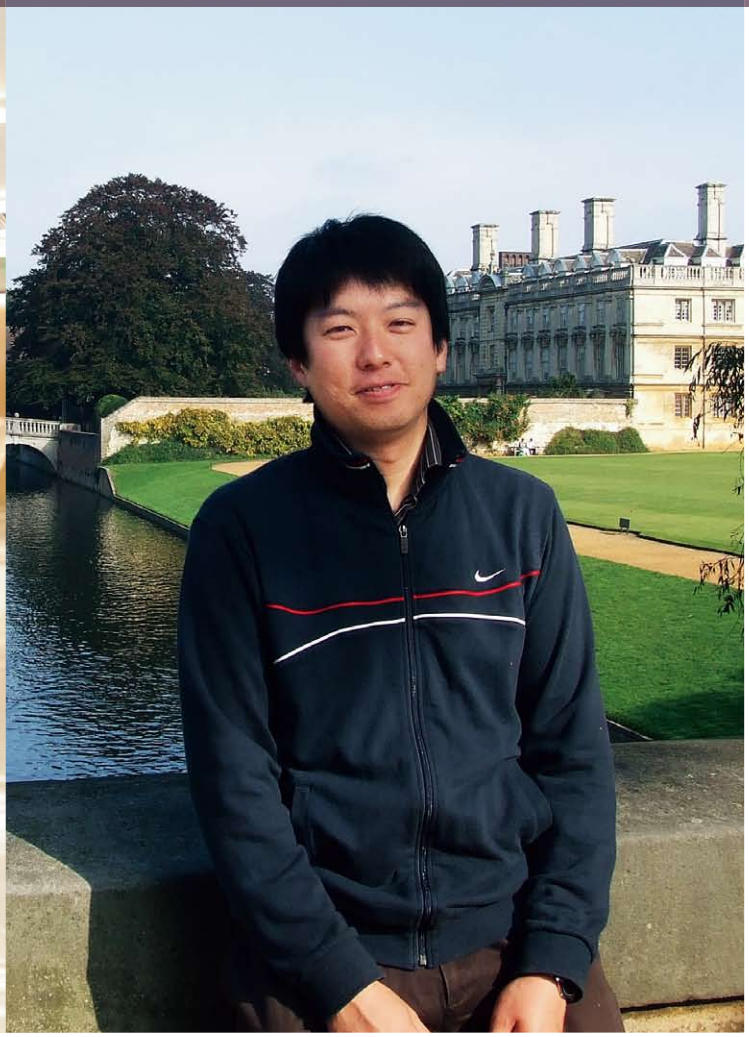
This sets up a delicate balance between the programs needed to drive otic and non-otic differentiation from a common progenitor region that is induced by FGF. Whereas otic differentiation relies on the combination of Wnt signaling and downregulation of FGF, epibranchial differentiation requires FGF to be maintained and Wnt shut off.

“This idea that a factor required for one stage in a multistep developmental process can actually have an inhibitory effect on subsequent differentiation of certain lineages was surprising to us at first, but now seems to make a great deal of sense,” says Freter. “It will be interesting to see if this is a common feature in the genetic control of other developmental routines.”



Effects of FGF overexpression (left) and suppression (right) on *Pax2* and *Soho1*

Division of duties in heterochromatin assembly



Rika KAWAGUCHI, Mahito SADAIE

Gene expression is controlled not only by regulatory elements that bind to the DNA, but by the way the DNA itself is packed away in chromosomes. These structures are composed of a combination of DNA and proteins known as chromatin, in which the genetic material is wrapped up into tight coils. In its "open" conformation (euchromatin), genes tend to be more transcriptionally active than in heterochromatin, where gene silencing is common. This transcriptional silencing in heterochromatic regions is controlled by various factors, including members of the HP1 protein family, but whether and how these proteins interact to achieve this function remains unknown.

Mahito Sadaie (now at the Cambridge Research Institute Cancer-Research UK), Rika Kawaguchi and colleagues in the Laboratory for Chromatin Dynamics (Jun-ichi Nakayama; Team Leader) demonstrated how, in fission yeast, the two HP1 proteins Swi6 and Chp2 play distinct, but cooperative, roles in heterochromatin assembly. In an article published in *Molecular and Cellular Biology,* the team revealed that while Swi6 contributes to the repressive structure through the ability of these molecules to self-associate, Chp2 functions by recruiting other chromatin-modulating factors.

Both Swi6 and Chp2 had previously been linked to heterochromatin assembly, but it was not clear whether their roles were discrete, or simply redundant. Taking advantage of yeast genetics, Sadaie first tested whether there was functional overlap by complementarily expressing Chp2 in a *swi6*-negative mutant strain, and Swi6 in a *chp2* mutant. In neither case was the transcriptional silencing defect rescued, suggesting that both were required in this mechanism, but each in a different way.

Sadaie M, et al. Balance between distinct HP1 proteins controls heterochromatin assembly in fission yeast. *Mol Cell Biol* 28, 6973-88 (2008)

One clear difference between the two is their levels of expression ; Western blot analyses showed that the Chp2 protein was only about 1/80 as abundant as Swi6, indicating differential regulation. The team found that this asymmetric balance was functionally important, for when they overexpressed Chp2 in cells expressing normal levels of Swi6 it disrupted heterochromatin assembly. Seeking possible molecular interactions, Sadaie performed pull-down assays and found that while Swi6 proteins bind with other proteins of the same, Chp2 showed no such self-association.

The team turned next to studying how Swi6 and Chp2 are involved in the recruitment of other chromatin-regulating factors to heterochromatic regions. Swi6 was known from previous reports to influence the binding of a pair of factors, Epe1 and Clr3, to heterochromatin; Sadaie et al. showed that Chp2 also physically associates with Epe1, and that it has a higher affinity for Clr3 than does Swi6, meaning that it is likely to play the dominant role in recruiting this protein to heterochromatic regions. The case for this interaction was further strengthened by experiments showing that the overexpression of Clr3 rescued the silencing defect in *chp2* mutants.

The picture that emerged from these findings is that, in fission yeast at least, heterochromatin organization relies on the proper balance between two members of the same protein family, each of which plays a distinct role. “It may not be possible to extrapolate these results directly to other species,” says Nakayama, “but the mechanisms we have found for the dynamic regulation of heterochromatin and gene silencing in fission yeast may enable a deeper understanding of how similar functions are achieved in mammals.”

Figure 1

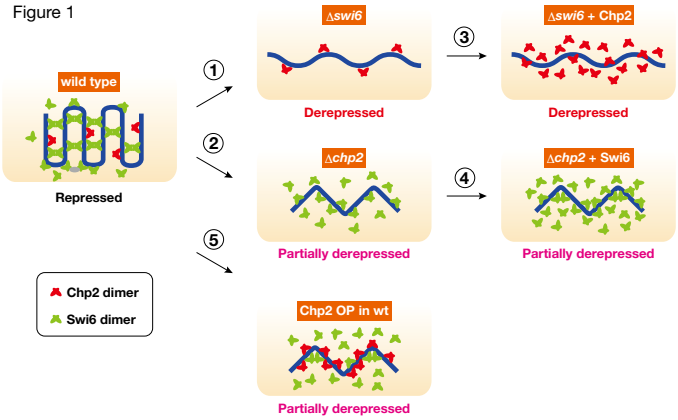


Figure 1
Cartoon showing *swi6* and *chp2* mutant phenotypes.

Figure 2

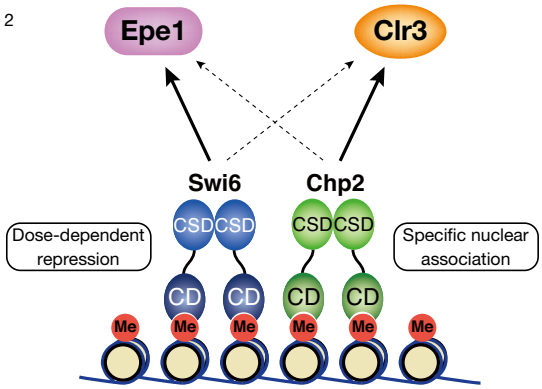
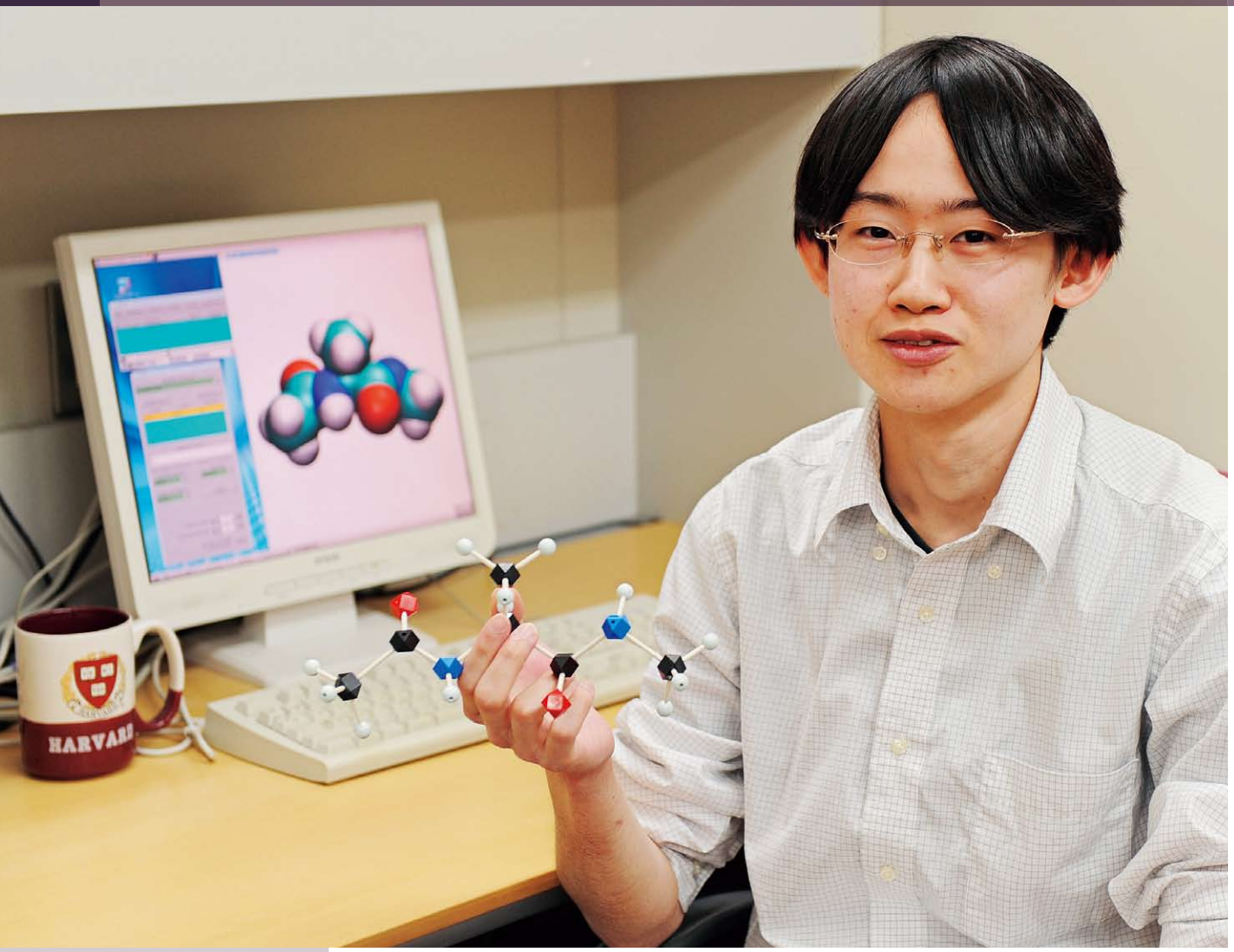


Figure 2
Discrete functions of Swi6 and Chp2.

An atom-by-atom approach to molecular conformational change



Yohei M. KOYAMA

For proteins and other biological molecules, function follows on structure. Scientists have simulated the molecular dynamics by calculating interactions between atoms and determining their stability in different conformations and the likelihood of transitions between states. One method for analyzing simulated data, known as principal component analysis (PCA), which uses the individual atoms' positions (atomic coordinates) works well at extracting conformational fluctuation and predicting changes in response to perturbations, but runs into difficulties when dealing with major transitions, such as those involving multiple stable conformations.

Yohei M. Koyama of the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader) introduced a version of PCA that takes advantage of the potential energy in atom-atom interactions, an approach he has named PEPCA (for potential energy PCA). The work, which allows for the analysis and prediction of major conformational changes, was published in *Physical Review E*.

PCA using atomic Cartesian coordinates works well in analyzing conformational fluctuations around a single stable conformation. But since these coordinates depend on the overall molecular motion, the confounding factors of translational and rotational motion must first be eliminated. And while this is possible even in large molecules for translational motion, factoring out rotational motion has proven to be a knottier problem.

Koyama Y M, et al. Perturbational formulation of principal component analysis in molecular dynamics simulation. *Phys Rev E Stat Nonlin Soft Matter Phys* 78. 046702 (2008)

Koyama's approach was first to look at the traditional method and make some generalizations, in which he determined that PCA can be used as a means of finding perturbations that cause the largest conformational change. In order to avoid the problems with conventional PCA, he developed an alternative to atomic coordinates, using the potential energy of interactions between atoms as a surrogate. And, as this energy is determined solely by the relative positions of a pair of interacting atoms, it is unaffected by overall molecular motion, meaning that the problematic removal of rotational motion does not come into play.

To show the utility of PEPCA method, Koyama focused on a conformational transition in the alanine dipeptide as his first test bed. He discovered in his analysis that the perturbation that induced the largest conformational change involved a change in the distribution of a pair of stable states, and identified important interactions in determining the stability of and transitions between these two conformations, demonstrating the value of the new approach in both identifying and predicting molecular conformational change.

"With the development of high-speed computational facilities specializing in molecular dynamics simulations, we're now increasingly able to simulate physiologically significant conformational changes," says Ueda. "And I think that the PEPCA method will help in determining the atomic interactions that are critical to molecular function, which could be of value, for example, in rational drug design."

Figure 1

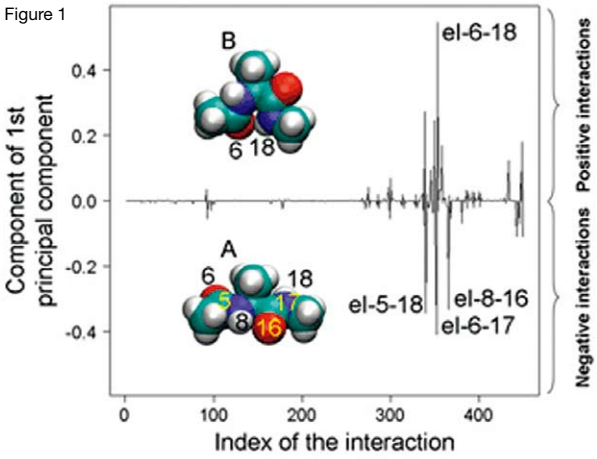


Figure 1
Combination of interactions resulting in largest conformational change in PEPCA analysis.

Figure 2

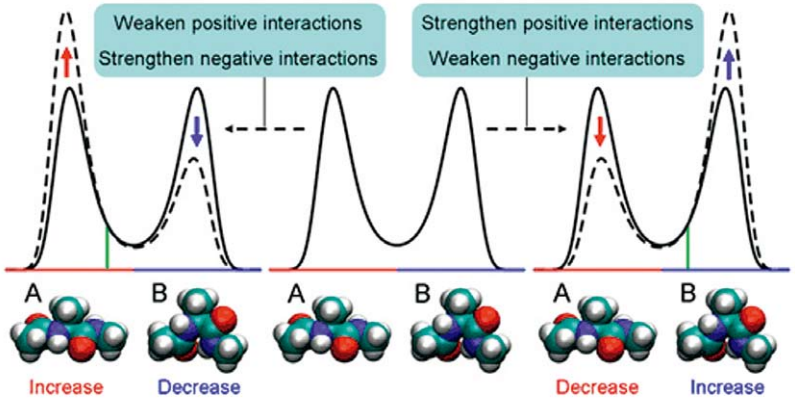


Figure 2
Modeling of the effects of differing strengths of positive and negative interactions affects probabilistic outcomes.

Clones from dead, frozen mice

Sayaka WAKAYAMA

The cloning of animals by nuclear transfer has been shown in a wide range of species from laboratory standards such as mice and rats, to agriculturally important sheep, pigs, horses and cows. The cloning procedure typically involves extracting the nucleus from a somatic cell taken from one animal (the donor) and injecting into a fertilizing egg from which the nucleus has been removed. The ability of the oocyte to reprogram the transferred nucleus from a somatic to a totipotent state remains one of the great mysteries of cell and developmental biology. But the fact that cloning by somatic cell nuclear transfer remains a very low efficiency process has led many to believe that the integrity of the donor cells providing the genome may be critical to its success.

This wisdom has been turned on its head by a study by Sayaka Wakayama and colleagues in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama; Team Leader) in which they report the cloning using nuclear material from mice that had been dead and frozen for 16 years. Their report, published in *the Proceedings for the National Academy* of Sciences, showed the possibility of nuclear transfer using genomic material from cells that had been frozen at a relatively high temperature and without cryoprotectants, which has implications for species conservation and the possibility of “resurrecting” extinct species.

The team began by confirming that tissues frozen for a week could be used as donors in cloning experiments, and found that, interestingly, brain cells yielded the highest efficiencies as genomic donors; higher, in fact, than seen in previous experiments that had used healthy, living cells. The researchers speculate that this


Production of healthy cloned mice from bodies frozen at −20°C for 16 years

Wakayama S, et al. Production of healthy cloned mice from bodies frozen at -20 degrees C for 16 years. *Proc Natl Acad Sci U S A* 105. 17318-22 (2008)

may be due to the high levels of sugars in brain tissue, or denaturing, in which the genomic tightly packed genome comes slightly uncoiled. Wakayama next tried using samples from two male mice that had been frozen at -20° C for 16 years without any chemical protectants, but was unable to generate clones using the standard SCNT procedure.

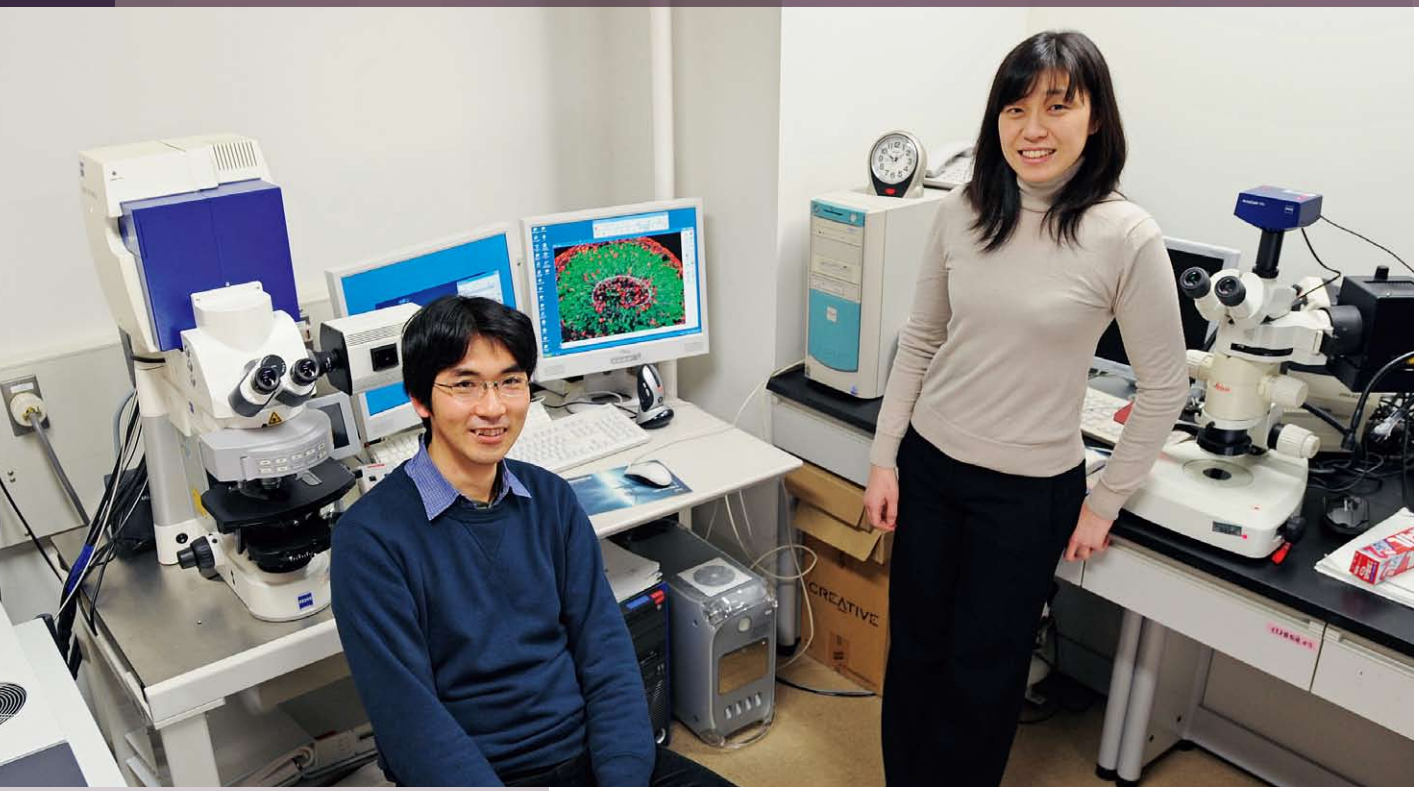
In addition to straight somatic cell nuclear transfer, a second strategy is available to cloners, in which SCNT is used to generate early stage embryos and these embryos are allowed to develop to the blastocyst stage, by which point they have a small, internal clump of cells, known as the inner cell mass; this inner cell mass is removed and cultured as embryonic stem (ES) cell colonies. These “ES by nuclear transfer” (ntES) cells can then be injected into other blastocysts and, through a process known as “tetraploid complementation,” giving rise to an individual in which nearly 100% of its nuclei carry the donor genome.

Mouse cloned from dead, frozen donor.

The Wakayama team first determined that the 16-year old frozen tissue could serve as a donor source, establishing 46 ntES lines from brain cell nuclear material. Using these pluripotent cells, they created tetraploid embryos and allowed them to develop in surrogate mothers. This time, the process worked, producing four true clones and nine chimeras, as determined by genotype and coat color.

Although one previous report had shown cloning from frozen material, those samples had been stored at the much lower temperature of -80° C, and in the presence of agents that may have some cryoprotective effect. “This is the first time a mammal has been cloned from a sample stored at conditions reasonably close to what might be expected in permafrost, making which gives some hope for those who might seek to clone extinct species from frozen carcasses” says Wakayama. “The higher temperature also puts this approach more closely in the reach of people working in the field to preserve samples from rare or endangered species.”

Cerebral cortical tissue from ES cells



Mototsugu EIRAKU , Masako KAWADA

The cortical regions of the brain are structurally complex and functionally important, providing the neural substrate for many of the brain's highest functions, such as language, memory, emotion and thought. The cortex begins to form from the anterior end of the neural plate, and is characterized by its laminar structure in which neurons born at different developmental stages migrate to different cortical layers, each with its own functions and circuitry. Given this highly stratified and interconnected three-dimensional architecture, corticogenesis would at first blush seem to require involve myriad, complex processes difficult to reproduce in vitro.

Work by Mototsugu Eiraku and others in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) revealed a surprisingly straightforward method for generating three-dimensional aggregates of multiple, spatiotemporally organized cortical neuron types from embryonic stem (ES) cells. The report, published in *Cell Stem Cell,* opens new insights into the genetic control of cortical development, but also provides a technique for generating different types of cortical neurons for use in the study of pathogenesis and drug discovery.

The Sasai group had previously developed an ES cell culture method, known as serum-free culture of embryoid body-like aggregates (SFEB), to induce the generation of forebrain neuronal precursors that expressed cortical marker genes from mouse embryonic stem cells. By tweaking the SFEB protocol, causing the cells to re-aggregate more quickly after dissociation, Eiraku found he could increase the efficiency of cortical differentiation. Naming the modified method, SFEBq (for quick-aggregation), he found that not only did the technique yield higher percentages of cortical precursors, but also that these reliably went on to form polarized neuroepithelial structures.

The group next cultured the mouse ES cell-derived cortical cell masses within forebrain slices, and found that the explants preferentially migrated into pallial regions, as would be expected from the normal destination of such cells in vivo. When they transplanted the same cells into the brains of newborn mice, they found that within four weeks they had developed into pyramidal neuron-like cells with typical morphology and patterns of axon extension.


Self-Organized Formation of Polarized Cortical Tissues from ESCs and Its Active Manipulation by Extrinsic Signals

Eiraku M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 3. 519-32 (2008)

To study the functionality of the cells, Eiraku looked at spontaneous neuronal activity (as measured by surges of calcium ions) in the cells, and found that this was sensitive to known chemical activators and inhibitors of such activity, indicating that it depended on local synaptic connections. Neonatal cortex is known to exhibit a unique form of coordinated activity, characterized by an oscillating wave of Ca^{2+} that washes across relatively large fields of neurons. Surprisingly, even this calcium wave is recapitulated in the ES cell-derived cortical tissues, showing that they mimic the properties of the neonatal cortex to a remarkable extent.

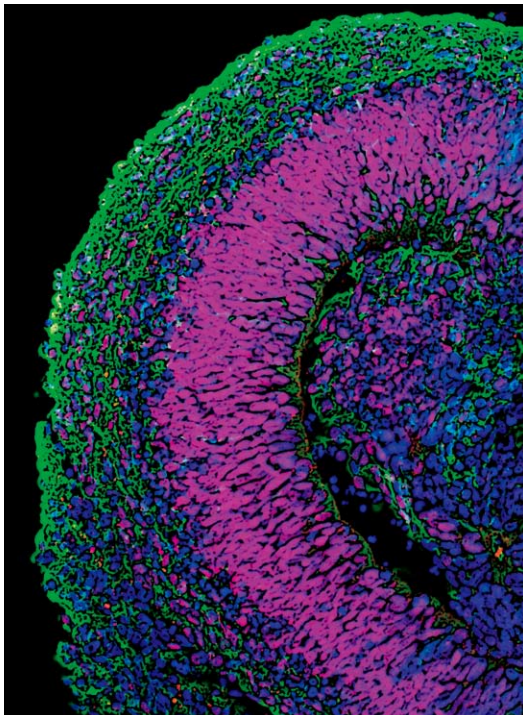
During embryogenesis, the cortex undergoes regional specialization as the result of molecular signals emitted by surrounding tissues, such as FGF, which induces rostral cortical regions, and Wnt and BMP, which are required for the specification of the choroid plexus and cortical hem. When treated with these factors in culture, the SFEBq-differentiated cortical progenitor-like cells responded to these signals similarly to their embryonic counterparts, giving rise to the apposite neuronal types.

Eiraku and colleagues next sought to find out whether the mouse ES-cell derived cells would follow the stereotypical "inside early, outside late" birthing pattern observed for cortical neurons in vivo. Watching the sequence of marker expression in their cultured cortical-like cells, they found that they closely mirrored the temporal changes in gene expression seen in the embryonic cortex.

After examining the temporal aspects of birthday-dependent differentiation, the Sasai group turned to the structure of the cells, which form hollow rosette-like clusters in suspension culture. Here again, they found that these rosettes comprised at least four distinct zones (as shown by gene expression), mimicking certain structural aspects of the early cortex.

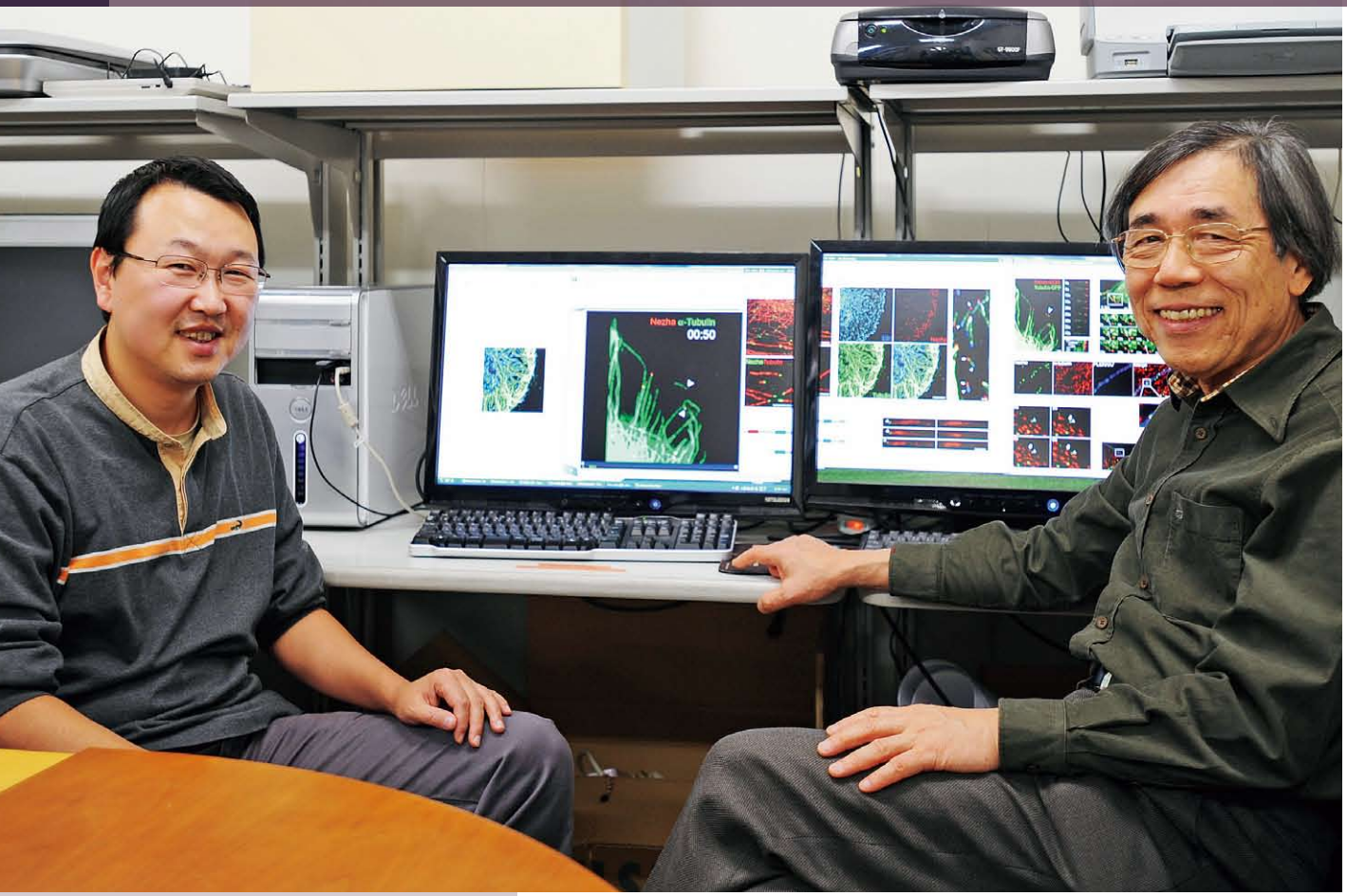
Having extensively studied the ability of mouse ES cells to give rise to cells closely resembling cortical neurons, Eiraku et al. tested whether the SFEBq approach would work for human ES cells as well. Although the cells did not cluster into rosettes, forming larger, mushroom-shaped aggregates instead, the resulting continuous neuroepithelia expressed genetic markers characteristic of cortex and showed some of the regionalization seen in the mouse ES-derived structures.

"The most surprising finding for us was that a patternless clump of cells can reorganize itself into something that so closely resembles cortical tissue, so we're eager to find out more about the role of such self-organization in brain development," says Sasai. "What's more, the fact that we were able to derive not just individual cell types, but functional tissue from ES cells may have a real impact in terms of both its medical significance and its value in drug discovery."



Immunostaining of cross-sectioned cortical tissue generated from human ES cells in vitro.

Tracks to the junction: Microtubules tethered to zonula adherens



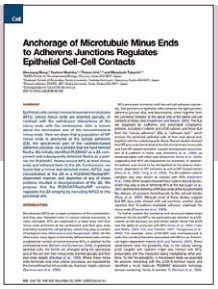
Wenxiang MENG, Masatoshi TAKEICHI

Microtubules are long polymer strands with functionally distinct plus- and minus-ends that function in a wide range of processes from morphogenesis to the shuttling of motor proteins through the cytoplasm. While many microtubules are anchored by their minus-ends to the centrosome, in epithelial cells some microtubules have non-centrosomal minus-ends, whose termination site has remained a mystery.

Research by Wenxiang Meng and others in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) revealed an important role for microtubules in the formation and maintenance of the zonula adherens, a lateral region in epithelial cells in which cell-cell adhesion proteins, such as cadherins, are highly concentrated. In an article published in *Cell,* Meng and colleagues identified a pair of novel proteins, PLEKHA7 and Nezha, which are involved in recruiting and dynamically anchoring the microtubules to the cadherin machinery.

The study began with a search for proteins that associate with p120 catenin, one of the cytoplasmic factors that binds to the cadherin tail in the cell's interior. PLEKHA7 emerged from a pulldown assay for p120 catenin binding partners, and on immunostaining, the group determined that the protein localized at cell-cell junctions in a p120 catenin-dependent manner. Testing for function, they found that overexpression of PLEKHA7 caused greater amounts of cadherin to accumulate at the zonula adherens, while its depletion by siRNA had the opposite effect.

The group next looked for other proteins that might interact with PLEKHA7 in a new pulldown assay, and came up with a second unknown protein, which they dubbed Nezha, after a god who appears in the Chinese adventure "Journey to the West." Immunofluorescence-tagged Nezha showed that it co-localizes with PLEKHA7 at the zonula adherens in a co-dependent fashion. Overexpression of Nezha in cultured


Anchorage of Microtubule Minus Ends to Adherens Junctions Regulates Epithelial Cell-Cell Contacts

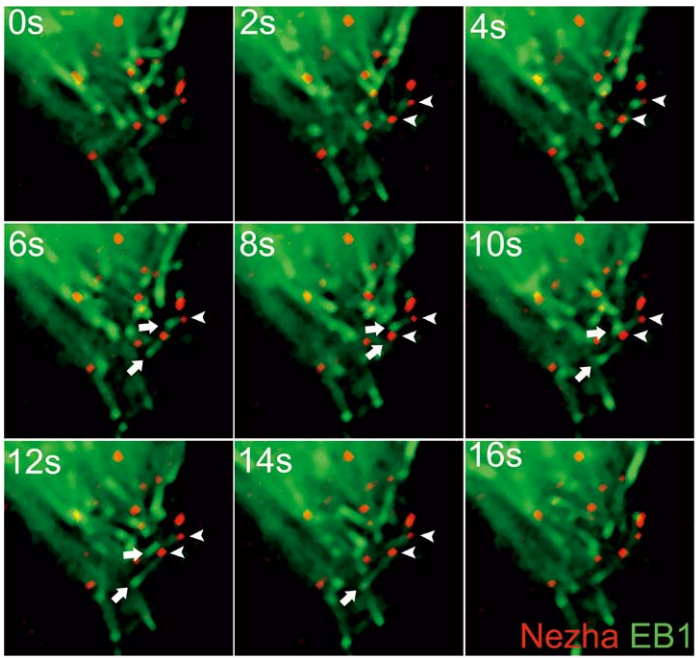
Meng W, et al. Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. Cell 135. 948-59 (2008)

cells highlighted a role in microtubule bundling, prompting Meng et al. to look more deeply into this interaction. Double-immunostaining of Nezha and microtubules revealed a tendency for Nezha to associate with the minus-ends, a relative rarity in contrast to the many proteins that interact with microtubule plus-ends. A capping experiment using chemically tagged Nezha confirmed the association between this protein and microtubule minus-ends.

Wanting to know more about the dynamics of the Nezha/minus-end interaction in living cells, Meng watched the behavior of fluorescence-labeled Nezha and α-tubulin using time-lapse microscopy. He found that Nezha formed concentrated points from which single microtubule strands emerged and retracted, suggesting it was serving as a kind of anchor. These Nezha-microtubule complexes were themselves situated in PLEKHA7-positive regions in the zonula adherens, lending further weight to a functional relationship among these players.

In addition to the roles in dynamically stabilizing cell structure, microtubules serve an important role as tracks for motor proteins moving through the cytoplasm. Thinking that Nezha might tether microtubules to provide a route for minus-end-directed motor proteins to the cell junction, Meng tested a number of such candidates and found that the minus-end-directed protein KIFC3 was indeed localized at the zonula adherens. Loss-of-function experiments showed that its role there was important, as KIFC3 knockdown resulted in disorganization of E-cadherin in this zone. When the group interfered with microtubule polymerization in these cells using an inhibitor, the junctional localization of KIFC3 gradually faded before disappearing altogether, suggesting that PLEKHA7, Nezha, KIFC3 and microtubule minus-ends work together in maintaining zonula adherens integrity, which is essential for preserving the typical epithelial morphology of these cells.

“The minus-end specificity of Nezha is an intriguing discovery,” says Meng. “It’s going to be exciting to learn more about how this PLEKHA7/Nezha/KIFC3 system relates to the processes of embryogenesis and proliferation and invasion in cancers.”



Nezha-Kusabira Orange and EB1-GFP are doubly expressed in a Caco2 cell. Arrowheads indicate Nezha signals, and arrows point to EB1 signals growing out of the Nezha signals.

Tead plays a part in cell contact inhibition of proliferation

Mitsunori OTA

Controlling organ growth, or cell proliferation, is one of the keys to ensuring that the body's parts grow to the appropriate size and no bigger. When this control is dysregulated by, for example, aberrances in the cell cycle, it can lead to hypertrophy or cancer. In cultured cells, a phenomenon known as cell contact inhibition of proliferation is one means by which groups of cells self-regulate their growth; at higher densities proliferation is slowed as cells come into closer contact with their neighbors. Cancerous cells, however, lose this inhibitory control and growth runs rampant as a consequence. Contact inhibition is thought to be a crucial mechanism in development as well, but the details of how it works on the molecular level have remained obscure.

A study by Mitsunori Ota of the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) revealed new insights into the molecular mechanisms that underlie cell contact inhibition in mouse. Reporting in the journal *Development,* the team showed that a transcription factor, Tead and its co-activator protein Yap1, exert control over proliferation by mediating Hippo signaling, a pathway associated with tumor suppression in *Drosophila.*

The work began almost by accident, when Ota was investigating expression of Tead and Yap1, which have been previously identified in Sasaki's laboratory as key regulators for development of the node and notochord inductive signaling centers in the mouse embryo. The turning point came when he tested Yap1 in cultured cells, and found a link between cell density and Yap1 localization. In low-density culture conditions in which cells divide rapidly, Ota found that Yap1 remained in the nucleus, but in higher-density conditions where growth is inhibited, the protein moved into the cytoplasm. "That was the first time I suspected there might be a link between Tead, Yap1 and cell contact inhibition of proliferation," says Ota. Tests of Tead activity in cells cultured at different densities confirmed those suspicions.


Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling

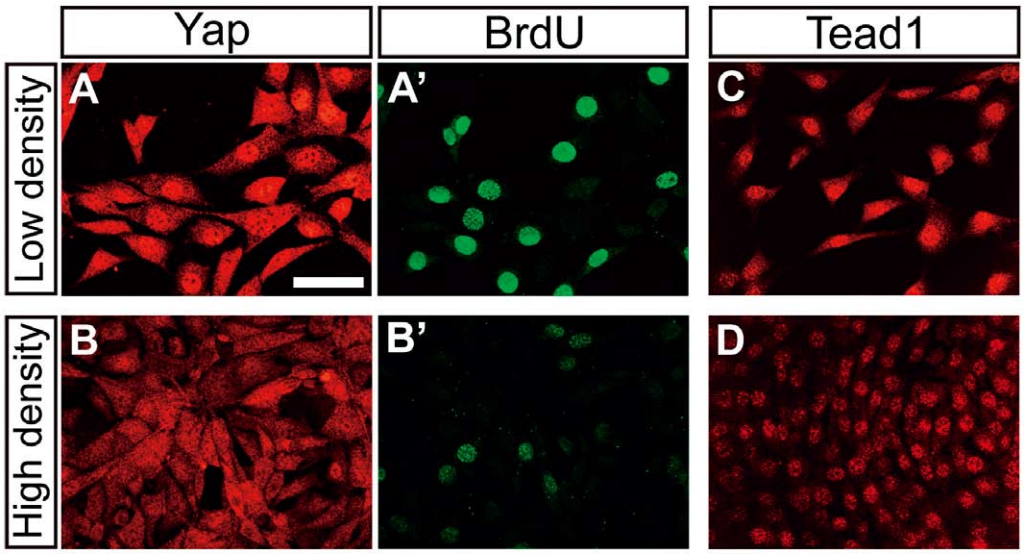
Ota M and Sasaki H. Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling. *Development* 135. 4059-69 (2008)

Knowing of the role of Hippo signaling in cell proliferation, Ota next examined whether it might be involved in the regulation of Tead. Constitutive expression of Hippo pathway components caused the downregulation of both nuclear Yap1 and Tead activity, indicating that cell contact modulates the activity of Tead proteins through the Hippo pathway.

The converse experiment, in which Tead was activated by misexpression of Yap1 or activated forms of the Tead protein, resulted in upregulation of growth, suppression of apoptosis, and promotion of tumorigenesis, supporting the case for roles for Yap1 and Tead in controlling cell proliferation. This prompted them to look for the gene targets of these factors, using microarrays to analyze expression profiles. Their tests showed considerable overlap in the genes controlled by the two factors, suggesting that both work on similar targets to regulate growth.

But on looking more closely in vivo, a more nuanced picture emerged. Using embryos with a homologous deletion of the two major *Tead* genes, or of *Yap1,* they found that the expression of only a small fraction of the genes induced by Tead in their cultured cells was affected in the knockout embryos, suggesting that these factors regulate different targets in a cell type-specific manner. Protein distribution patterns showed diversity as well. While Tead1 was expressed in all tissues in embryos between days 8.5 and 10.5, it was particularly strong in heart muscle. Similarly, Yap1 was widely expressed, with the highest levels in the node and notochord, as well as in myocardium, where it co-localized with Tead. These findings point to cell type-dependent differences in the Hippo pathway, which regulates contact inhibition of proliferation by its effects on Yap1 and, consequently, Tead.

“We knew from studies in fly that Hippo was linked to the control of organ size,” says Sasaki, “what we’ve done here is show not only that it plays a role in cell contact inhibition of proliferation in mouse, but that the pathway involves Tead proteins. We have been studying other roles for Tead as well, so we’ll be interested to see how the Hippo pathway and cell contact information as well fit into the greater picture of mammalian embryogenesis.”



Yap1 localizes in the nuclei in cells proliferating at low density (top left), but diffuses into the cytoplasm in high-density culture, in which growth is slowed (bottom left). Tead shows a similar pattern of localization (right panels).

Protein network reshapes the ECM

Kayo NAGATA, Yukihiko KUBOTA (back), Asami SUMITANI, Kiyoji NISHIWAKI

Studies of how the body is put together tend to focus on cells as the building blocks of tissues and organs, but the extracellular matrix, the collective name for various meshworks of proteins and complex carbohydrates that play a vital role in holding the body's diverse cells together. Basement membranes are one such extracellular matrix, comprising layers of collagen and other fibrous proteins and proteoglycans, which serve the important functions of anchoring epithelial tissue and providing a barrier between the body's compartments. The migration of distal tip cells (DTCs) during gonadogenesis in *C. elegans* has been used as a relatively simple system for studying the part played by basement membranes in organ formation. But though it has been shown that an ADAMTS family protease known as MIG-17 is required for controlling the direction of the distal tip cells as they followed their U-shaped path through the larval body, possibly by remodeling the basement membrane, but the picture downstream of MIG-17 remained unclear.

A new study by Yukihiko Kubota, Kiyotaka Ohkura and colleagues in the Laboratory for Cell Migration (Kiyoji Nishiwaki; Team Leader) has now revealed a new network of proteins which, in mutant form suppressed the defects induced by mutation of MIG-17. Interestingly, these suppressor mutations (in the genes *let-2* and *fbl-1*) appear to work by distinct mechanisms.

Kubota Y, et al. MIG-17/ADAMTS controls cell migration by recruiting nidogen to the basement membrane in *C. elegans*. *Proc Natl Acad Sci U S A* 105. 20804-9 (2008)

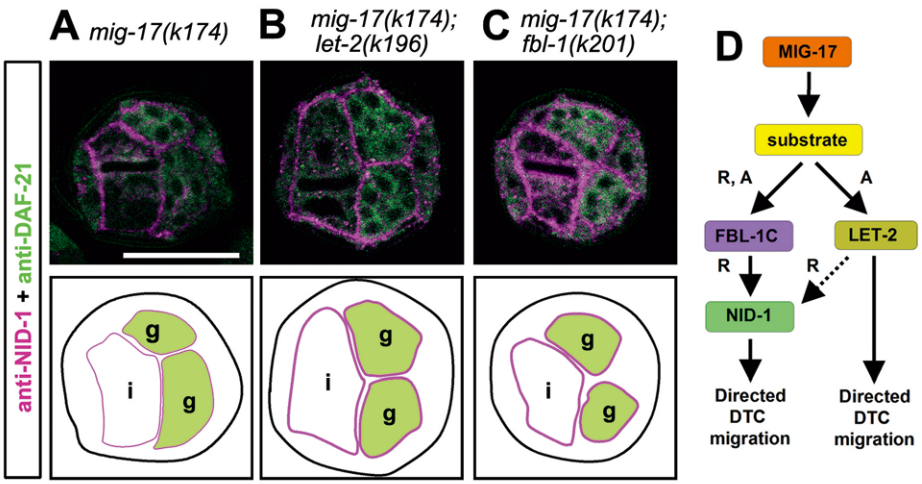
The study began with a screen for genetic defects affecting gonadal migration, using *mig-17* mutant worms as a starting point. Previous work by the Nishiwaki lab had identified one such suppressor mutant, in which a mutant form of the fibulin-like protein encoded by *fbl-1* suppresses MIG-17 defects in a dominant gain-of-function manner. The mutagenesis screened turned up two new mutations in the gene *let-2* (which encodes a *C. elegans* version of collagen IV), both of which bypass the *mig-17* migration defect.

Although neither *let-2* nor *fbl-1* mutants alone show significant migration phenotypes, when Kubota and Ohkura introduced mutations into both genes, they found severe gonadal defects, suggesting a genetic interaction. They next looked at another basement membrane protein, nidogen, which is known to bind to type IV collagen, in order to determine whether it played a part in the equation. But although nidogen and MIG-17 appeared to be at least partially redundant, the combination of mutant *nid-1* with either of *let-2* collagen mutants had no effect on their suppression of *mig-17*-induced migration defects.

The loss of *nid-1* had a strong effect, however, on the ability of the *fbl-1* mutation to suppress the *mig-17* phenotype, indicating that the two suppressor mutations of *let-2* and *fbl-1* operate respectively by nidogen-independent and -dependent mechanisms. As nidogen normally binds to collagen in the basement membrane, the team checked whether this was affected in the various migration-related mutations, alone or in combination. Using immunohistochemistry, they found that mutations in *mig-17* and *let-2* had weakened on nidogen localization, while loss of *fbl-1* function caused a more dramatic deficit. Intriguingly, the introduction of either of the suppressor mutations in a *mig-17* mutant background amplified the accumulation of NID-1. Overexpression of NID-1 in *mig-17* mutant worms significantly rescued the gonadal defect, suggesting that its accumulation in the basement membrane plays an important role in *mig-17*-dependent control DTC migration.

The picture that emerges is complex, and still incomplete. Although the details of the relationship between FBL-1 and NID-1, and the precise role for LET-2 in the regulation of migration along the basement membrane remain for further study, the findings of Kubota, Ohkura, et al. point to a much more elaborate role for the extracellular matrix in organogenesis than previously suspected.

“We know of a number of genetic diseases involving ADAMTS proteases associated with changes in the extracellular matrix, but how these proteins affect the ECM is largely unknown,” says Nishiwaki. “We have now found that in *C. elegans*, MIG-17/ADAMTS recruits factors to the basement membrane in a determined order, giving us one of the first insights into the molecular control of organogenesis by this system.”



(A-C) Cross-sections of worms. Weakened NID-1 localization (magenta) in *mig-17* mutants (A) is recovered to wild type levels when combined with *let-2* (B) or *fbl-1* (C) suppressor mutants. (D) Model for the protein cascade downstream of MIG-17. MIG-17-dependent proteolysis of an unknown substrate recruits and activates FBL-1C, which then recruits NID-1 to the basement membrane to control DTC migration. MIG-17-dependent proteolysis also activates LET-2 to induce NID-1-dependent and -independent MIG-17 pathways.

Educational activities

While the CDB is not a teaching institution, we maintain close links with the local academic community and work to make a contribution by supporting active educators and students with an interest in learning more about developmental biology and experimental approaches.


Intensive lecture program for grad students

The RIKEN CDB hosted its fifth intensive lecture program for graduate school affiliates on July 17 and 18. The program drew more than 150 participants from the Center's affiliated graduate and medical schools. The two-day event featured scientific talks by CDB lab leaders, exhibitions of model organisms, and demonstrations of research tools and techniques.


Summer school for high school students

The CDB held a pair of single day courses for local high students interested in the life sciences on August 5 and 7, building on the success of last year's summer school initiative. Each day's program featured a talk by Shigenobu Yonemura, head of the CDB's Electron Microscopy Laboratory, followed by a visit to his lab, as well as a chance to do some hands-on bench work (staining cultured cells by immunofluorescence) and a tour of the Kobe Medical Industry research park in which the CDB is located.


Workshop for high school teachers

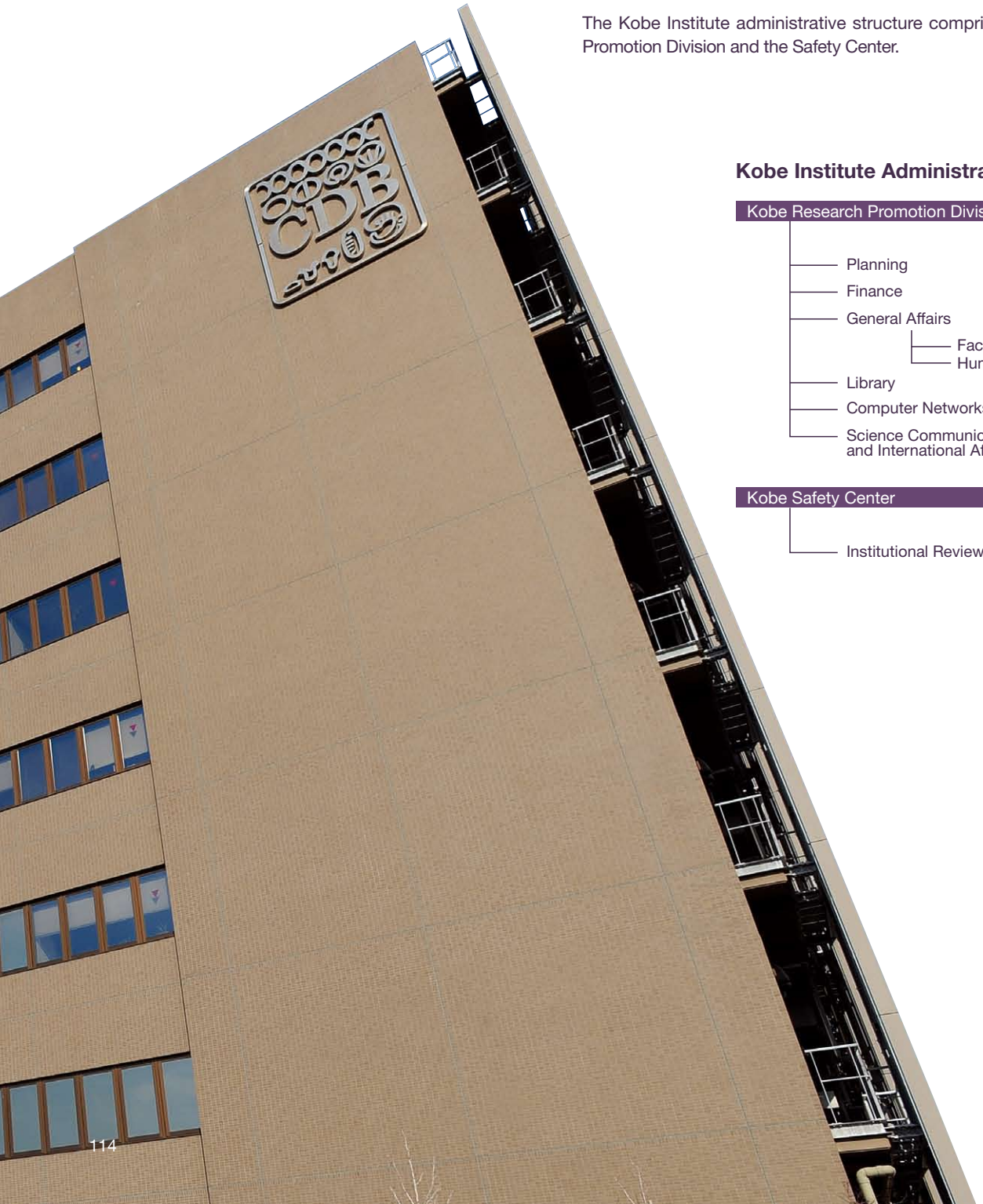
The CDB hosted a two-day educational program for area high school teachers on October 4 and 5, with support from the Japanese Society for Developmental Biology (JSDB), the Kobe Educational Council and the Japan Science and Technology Agency (JST). Thirty teachers of biology from the Kansai region of Japan joined the course, which featured hands-on experiments in *Xenopus* (African clawed frog), zebrafish and chick embryology, and lectures by Masaki Taira (Associate Professor, University of Tokyo) and Hitoshi Niwa (CDB Laboratory for Pluripotent Cell Studies).

Kobe night view. Photography: Natsuhiko KUSUMOTO

RIKEN Kobe Institute

The RIKEN Kobe Institute was established in April 2002 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 work with the potential to make a contribution to the field of regenerative medicine. In April 2007, the Kobe Institute welcomed a new institution, the Molecular Imaging Research Program (MIRP). In autumn 2008, the MIRP was re-designated as the Center for Molecular Imaging Science.

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 (IBRI) and other neighboring institutions, to help bridge basic science to applications in medical therapy and drug discovery and thereby to contribute 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
    - Facilities
    - Human Resources
  - Library
  - Computer Networks
  - Science Communications and International Affairs
- Kobe Safety Center
  - Institutional Review Board

Research Promotion Division

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

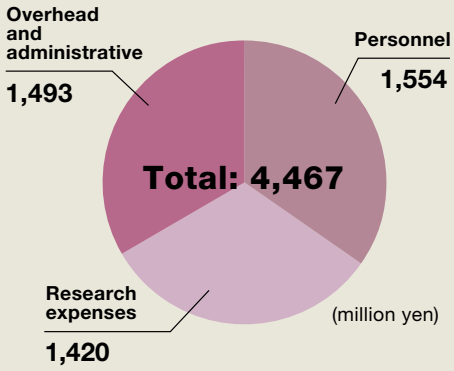
Safety Center

The KI Safety Center provides training and supervision for lab and workplace safety, implements and monitors disaster prevention measures, and educates staff on and ensures compliance with national and institutional regulations governing laboratory practice and technology. The office also handles all administrative affairs of the Institutional Review Board, the CDB's research ethics review committee, and administers the Institute's medical center.

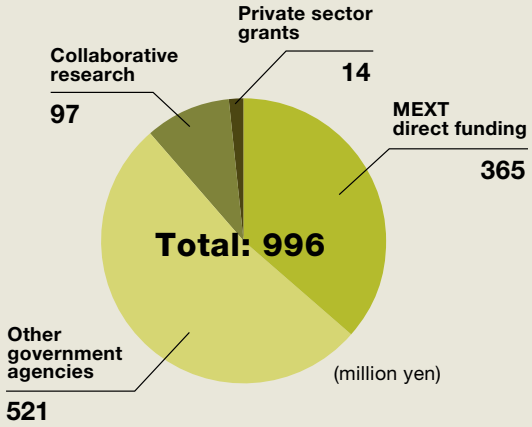
Center for Molecular Imaging Science

In 2005, the Molecular Imaging Research Program was selected by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) as an important part of its efforts to promote research and development in life sciences that respond to real societal needs. Since then, RIKEN has been striving to establish a core center for drug development molecular imaging. In the fall of 2006, the Kobe MI R&D Center Building was completed with the support of the Kobe municipal government and others, in which RIKEN's facility for the Molecular Imaging Research Program (MIRP) was now located. In October 2008, to further advance research and improve its research system, the MIRP was reorganized and inaugurated as the Center for Molecular Imaging Science (CMIS). The CMIS has developed many novel probes for molecular imaging, contributing to drug discovery and the development of diagnostic and therapeutic indicators.

2008 CDB Budget



In addition to these intramural funds, individual labs and investigators are encouraged to compete for external funding as well, from sources such as the Japan Society for the Promotion of Science (JSPS), other government agencies, private foundations and industry. These external funds form an important component of the CDB's overall funding mix, representing more than 1 billion yen in external funding for CDB research programs in the fiscal year ending in March 2008.



2008 CDB Staff

Laboratory heads	29
Research scientists	107
Research associates	6
Technical staff	102
Assistants	27
Visiting scientists	63
Student trainees	43
Part-time staff	30
Research Promotion Division	51
Other	52
Total	**510**

2008 CDB Symposium

Turning Neurons into a Nervous System

March 24-26, 2008

The RIKEN CDB held its sixth annual symposium on the theme "Turning Neurons into a Nervous System," from March 24 to 26. The three-day program featured a broad spectrum of talks ranging from the genetic and molecular activities that guide the morphological and migratory behaviors of individual neurons, to the complex networks that allow huge numbers of neurons to work together in a coordinated fashion to achieve higher brain functions. In addition to the nearly 30 invited talks, each day included a poster session in which more than 80 presenters discussed their work. The annual symposium series, which was launched in 2002, 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

William H. KLEIN
(The University of Texas,
M. D. Anderson Cancer Center, USA)
Matthew KELLEY (NIDCD/NIH, USA)

Session 2

Elizabeth GROVE (University of Chicago, USA)
Yoshiki SASAI
(RIKEN Center for Developmental Biology, Japan)

Session 3

Adrian W. MOORE
(RIKEN Brain Science Institute, Japan)
Kozo KAIBUCHI (Nagoya University, Japan)
Vivian Yi Nuo POON (Stanford University, USA)

Session 4

Hideki ENOMOTO
(RIKEN Center for Developmental Biology, Japan)
Takako MAKITA
(Howard Hughes Medical Institute,
Johns Hopkins University, USA)
Hideaki TANAKA (Kumamoto University, Japan)

Session 5

Barbara HEMPSTEAD
(Weill Medical College of Cornell University, USA)
Raj LADHER
(RIKEN Center for Developmental Biology, Japan)
William SNIDER (UNC Neuroscience Center, USA)

Session 6

Hitoshi OKAMOTO
(RIKEN Brain Science Institute, Japan)
Silvia ARBER (University of Basel, Switzerland)

Session 7

Masatoshi TAKEICHI
(RIKEN Center for Developmental Biology, Japan)
Takeshi IMAI (The University of Tokyo, Japan)
Mineko KENGAKU (RIKEN Brain Science Institute, Japan)

Session 8

Dietmar SCHMUCKER (Harvard Medical School, USA)
Larry ZIPURSKY
(Howard Hughes Medical Institute/ UCLA, USA)

Session 9

Thomas C. SÜDHOF
(Howard Hughes Medical Institute, USA)
Mei ZHEN (Samuel Lunenfeld Research Institute, Canada)

2009 CDB Symposium

Shape and Polarity

March 23-25, 2009

The seventh CDB symposium on "Shape and Polarity" will be held on March 23 to 25 in the CDB Auditorium. The focus will be on questions such as how cells become polarized and the roles of cell polarity, and how cell populations organize into complex tissue and organs through rearrangement of polarized cells. The function of anisotropic organization and distribution of components such as proteins at the cellular level will also be addressed, along with its function in asymmetric cell division and other developmental processes.

Session 10

Joshua R. SANES (Harvard University, USA)
Aaron DIANTONIO
(Washington University School of Medicine, USA)

Session 11

Mario R. CAPECCHI (University of Utah, USA)

Session 12

Joseph G. GLEESON
(University of California, San Diego, USA)
Christopher A. WALSH
(Howard Hughes Medical Institute, BIDMC, USA)
Li-Huei TSAI
(Picower Institute for Learning and Memory, USA)

Session 13

Yi Eve SUN (UCLA Medical School, USA)
Hideyuki OKANO
(Keio University School of Medicine, Japan)
Arturo Alvarez-BUYLLA
(University of California, San Francisco, USA)

Session 14

Ikue MORI (Nagoya University, Japan)
Edwin W. RUBEL (University of Washington, USA)
Eiji MATSUNAGA (RIKEN Brain Science Institute, Japan)

Session 15

Herwig BAIER (University of California, San Francisco, USA)
Takao K. HENSCH (Harvard University, USA)

Session 16

Tobias BONHOEFFER
(Max-Planck-Institut für Neurobiologie, Germany)
Barry J. DICKSON
(IMP-Research Institute of Molecular Pathology, Austria)

Invited speakers

Julie AHRINGER (University of Cambridge, UK)
Jeffrey D. AXELROD (Stanford University, USA)
Ping CHEN (Emory University, USA)
Jon CLARKE (King's College London, UK)
Anne EPHRUSSI (EMBL, Germany)
Jiri FRIML (Ghent University, Belgium)
Bob GOLDSTEIN (University of North Carolina at Chapel Hill, USA)
Hiroshi HAMADA (Osaka University, Japan)
Shigeo HAYASHI (RIKEN CDB, Japan)
Carl-Philipp HEISENBERG (MPI-CBG, Germany)
Robert INSALL (Beatson Institute for Cancer Research, UK)
Christopher KINTNER (The Salk Institute, USA)
Juergen KNOBLICH (IMBA, Austria)
Thomas LECUIT (IBDML, France)
Fumio MATSUZAKI (RIKEN CDB, Japan)
Roberto MAYOR (University College London, UK)
Hiroki NISHIDA (Osaka University, Japan)
Shigeo OHNO (Yokohama City University, Japan)
Sarah RUSSELL (Peter MacCallum Cancer Centre, Australia)
Hitoshi SAWA (RIKEN CDB, Japan)
Rudolf WINKLBAUER (University of Toronto, Canada)
Zhenbiao YANG (University of California, Riverside, USA)

CDB Seminars

The CDB has made special efforts to develop a full and diverse program of invited seminars by scientists from around the world. To date, the Center has hosted more than 400 such talks, in addition to numerous meetings, internal forums and colloquia. The following speakers presented CDB Seminars in the period from January to December 2008.

date	title	speaker
01-09	FANTOM and genome-wide technologies	Yoshihide HAYASHIZAKI
02-01	*Drosophila* as a model for investigating obstructive pulmonary diseases: Suppression of NF-kB-mediated innate immune responses in flies and humans by elevated CO_2 levels (hypercapnia)	Greg BEITEL
02-01	Genome evolution and the origins of the innate immune repertoire: insights from the coral Acropora and other "lower" animals	David J. MILLER
03-10	Kruppel-like factors: Key molecular regulators of cardiovascular biology	Mukesh K. JAIN
04-01	RNAi screens for novel candidate insulin receptor-like proteins turned up an unexpected role of EGF signaling in aging	Hiroaki IWASA
04-01	Getting published in *Nature Neuroscience*	Annette MARKUS
04-15	From stem cells to neuronal networks, from mouse to man: Genetic mechanisms of development and evolution of the cerebral cortex	Pierre VANDERHAEGHEN
04-17	Molecular motors, adaptor proteins and Rab GTPases control vesicle movements along microtubules	Ilya GRIGORIEV
04-17	Live imaging analysis of morphogenesis and lineage formation in the early mouse embryo	Yojiro YAMANAKA
04-23	Kid-mediated anaphase chromosome compaction safeguards mouse early embryos against multinuclear formation	Miho OHSUGI
04-30	Mechanosensory Tip-link is an asymmetric filament with Cadherin 23 and Protocadherin 15 at opposite ends	Hirofumi SAKAGUCHI
05-08	Nuclear transport as a regulator of cell differentiation	Noriko YASUHARA
05-08	Analysis of axis formation by observing the cell behaviors during the early mouse development	Toshihiko FUJIMORI
05-08	Spermatogenic-stem-cell System and its spatiotemporal regulation in the mouse testis	Shosei YOSHIDA
05-12	Macro- and micro-environmental regulation of ectodermal organ stem cells	Cheng-Ming CHUONG
05-19	Physical forces generated by cells and sensed by cells	Toshihiko OGURA
06-02	Integrating positional information in *Xenopus* and *Drosophila* embryos	Eddy M. DE ROBERTIS
06-02	The role of transcription factor Six3 in forebrain development	Lila Solnica-KREZEL
06-06	Multipotency of early and late Isl1$^+$ cardiac progenitors	Atsushi NAKANO
06-12	Characteristics of epithelial intercellular adhesion and cell proliferation	Sachiko TSUKITA
07-02	Sculpting cell membranes: Understanding pathways of endocytosis and exocytosis	Harvey T. McMAHON

date	title	speaker
07-07	Trophic factor signaling in the nervous system	Carlos IBANEZ
07-15	Life and death in dopamine neurons - Insights from TGFbeta-HIPK2 signaling pathway	Eric HUANG
07-16	Biosynthesis and processing of brain-derived neurotrophic factor (BDNF) in CNS neurons	Tomoya MATSUMOTO
07-22	Regulation and function of *Hox* genes during chick paraxial mesoderm development	Tadahiro IIMURA
09-05	Chemicals and neurons controlling nematode behavior	Paul STERNBERG
09-05	Scientific publishing, choices and challenges	Katrina KELNER
09-11	Sex determination and the role of SRY	Ryohei SEKIDO
09-22	Intrinsic factors controlling neural stem cell properties: a tale of two *Sox* genes	Robin LOVELL-BADGE
10-31	Nuclear regulation during newt lens regeneration	Nobuyasu MAKI
11-10	The role of lineage and cell interactions in the specification of blood stem cell fate during embryonic development of the zebrafish	Robert HO
11-10	Using zebrafish to study pancreas development	Victoria PRINCE
11-13	Translational control of cellular senescence	Joel D. RICHTER
11-14	Understanding Wnt/beta-catenin signaling in development and disease	Xi HE
11-19	Meganucleases with tailored specificities for genome engineering purposes	Julianne SMITH
12-02	Defining the spatiotemporal origins of GABAergic cortical interneuron subtypes	Goichi MIYOSHI
12-03	Population dynamics of human ES cell cultures: Self-renewal, adaptation and cancer	Peter W. ANDREWS
12-03	Neural stem cells and neurogenesis in the adult central nervous system	Jonas FRISÉN
12-05	Molecular control of cell fate specification in the cerebral cortex	Victor TARABYKIN
12-08	Microtubules, microfluidics, and cell shape	Phong T. TRAN
12-08	Adenomatous Polyposis Coli (APC) protein directly nucleates actin assembly by a novel mechanism	Kyoko OKADA
12-08	Biologists in the director's chair: Communicating with Maya animation software	Dan W. NOWAKOWSKI
12-09	Human embryonic stem cell Research at Royan Institute	Hossein BAHARVAND
12-24	Nematode dauer formation as a case study for evolution of developmental plasticity	Akira OGAWA

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 has seven campuses nationwide, comprising fifteen research centers and programs. It also operates three joint laboratories with counterpart organizations overseas, and has established relationships with research institutions, universities and science promoting agencies in countries around the world, including liaison offices in Singapore and Beijing.

RIKEN Website

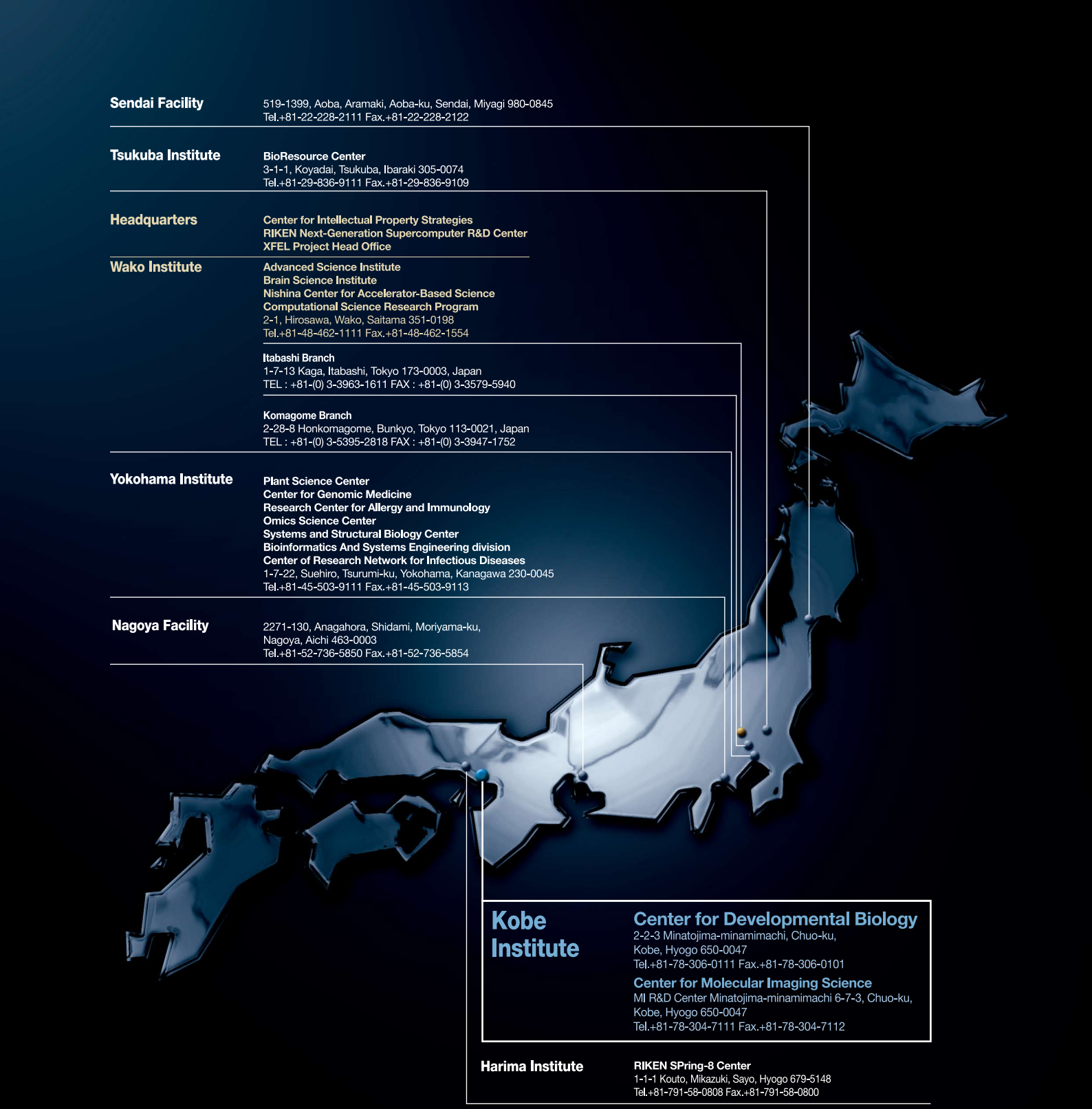
The RIKEN website provides a full, rich experience for online visitors to the institute. The site contains important links to all RIKEN institutes nationwide, a full range of public information and materials, as well as databases and other electronic resources developed by RIKEN labs. We encourage those with an interest in learning more about RIKEN's organization, activities and history to visit the new site.

URL: http://www.riken.jp/

RIKEN Research

RIKEN publishes the monthly print and online newsletter RIKEN RESEARCH to draw the world's attention to some of RIKEN's best research in a timely and easy to understand fashion. This magazine provides a central resource for up-to-date information on key achievements of the numerous RIKEN institutes and research centers, along with related news and retrospectives on the history of the 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.

URL: http://www.rikenresearch.riken.jp/



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

RIKEN was first founded in 1917 as a private research foundation, *Rikagaku Kenkyuusho* (The Institute of Physical and Chemical Research). In 2003, the Institute was reorganized as an Independent Administrative Institution under the Ministry of Education, Culture, Sports, Science and Technology (MEXT), since which time it has engaged in wide-ranging research activities spanning the basic and applied sciences.

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