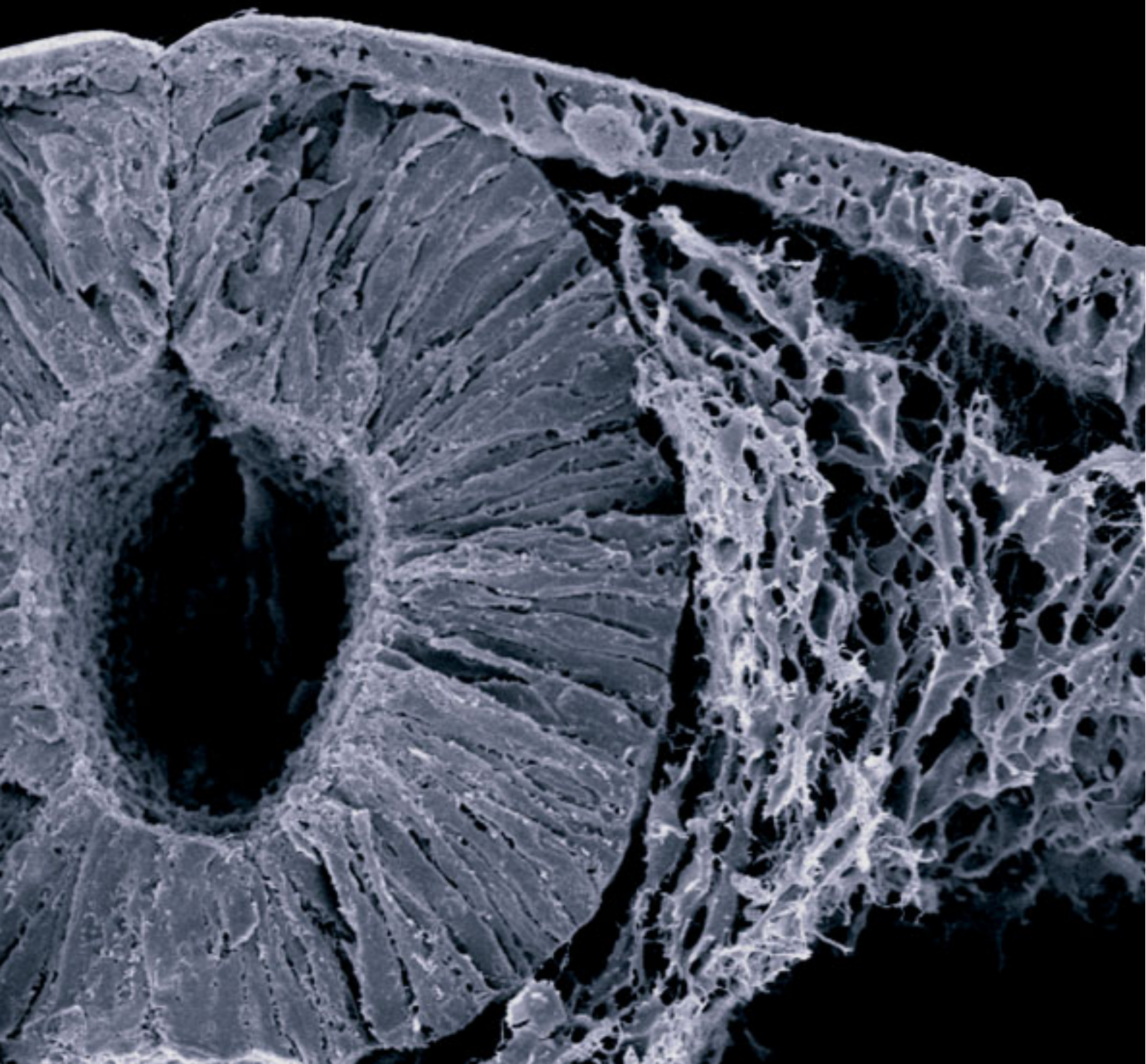
RIKEN Center for Developmental Biology

2007 Annual Report



RIKEN Center for Developmental Biology

2007 Annual Report

Contents


RIKEN
RESEARCH

Message from the CDB Director

As director of the RIKEN Center for Developmental Biology, I am delighted to introduce our institute's 2007 annual report with the knowledge that the investment of time and effort into the establishment of our research programs and infrastructure in the first half of this decade is now returning such a rich scientific dividend. Previous years have seen a bounty of new findings in the fundamental mechanisms of development and regeneration emerge from so many of the CDB laboratories, and once again in 2007 we are proud to have been able to contribute to this exciting and rapidly evolving field of science.

Developmental biology remains the cornerstone of CDB research, and the strength and breadth of our emphasis there is reflected in our many publications focused on embryological phenomena at the molecular and cellular levels. This past year has seen particularly strong output from labs studying questions of mitotic cell division, the organization of neural tissues, and the emergence of the blood and vascular systems. We were also proud to open new inroads into such areas as the *Drosophila* olfactory system and cyclostome embryology. The concentration of talent and accumulated expertise of the research community here, and the collegial environment we seek to foster makes working at the CDB a continually exciting and rewarding experience.

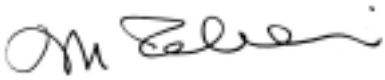
We also believe that it is that solid scientific foundation that has enabled our biomedically-oriented labs to make important new strides as well. Many of the CDB's researchers now work increasingly in areas with potential direct impact on human healthcare, particularly in the field of regenerative medicine, which represents a source of hope for patients suffering from degenerative diseases, and their families. Advances in techniques for the culturing of human embryonic stem cells and the boosting of the natural regenerative powers of the mammalian retina reported by CDB labs over the past year stand as important new steps toward the realization of our mission to provide surer footing for new therapies clinical applications.

That mission, however, must continue to evolve with the changing times. The explosive progress in cell and molecular biology over the past several decades has led us to a fairly sophisticated understanding of the mechanisms that make cells tick, and enable them to cooperate in the construction and maintenance of a body. The recent paradigmatic discovery by Shinya Yamanaka at Kyoto University and others that human differentiated cells can be reprogrammed to a state of pluripotency represents the culmination of one long quest, but perhaps the first step on another new quest as well. To support the exploration of these new horizons in stem cell biology, the CDB established a Division of Human Stem Cell Technology in 2007 to provide technological expertise, training and support in the culture, maintenance, distribution and management of human pluripotent cells. Although we now know understand many aspects of, and can indeed manipulate, cells, the question of how diverse populations of cells orchestrate and arrange themselves into the intricate organs, limbs and other systems of the body remains beyond our ken. We now must ask how such wonderful designs of such profound function can emerge autonomously, and seek ways of applying that knowledge to the betterment of the human condition.

As a research center funded primarily by the Japanese government, it is pleasing to see that this country has attained such a prominent position in the fields of developmental and stem cell biology. But, as members of that community, we also see it as part of our role to encourage the development of the scientific endeavor worldwide. CDB scientists continue to support and develop global interactions with a particularly strong focus in Asia, with ongoing regional networking efforts in developmental biology and reproductive biotechnology, and new support in 2007 for the establishment of a Stem Cell Network for the Asia-Pacific region (SNAP). It is our sincere hope to cultivate relationships with scientists from across the region and around the world, through collaboration, discussion, and programs intended to foster the careers of the next generation of researchers.

After seven years of the privilege of serving as director of the CDB, I can say that we have been fortunate in experiencing the challenge of being born in interesting times. And as those times change, the CDB will strive to keep pace with the evolution of our science, the needs of our society, and the curiosity that drives us to follow wherever nature leads.

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 33 laboratories in its Core Research (7 groups), Creative Research Promoting (21 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 regeneration, and the scientific bases of regenerative medicine.

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.

Leading Project Research Units

Research Promotion Division

Safety Center

Molecular Imaging Research Program

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.

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.

Stem Cell Translational Research
Takayuki ASAHARA M.D., Ph. D.

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

Neural Network Development
Chihiro HAMA 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.

Research Unit for Cell Plasticity Mitsuko KOSAKA Ph. D.
Research Unit for Organ Regeneration Hideki TANIGUCHI M.D., Ph. D.

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

Adding Notch to the tally of olfactory neuronal patterning determinants

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Endo K, et al. Notch signal organizes the *Drosophila* olfactory circuitry by diversifying the sensory neuronal lineages. *Nat Neurosci* 10. 153-60 (2007)

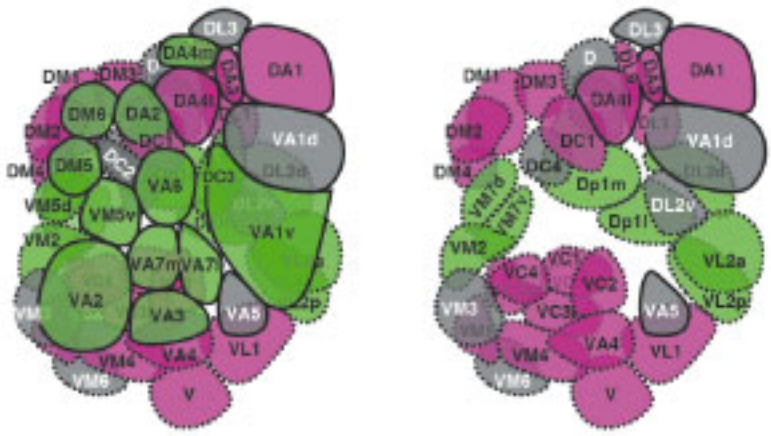
Olfaction is an ancient and highly adapted sensory system that enables animals to survey their chemical environment by detecting and discriminating between odorant compounds in the air. The importance of the olfactory system is evident from the amount of genomic space it occupies – as much as 5% of some species' genomes is devoted to genes involved in the sense of smell. But the question of how that genetic diversity is translated into the neuronal diversity of the olfactory system, comprising a variety of olfactory receptor neurons (ORNs), each expressing a single molecular receptor and connecting to a specific glomerulus in the brain, has eluded a clear solution.

Studying the comparatively simple olfactory network of the fruit fly, *Drosophila melanogaster*, Keita Endo and colleagues in the Laboratory for Neural Network Development (Chihiro Hama; Team Leader) discovered a new role for an important molecular pathway in organizing this neuronal circuitry. In work published in *Nature Neuroscience*, Endo (now at the University of Tokyo) showed that the differentiation of olfactory neurons deriving from the same precursor varies depending on the activity level of the Notch signaling pathway.

During the metamorphosis stage of fly development, approximately 50 different types of olfactory receptor neurons are generated from a smaller number of precursors. Each of these neurons expresses a specific olfactory receptor, enabling it to detect a given chemical stimulus, and projects axons to a specific target in the brain. Several molecules have been implicated in later processes, such as axonal guidance and the establishment of neuronal connections, but the means by which the diversity of the olfactory receptor neuron population is generated are poorly understood.

Endo et al. conducted a screen for mutations that disturbed the normal axonal patterning of olfactory receptor neurons in the fly, and identified a phenotype in which the axonal projection of a pair of neurons was disrupted. In this mutant, the neurons, which normally target separate glomeruli, both projected axons to the same destination. They identified the gene responsible as an allele of *mastermind* (*mam* – the new allele was christened mam^{k1514}), which encodes a nuclear protein involved in the Notch signaling pathway. Notch signaling functions in numerous forms of cell differentiation, translating signals from the cell's exterior via a Notch receptor to the nucleus, where they influence gene expression.

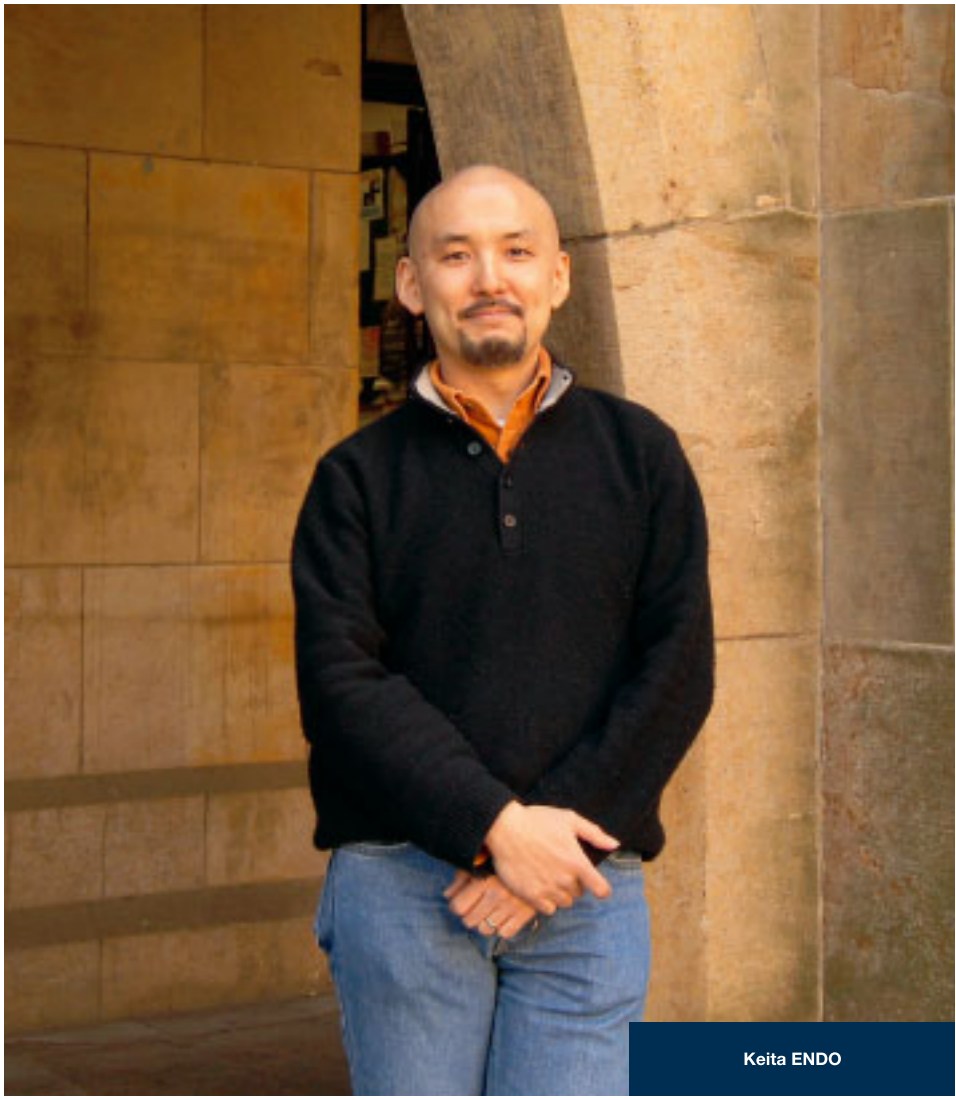
Knowing that Notch signaling had been shown previously to work in asymmetric cell divisions (in which two daughter cells of different character are generated from a single precursor) via the accumulation of the Notch antagonist Numb, the team next looked at a *numb* (*nb*) mutant, and found that the phenotype was the precise opposite of the unilateral axonal projection seen in the mam^{k1514} fly. To



Individual glomeruli receive projections from either Notch-ON (magenta) or Notch-OFF (green) ORNs, creating a domain organization in the antennal lobes, the first olfactory processing center in the *Drosophila* brain. Left and right figures show the anterior and posterior regions of the antennal lobe, respectively.

develop a more comprehensive picture of the role of these Notch factors, they first surveyed the organization of glomerular clusters in wildtype flies, and identified 52 glomeruli grouped in 24 clusters, each of which receives axonal projections from a single clonal cluster of olfactory receptor neurons. Their map showed that all ORNs housed within the same sensillum (a sense organ comprising one to four ORNs) are clones, deriving from the same precursor.

To identify which glomeruli in each cluster receive projections from ORNs in the *mam* and *nb* clones, flies were engineered to contain small mutant clones of cells that included ORNs. This experiment revealed that each glomerular cluster received projections from a combination of ORNs present in *mam* clones and *nb* clones. This was the first evidence that the differentiation of olfactory neuronal clusters could be determined by the activation or absence of Notch signaling. An examination of the Notch state across all parts of the olfactory system show that this effect functions globally – asymmetries in Notch signaling in olfactory receptor neurons within a clonal cluster determine the patterns of axonal projections to specific glomeruli throughout the system.



Keita ENDO

To determine how the Notch "On" and "Off" states arise during the development of olfactory receptor neurons, Endo et al. studied cells from single sensory organ precursors, and found that daughter cells expressed one or the other of a pair of proteins, Senseless (Sens) and Cut, in a mutually exclusive fashion. This Sens-Cut dichotomy is perturbed in the *mam* and *nb* mutant clones; in the Notch-ON *nb* mutant, inner cells failed to express Sens, while in mam^{k1514} mutants, all daughter cells stained for Sens. Studies of olfactory receptor neurons expressing Gal4 drivers of specific olfactory receptor genes showed that Notch status determines both receptor gene expression and axonal targeting in olfactory receptor neurons.

This clean segregation of olfactory receptor expression and axonal projection of clonal ORNs based on their Notch activity state represents a major new insight into the understanding of an important and highly complex sensory system. "What we have revealed here is only one element of the mechanisms used for generating a diverse array of ORNs derived from sensory organ precursors," notes Hama. "It raises a number of intriguing questions concerning the genes downstream of Notch and Notch-independent mechanisms for ORN diversification. We are also curious as to whether the domain organization of glomeruli created by Notch-ON and –OFF classes of ORNs has any functional or behavioral relevance in olfaction."

Catenin cleft: Calpain-mediated cleavage of ß-catenin in novel signaling pathway

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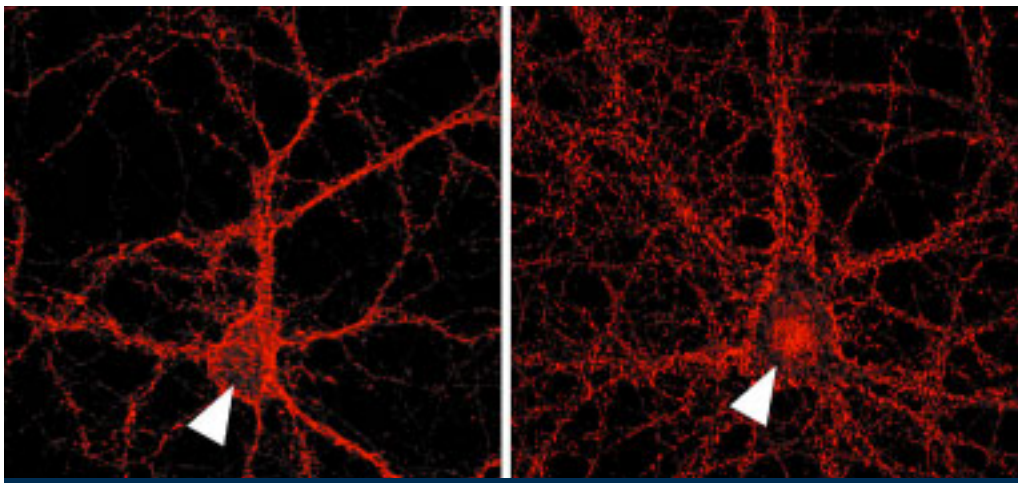
Abe K and Takeichi M. NMDA-receptor activation induces calpain-mediated beta-catenin cleavages for triggering gene expression. *Neuron* 53. 387-97 (2007)

The molecule β-catenin famously plays a dual role as a binding partner of the classic cadherin machinery in cell-cell adhesion, and as a critical component in the Wnt signaling pathway, which features in so many developmental and differentiative processes. Work from the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) suggests that this same protein participates in a hitherto unreported signaling pathway in the nervous system. The study, performed by Kentaro Abe, a Ph. D. candidate at the Kyoto University Graduate School of Biostudies at the time, revealed that post-synaptic activation of the NMDA receptor triggers a novel pathway in which β-catenin is cleaved by the enzyme calpain before entering the nucleus to initiate gene transcription.

"The study actually began when I was looking into possible interactions between neuronal activity and cadherin adhesion," says Abe. "But what I found was that when I treated neurons in culture with an agent that activates the NMDA receptor, truncated fragments of β-catenin began appearing in the cells." This sparked a search for a mechanism by which the full-length catenin could be cut into smaller lengths, which ultimately led to the identification of calpain, a proteolytic enzyme known for its roles in cell cycle progression and apoptosis.



In cultured hippocampal neurons, β-catenin could be found only at synapses (left) but, after stimulation with glutamate (right), β-catenin signal was also observed in the nucleus (indicated by arrowheads).

Abe found that after NMDA-R activation, calpain cleaves the N-terminus of the β-catenin protein, protecting it from degradation within the cytoplasm. Ordinarily, full-length β-catenin is phosphorylated by the kinase GSK3β, marking it for ubiquitination and digestion in the proteasome. Activation of the Wnt pathway protects against this degradation, allowing β-catenin to make its way into the nucleus, associate with the transcription factor Tcf/Lef, and activate the transcription of various genes. Interestingly, β-catenin cleaved by calpain is also safeguarded from destruction in the cytoplasm and thus able to reach the nucleus and engage transcription. "While the evidence is not yet conclusive, it appears possible that calpain even cleaves β-catenin that is bound to cadherin, which, if it proves to be true, would set up a link between neuronal activity and a loosening of the adhesion between cells at the synapse," notes Abe.

Additional tests confirmed that this cleavage of β-catenin could be blocked by the administration of a calpain inhibitor, and its effects on Tcf-dependent gene transcription could be mimicked by the transfection of β-catenin fragments of the same length. Abe next turned to the question of what genes might be activated by this pathway, and on analyzing transcripts amplified by RT-PCR, found that *Fosl1*, a gene also activated by the canonical Wnt pathway, was upregulated after NMDA-R stimulation by glutamate (which leads to calpain activation). Again, this effect was blocked by calpain inhibition and

Kentaro ABE

duplicated in experiments using exogenous β-catenin fragments, strongly indicating that a novel pathway in which NMDA-R induces calpain to cleave β-catenin was being used to initiate the transcription of genes in hippocampal neurons.

Looking ahead, Abe says, “For the next step, I’d like to try to work out what role this transcriptional activity plays in nervous system functions such as memory and learning. And, of course, a possible scenario in which neuronal activation sets off interactions between cellular adhesion and transcriptional pathways remains intriguing.”

New source of oocytes for SCNT?

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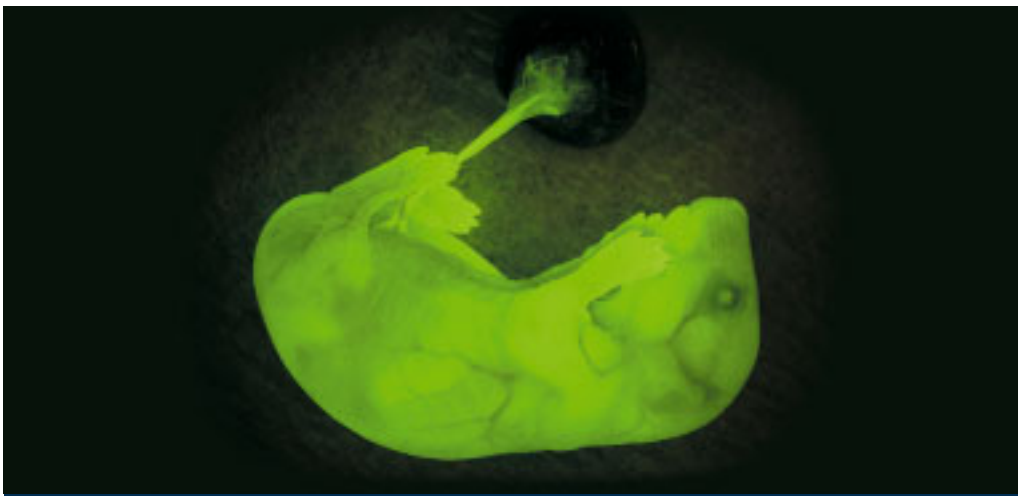
Wakayama S, et al. Establishment of mouse embryonic stem cell lines from somatic cell nuclei by nuclear transfer into aged, fertilization-failure mouse oocytes. *Curr Biol* 17. R120-1 (2007)

The study of human embryonic stem (ES) cells has enjoyed much attention for the promise these cells hold as a potentially limitless source of cells for use in drug development and regenerative medicine, but the field has also been dogged by controversy arising from a number of ethical issues. One of the most hotly debated of these involves how oocytes used in ES cell derivation by somatic cell nuclear transfer (SCNT; also known as therapeutic cloning) are obtained. Conventional wisdom has it that only "fresh" oocytes can be used, but research from the Laboratory for Genomic Reprogramming (Teruhiko Wakayama; Team Leader) suggests that even oocytes that fail to fertilize may be usable in SCNT. If it can be shown that the same holds true for human egg cells, this finding could help to resolve many issues surrounding oocyte donation, as large numbers of such oocytes are simply discarded in IVF clinics.

The Wakayama lab collected oocytes that had failed to fertilize after insemination, which they named "aged, fertilization failure" (AFF) oocytes, and used them as recipient eggs for nuclear transfer. The donor nuclei used in these experiments were taken from various strains of lab mice, including one engineered to express green fluorescent protein (GFP) ubiquitously, causing the animals to glow green under fluorescent light. While the efficiency of nuclear transfer and subsequent early development was lower for the AFF oocytes, they found that embryos that did reach the blastocyst stage could be used to derive ES cells at a success rate equivalent to that possible when starting from fresh oocytes. "We're hoping that this method can be optimized for use with human oocytes, which would open up a substantial new source of oocytes for use in some kinds of research and overcome a number of the objections that have been voiced over this promising technology," says Wakayama.

The cell lines established from the fertilization-failure oocytes expressed the molecular hallmarks of pluripotent cells, such as Oct4 and Nanog, and all cells examined were of normal karyotype (meaning their chromosomes were in the appropriate number and arrangement). Even more convincingly, these nuclear transfer ES cells contributed to the development of healthy, fertile offspring, with functional differentiation into both germline and all somatic germ layers.

In full-term cloning and intracytoplasmic sperm injection (ICSI) experiments, the AFF oocytes proved to be less amenable than their fresh counterparts, which the authors reckoned might be attributable to defects in genomic reprogramming. A partial deficiency in this mechanism could account for the fertilization-failure egg cells' ability to generate nuclear transfer ES cells, but not to support full-term development. This subtle but crucial difference could help to answer many of the standing criticisms about the trade-offs involved in dismantling a blastocyst-stage embryo in order to derive ES cells for



Chimeric mouse generated from NT-ES cells derived from aged, fertilization failure oocyte.
The donor nuclei were from mice engineered to express GFP, which can be seen as a green glow in all tissues.

research, for if the blastocyst is naturally destined never to reach later stages of embryonic development, arguments regarding the sacrifice of individual potential largely become moot. Similar concepts underlie a number of other methods, sometimes referred to as altered nuclear transfer, in which the embryo is genetically engineered to prevent its development into an individual, while allowing it to grow to the blastocyst stage.

“What’s important about this demonstration is that it shows that even aged oocytes that failed to fertilize on an initial attempt can be used to generate ES cells, which are extremely useful in basic research and show great future promise in medical applications,” says Wakayama. “While past attempts to use fertilization-failed human oocytes have been unsuccessful, we hope that this work shows that that may have been due to the nuclear transfer technique, rather than any inherent biological deficiency.”

Sayaka WAKAYAMA

Beta version: New role for cortical WRM-1 in asymmetric cell division

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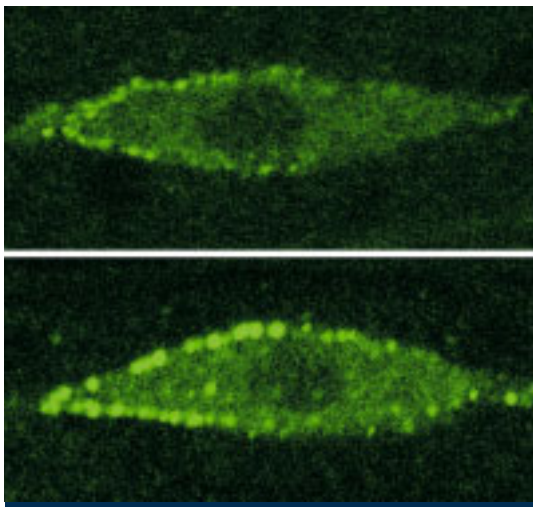
Mizumoto K and Sawa H. Cortical beta-catenin and APC regulate asymmetric nuclear beta-catenin localization during asymmetric cell division in *C. elegans*. *Dev Cell* 12. 287-99 (2007)

In most species, β-catenin works a double shift, functioning in both cadherin-mediated cell adhesion and at a critical step in the canonical Wnt pathway. But in *C. elegans*, these roles are played by distinct molecules: HMP-2, which binds cadherin, and WRM-1, which participates in Wnt signaling. The Wnt pathway is critically important throughout roundworm development, regulating the polarity of the majority of asymmetrically dividing cells via the unequal localization of cortical and nuclear factors. The function of WRM-1 at the cellular cortex, however, remains unknown.

Work from the Laboratory for Cell Fate Decision (Hitoshi Sawa; Team Leader) is set to change that. In an article published in *Developmental Cell*, Kota Mizumoto, a grad student in the Sawa lab, proposed a general model of how cortical WRM-1 interacts with other factors in the Wnt pathway, such as the APC homolog APR-1, to regulate the localization of WRM-1 in the nucleus, where it would otherwise regulate the activity of TCF transcription factor.

"The nematode is a really nice system for studying β-catenin in Wnt signaling, because unlike most species, where you have one molecule interacting with both the Wnt pathway and the cadherin machinery, in *C. elegans* you can look at them in isolation," says Mizumoto. "We know that WRM-1 localizes to the anterior cortex and the posterior nucleus during mitosis, and here we tried to work out what role it might be playing there."

The first step toward answering that question came when the team caused WRM-1 to localize throughout the entire cortex, rather than only the anterior, in the T cell lineage that appears in postembryonic development. This strategy involved forcing the expression of a fusion protein (WRM-1::GFP::CAAX); in this construct, CAAX effectively tethers WRM-1 to the cortex, while GFP aids in visualization. Worms engineered in this way showed the same phenotype as mutants in which *wrm-1* function is lost (i.e., on division, both daughter cells shared a hypodermal cell fate, in contrast to one hypodermal and one neural cell in wildtype), showing that the defect was an outcome of the uniform localization. Mizumoto further found that LIT-1, the *C. elegans* homolog of MAPK, also localized symmetrically at the cell surface. This is significant, as MAPK has also been shown to play a role in mediating asymmetric cell division.



Asymmetric cortical localizations of APR-1::GFP (upper panel) and PRY-1::GFP (lower panel) in the V5.p cell

Given the central role of WRM-1 in Wnt signaling, Mizumoto next looked at APR-1, the homolog of APC, a member of a group of factors that form what is known as the "destruction complex," which marks β-catenin for destruction in the absence of Wnt. In worms in which *apr-1* was knocked down by RNAi, the phenotype was opposite to that of the WRM-1::GFP::CAAX mutant; both daughters became neural, not hypodermal. The defect was a mirror image of that of other mutants in which the Wnt/MAPK pathway is upregulated, suggesting that *apr-1* negatively regulates this pathway. Inhibition of *apr-1* also disturbed the pattern of asymmetric WRM-1 nuclear localization, which normally causes it to localize in the posterior nucleus from about the telophase stage of cell division. In the *apr-1* knockdown phenotype, the distribution was symmetric to both nuclei.

"It appears that somehow APR-1 enables WRM-1 at the anterior cortex to prevent the accumulation of WRM-1 in the anterior nucleus," says Mizumoto. "When we looked at the movement of WRM-1 out of the nucleus, it appeared that more WRM-1 remains in the nucleus as a result of RNAi knockdown of *apr-1*, which we think has to do with its role as a mediator of nuclear export."

Kota MIZUMOTO

PRY-1, the homolog of Axin, another factor in the destruction complex, also localized asymmetrically to the anterior cortex, as did APR-1, suggesting that these may be components of a complex along with WRM-1. This asymmetry failed to occur in Wnt mutants, indicating that the effect is likely regulated by Wnt signaling.

“We still don’t have the molecular mechanism that explains the precise way in which APR-1 mediates the inhibition of WRM-1 accumulation in the anterior nucleus by WRM-1 at the anterior cortex,” Mizumoto admits, “but we do have a reasonable model of how it might work through the asymmetric export of WRM-1 from the nucleus. But as *apr-1* also appears to be required for the nuclear localization of WRM-1 in other cell types, there may still be some surprises ahead.”

Hagfish first: Elusive organism sheds light on the emergence of the neural crest

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

The possession of a hinged jaw is a trait shared by many, but not all vertebrates. The cyclostomes, such as lampreys and hagfish, are vertebrate taxa but feed by means of circular mouthparts, which can be used for either predation or scavenging. Even within the Cyclostomata, considerable diversity can be found; indeed the hagfish, in which many primitive traits are preserved, are sometimes considered to be markedly distinct from both the jawed vertebrates (gnathostomes) and the lampreys. It has long been suspected that an analysis of hagfish embryonic development would shed light into the question of its true phylogenetic position, which conceivably is near the branch point of the vertebrate divergence, but their seafloor habitat is little explored and hagfish eggs have proven extremely difficult to obtain. So difficult, in fact, that the last major research into hagfish development dates back to an 1899 treatise by Bashford Dean.

A major hurdle on the path to developing a more certain understanding of hagfish ontogeny and phylogeny was cleared with a report by Kinya Ota et al. of the Laboratory for Evolutionary Morphology (Shigeru Kuratani; Group Director) of the first significant new study of hagfish development in more than 100 years. The study, published in *Nature*, described the obtainment and analysis of multiple pharyngula-stage embryos of the hagfish, *Eptatretus burgeri*, yielding new insights into their early development, particularly that of the neural crest.

To collect viable specimens, the group placed eel traps in the waters off the coast of Shimane Prefecture, collecting around 50 live hagfish. “We were lucky to work with some local fisherman who knew the best spots to look for egg-carrying females,” says Ota. “It turns out that it may be easier to catch them in Western Japan, rather than the Pacific Coast, as the costal shelf slopes more gradually there.”

On returning to the CDB, they kept the animals in special aquarium tanks designed to replicate the deep-sea environment of the hagfish as closely as possible, and were rewarded for their efforts with a few dozen eggs, seven of which were fertilized. This in itself was something of an achievement, as hagfish have never successfully been bred in captivity. “We’re not sure what the critical factor in keeping

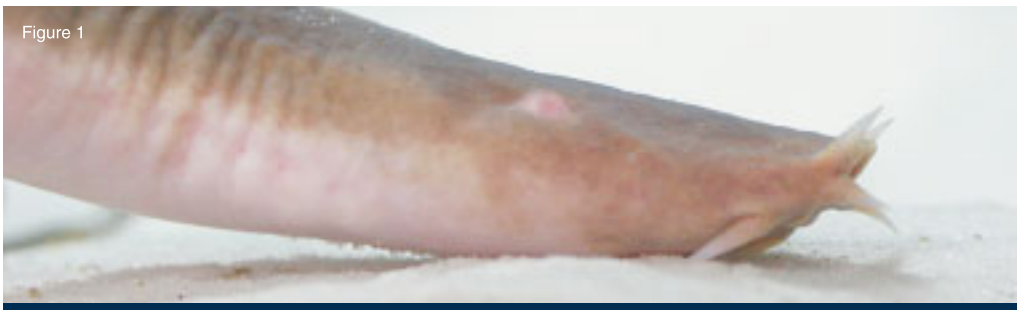
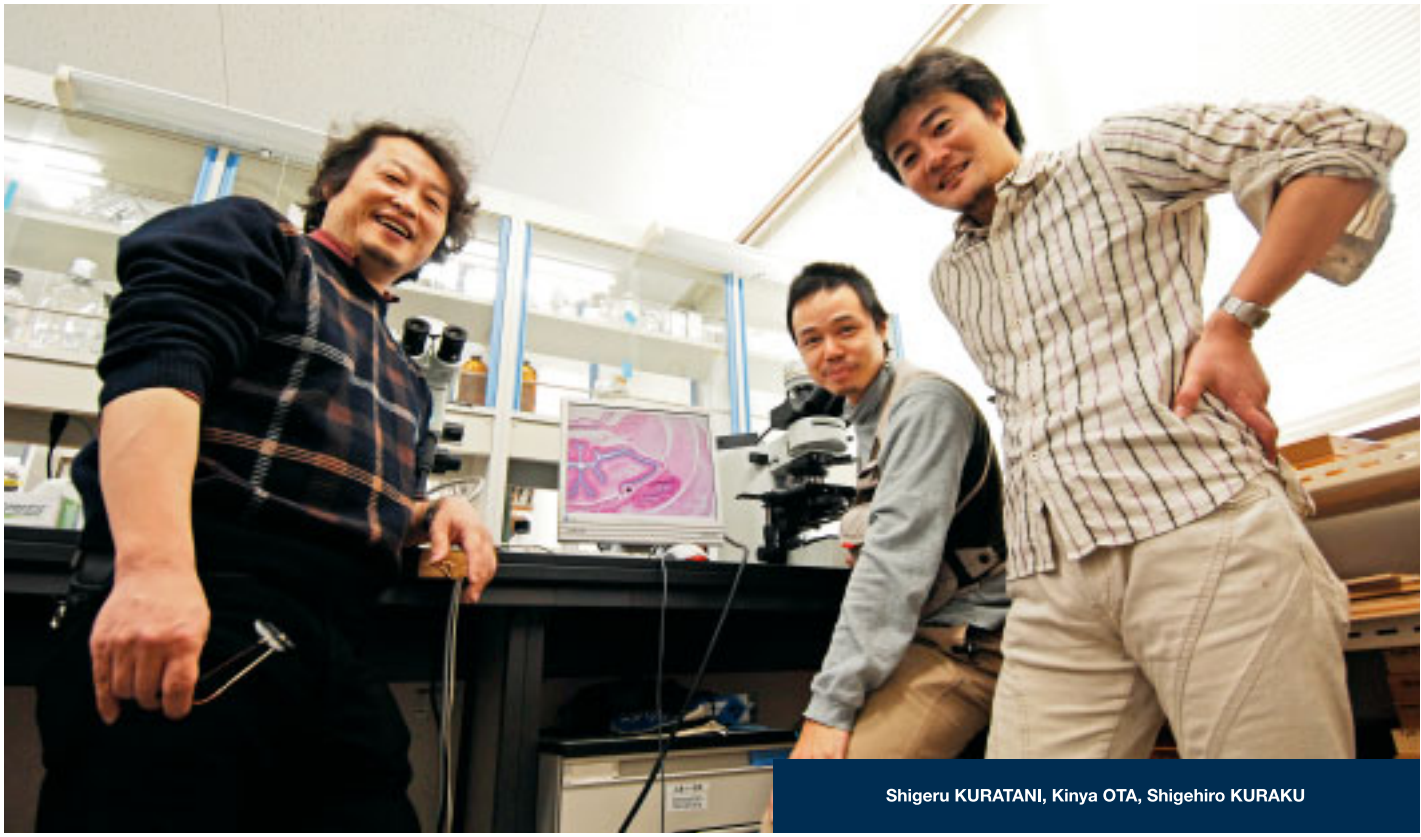


Figure 1
Hagfish maintained in laboratory aquarium

Figure 2
EFT: Hagfish pharyngula-stage embryo (anterior to left, gills visible); RIGHT: Sox9 gene expression in embryonic trunk. The stained Sox9-expressing region corresponded to neural crest.



Shigeru KURATANI, Kinya OTA, Shigehiro KURAKU

them alive in captivity is," Ota admits, "but we know that hagfish like cold water and are extremely sensitive to changes in salinity – even a small dilution of salt content can kill them. Their breeding habits are also still a complete mystery."

Histological staining and in situ hybridization of the embryos enabled the Kuratani group to address the question of neural crest development in these phylogenetically important animals. The vertebrate neural crest is a population of delaminated migratory cells, which serves as the source for such a variety of cell types that it is sometimes referred to as the fourth germ layer. Non-vertebrate chordates (such as tunicates and lancelets), however, develop only pockets of relatively immobile epithelial cells. The crucial question for the hagfish was whether this aspect of its development would more closely resemble that of the gnathostomes and lampreys, or that of the non-vertebrates (one previous study had speculated that it would be the latter). Looking at the expression of *Sox9* and several other neural crest maker genes, Ota found that the distribution patterns were closely matched to those seen in neural crest development, indicating that the genetic programs at work in the hagfish crest are similar to those in other vertebrates.

This new work, made possible by the first-ever artificial breeding of this elusive species, strongly supports the phylogenetic grouping of hagfish with the lampreys and other vertebrates. "Although they appear to be quite primitive, the hagfish actually use the same basic developmental program common to the vertebrates, including humans," notes Kuratani. "This genetic architecture has been preserved over at least 500 million years of evolutionary history, since the time of the divergence of the jawed gnathostomes from the more basal cyclostomes." The new availability of breeding techniques and viable embryos for this key organism opens up new inroads for molecular embryology toward a better understanding of vertebrate evolution.

Tracing bloodlines: An extraembryonic source of hematopoietic cells

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Samokhvalov I M, et al. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* 446. 1056-61 (2007)

The ultimate origins of the blood supply have been surrounded by scientific controversy. There are two recognized stages of hematopoiesis (blood formation) during embryonic development: an initial "primitive" stage, taking place in the extraembryonic yolk sac that provides nutrients to the early embryo, and a later "definitive" stage, which begins in a region of the embryo known as the aorta-gonad-mesonephros (AGM). But the debate over whether the source of the definitive hematopoietic population is local or represents some contribution by inwardly migrating precursors from the yolk sac remains unsettled. Importantly, knowledge into the blood supply's origins might be used for the generation of blood stem cells in culture, with the potential for therapeutic applications.

Evidence from *in vitro* studies had suggested that definitive hematopoiesis occurs independent of its primitive forerunners, but now, cell tracing studies conducted *in vivo* have conclusively shown that yolk sac cells expressing a hematopoietic marker gene contribute to both embryonic and adult blood development. This work was led by Igor Samokhvalov of the Laboratory for Stem Cell Biology (Shin-Ichi Nishikawa; Group Director), and reported in the journal *Nature*.

The group used a conditional genetic technique that allowed them to label yolk sac-derived blood precursors in a spatially and temporally specific manner. "I think the fact that we were able to study this process non-invasively and over the long term was critical," says Samokhvalov. "Anytime you take tissue out of its normal context, you introduce stresses on the system that can affect how it behaves, which can give you an inaccurate picture of what is really going on in the embryo."

The yolk sac-derived cells were found to contribute to both the erythroid (red blood) and lymphoid (white blood) lineages in embryos, but interestingly, their contribution to the formation of the yolk sac blood vessels was negligible. This stand counters some long-held claims for the existence of what has come to be known as the "hemangioblast," the presumed mutual origin of both the blood and the vascular system. The Nishikawa group instead found that, while the *Runx1*$^{+}$ yolk sac precursors play a part in definitive hematopoiesis, their contribution to blood vessel development is small to none.

Tight control of *Runx1*-directed inducible cell tagging.
Stained E10.5 embryos, control (left) and E7.5 activated (right)

"The picture that is developing from the cell tracing at different developmental time points is one of cells migrating from the extraembryonic region to the embryo proper," says Samokhvalov. "It may be that by E9.5 all commitments are made, with cells destined to become long-term stem cells associating with the endothelia of the embryonic vasculature, while those engaged in generating red blood cells for the embryo get recruited to the fetal liver where they begin proliferating. But by that point, the *Runx1*-positive cells are certainly already specified to the blood lineage."

The persistence of the cell tagging system used in this study even enabled the group to study the contribution of yolk sac precursors to adult hematopoiesis. Using flow cytometry to identify blood progenitors derived from the yolk sac, the group found a contribution of up to 10% of the adult. "We can't yet say quantitatively how much of a role the yolk sac precursors play in definitive hematopoiesis, only that they do," cautions Samokhvalov. "We still don't know how large a fraction of the *Runx1*+ cells is labeled by our method, so it's possible that the true contribution is even higher than what we were able to find."

Igor SAMOKHVALOV

Fifth CDB Open House

The RIKEN CDB opened its doors to more than 1,000 visitors on Saturday, April 21, hosting its fifth annual Open House with a new variety of seminars, exhibits and fun-with-science activities. This yearly event helps to introduce the Center's research into such areas as developmental biology, regeneration and stem cells to visitors of all ages and interests.

Among the highlights of the day's events were a science café-style chat session, in which members of the public could enjoy a relaxed and friendly discussion with CDB scientists on questions surrounding brain-machine interfaces; science craft workshops teaching how to make DNA papercraft and clay models of animals used in research; a gallery of fluorescent posters of biological images; and a themed exhibition hosted by four CDB labs on the biological properties and medical promise of stem cells.

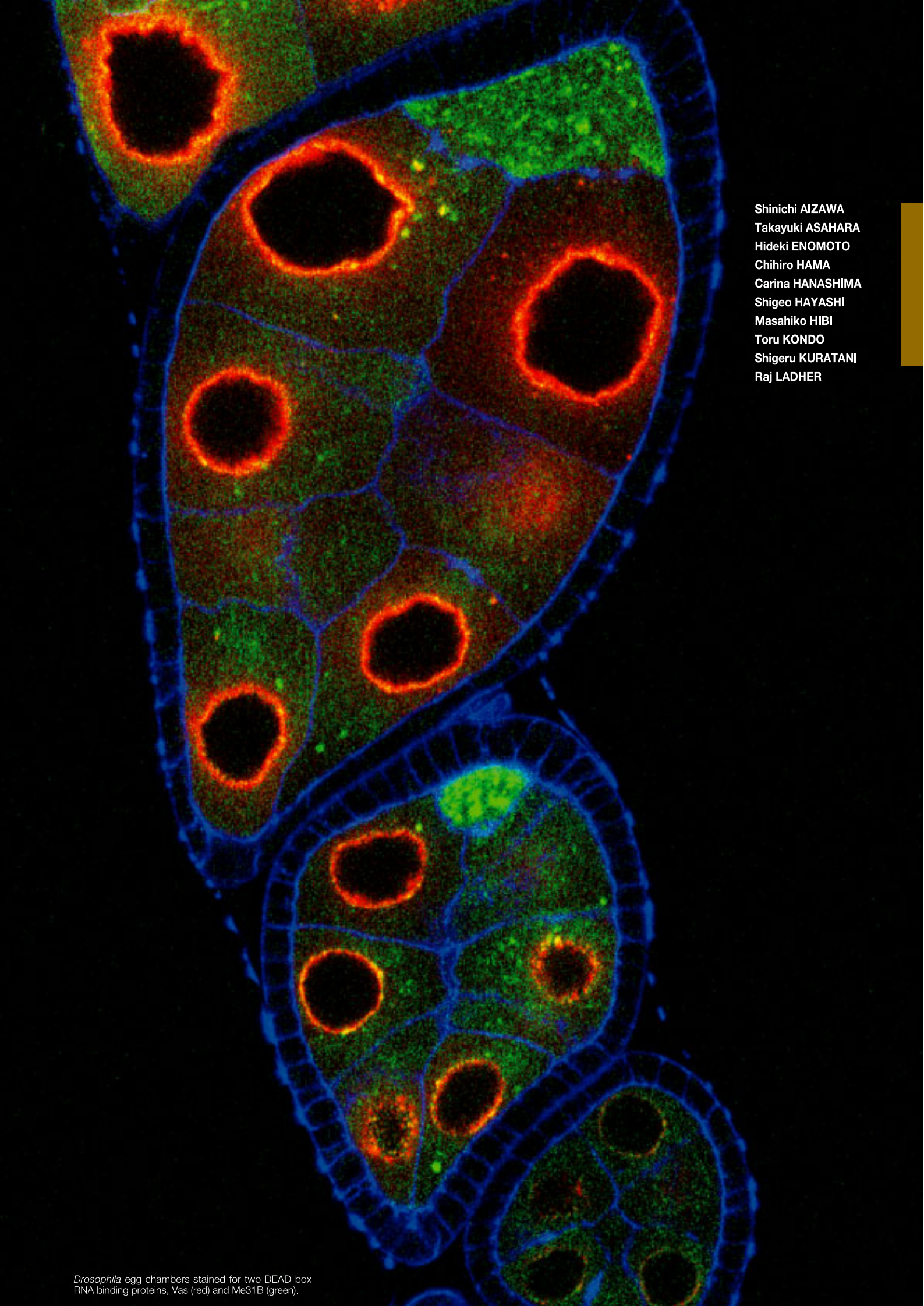
This year's public science lectures included talks by Masayo Takahashi (Team Leader; Laboratory for Retinal Regeneration) on the current realities and future potential of retinal regeneration and, marking the first Open House held by the Molecular Imaging Research Project (MIRP) that recently joined the RIKEN Kobe Institute, by MIRP Director, Yasuyoshi Watanabe.

A number of labs gave visitors the chance to take a peek inside a research unit, with explanations of experimental equipment and techniques by the scientists, galleries of microscopic images, and even areas where kids could have their photos taken in a real laboratory setting. Also for kids, the day featured the debut of a new interactive program, *CDB Lab Panic*, that lets players try their hands at scientific methods in a fast-paced and challenging game format.

CDB Lab Panic!

At its 2007 Open House, the RIKEN CDB announced the release of *CDB Lab Panic*, a new online game for kids featuring fast-paced simulations of experiments and phenomena. The software includes eight mini-game scenarios based on real lab activities like pipette-work and mutant screening, challenging players with three increasingly difficult levels in each module. After selecting a character and customizing his or her appearance, players can test their lab skills all 8 games and save their high scores to the game ranking website for posterity. Visitors to the CDB Open House held on April 21 enjoyed a sneak peek at a beta version of the game, which went live on May 1.

The game can be played on the CDB website at: **http://www.cdb.riken.jp/webgame-e**



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Takayuki ASAHARA
Hideki ENOMOTO
Chihiro HAMA
Carina HANASHIMA
Shigeo HAYASHI
Masahiko HIBI
Toru KONDO
Shigeru KURATANI
Raj LADHER

Drosophila egg chambers stained for two DEAD-box RNA binding proteins, Vas (red) and Me31B (green).

Vertebrate Body Plan

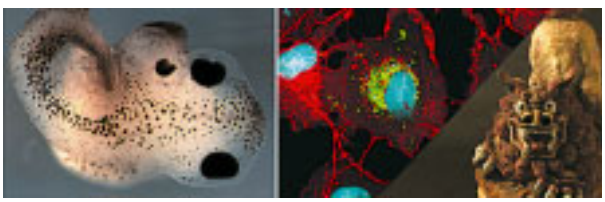
Shinichi AIZAWA Ph. D.

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. He also serves as 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. However, the mechanisms that instruct the brain's laminar regions to conform to anterior-posterior and dorsal-ventral axis patterning programs, and the means by which each individual region is formed, remain unknown.

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

A long road lies ahead in the search for the origins of the *Bauplan* of the vertebrate head, but application of powerful 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.



Shisa retains the Wnt-receptor Frizzled within the endoplasmic reticulum, thereby suppressing Wnt signaling (left). Shisa is a form of sculpture common to southern Japan, with a large head similar to the Egyptian sphinx (right).

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Stem Cell Translational Research

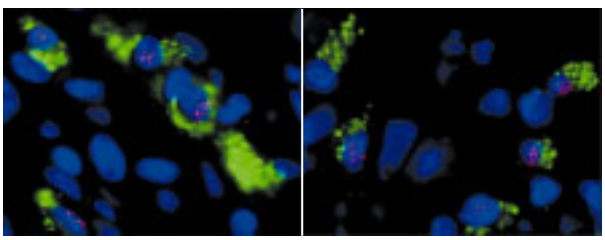
Takayuki ASAHARA M.D., Ph. D.

Takayuki Asahara received his M.D. from Tokyo Medical College in 1984, and performed residencies in cardiology and emergency medicine. He worked as a research fellow in cardiology at the Tokyo Medical College Hospital from 1989 to 1993 before moving to a fellowship in cardiovascular research at St. Elizabeth's Hospital in Boston. He was appointed assistant professor at Tufts School of Medicine in 1995, and associate professor at the Tokai University Institute of Medical Sciences in 2000. In addition to his current position as CDB team leader, Asahara serves as director of Regenerative Medicine and Research at the Kobe Institute of Biomedical Research and Innovation, and Professor of Physiology at the Tokai University School of Medicine.

The field of stem cell-based therapy continues to receive attention as one of the most promising frontiers of medical science. Building on previous work in which we identified bone marrow-derived endothelial progenitor cells (EPCs) and demonstrated their role in the generation of new blood vessels, our lab now seeks to characterize adult stem and progenitor cells with even greater differentiative potential, and simultaneously to translate that research into clinically relevant advances.

Our previous series of studies into endothelial progenitor cells (EPCs) and the preliminary data from investigations into post-natal pluripotent stem cells conducted by our lab challenge the conventional notion that postnatal neovascularization occurs exclusively as the result of sprouts derived from pre-existing, fully differentiated endothelial cells, i.e., angiogenesis. Our protocols were designed with the goal of determining the extent to which blood vessels derived at least in part from endothelial stem/progenitor cells, i.e., vasculogenesis, contribute to postnatal neovascularization.

Our lab also investigates the interactive mechanisms between vasculogenesis and organ regeneration. The elucidation of vasculogenesis signals in organ regeneration will contribute broadly to therapeutic application of human diseases. These studies are intended to translate basic research findings into clinically significant knowledge. We hope that our preliminary studies will elucidate the specific circumstances and mechanisms responsible for vascular development in organogenesis.



Bone marrow-derived cardiac lineage cells mobilized by PlGF gene transfer in myocardial infaction area. (green=bone marrow derived cells, red=GATA4 (in A), red=MEF2(in B))

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Neuronal Differentiation and Regeneration

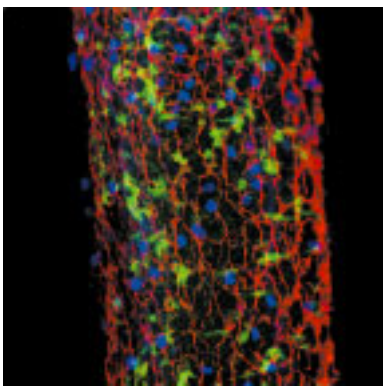
Hideki ENOMOTO M.D., Ph. D.

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

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 gut of conditional GFRα1-knockout embryos stained with anti-class III β tubulin (red) and anti-phospho-histone H3 (blue) antibodies. In this preparation, cells in which GFRα1 is conditionally inactivated are identifiable as GFP-positive cells (green).

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Publications

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Neural Network Development

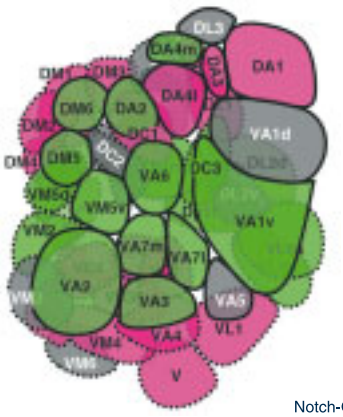
Chihiro HAMA Ph. D.

Chihiro Hama received his B. Sc. and M. Sc. from the University of Tokyo Department of Biophysics and Biochemistry and was awarded a Ph. D. from the same institution in 1985 for his work on the regulation of plasmid ColIb DNA replication by inc and repY. He spent the period from 1985 to 1988 as a post-doc in the laboratory of Thomas Kornberg at the University of California, San Francisco before returning to Japan to continue his post-doctoral work at the National Institute of Neuroscience, NCNP, Tokyo. He advanced to section chief in the Department of Molecular Genetics in 1991, and remained at the NCNP until 2001 when he was appointed to his current position at the CDB.

One of the most tantalizing questions in the field of neurobiology is how neural circuits of exquisite complexity are generated during nervous system development. Human brains consist of 10^{10} neurons, and each neuron projects an axon that extends along a predetermined pathway before finally finding its specific synaptic partner from myriad dendrites. These processes are regulated by a number of intrinsic factors and extracellular cues, which may be expressed in subsets of cells or localized to limited intracellular regions. To improve our understanding of the molecular mechanisms underlying this circuit formation, we have conducted a mutant screen using fruit flies to identify the regulatory factors involved.

We chose to study the olfactory sensory system in our mutant screen, as this system exhibits beautiful organization in its structure. *Drosophila* carries 1300 olfactory receptor neurons (ORNs) on its head appendages. Each of these neurons projects an axon into one or two out of 50 glomeruli in the antennal lobe, which is the first centralized olfactory processing region in the brain. The *Drosophila* genome encodes about 60 odorant receptors (ORs), and each olfactory receptor neuron expresses only one or two ORs. Interestingly, axons from neurons that express a given OR precisely converge at one glomerulus, suggesting that olfactory codes in the brain are generated by a combination of glomeruli stimulated through ORs.

To study the question of how olfactory receptor neuronal axons are specifically targeted to the correct glomerular positions, we have isolated several mutations that impair the projection of ORN axons into glomeruli. One of the mutations is a new allele of *mastermind* (*mam*), which is a transcription factor mediating Notch signaling. Analysis of clones mutant for either *mam* or *numb*, which antagonizes Notch signaling, indicates that asymmetric Notch activity diversifies the cell fates of secondary and tertiary precursors and thereby contributes to the generation of 'Notch-ON' and 'Notch-OFF' ORNs. We revealed that the asymmetric cell fate specification of ORNs mediated by Notch signaling affects axonal pathway, synaptic targeting and choice of OR expression. Thus, Notch signaling serves as a crucial mechanism for the organization of the olfactory circuitry.



Notch-ON (magenta) and Notch-OFF (green) olfactory receptor neurons target distinct glomerular regions.

We will continue to analyze other mutations, seeking to identify factors that control the projection of ORN axons and ultimately to understand more completely the molecular mechanisms underlying glomerular organization. An appreciation of basic mechanisms uncovered in the *Drosophila* brain may also help us to explain how the elaborate wiring of the human brain is established early in life.

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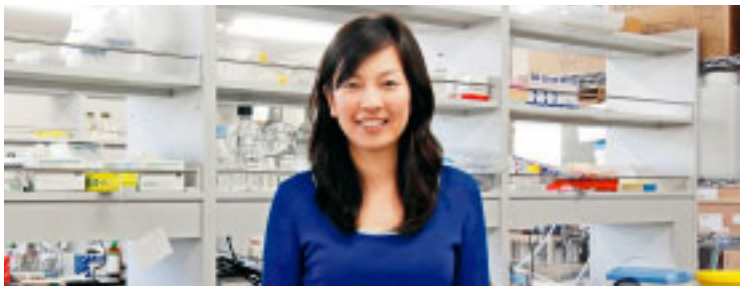
Neocortical Development

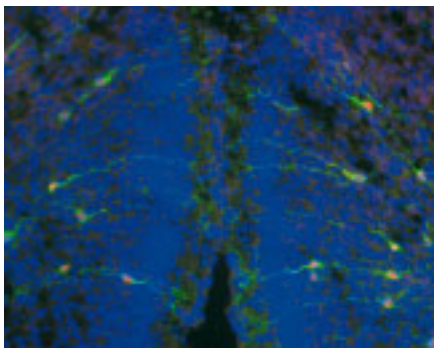
Carina HANASHIMA Ph. D.

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 pre-determined within the progenitor cells. We have found that specification of deep-layer projection neurons is dependent on transcriptional repressor that function cell-autonomously to prevent neurons from acquiring an earlier neuronal phenotype. These results imply cortical intrinsic programs 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 anterior-posterior (AP) and medial-lateral (ML) axes. We are exploring the extent to which intrinsic determinants control the specification of neuronal subtypes within discrete regions of the neocortex, as well as the extrinsic influences that refine the boundaries between functional areas of neocortex. To further these studies, we employ genetic manipulations in mice that will enable conditional loss of gene and cellular functions, recombination mediated cell-lineage tracing, and systematic approaches to identify novel molecules responsible for precise areal specification. Through these studies we wish to understand the mechanistic basis by which unique sensory perceptions and functional circuitries develop in the human neocortex.



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

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Morphogenetic Signaling

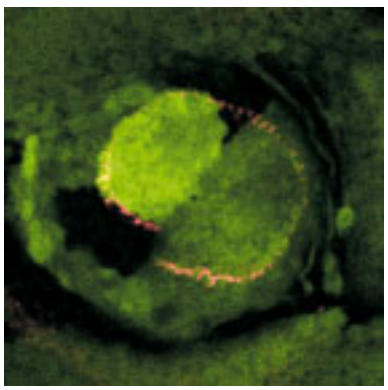
Shigeo HAYASHI Ph. D.

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.

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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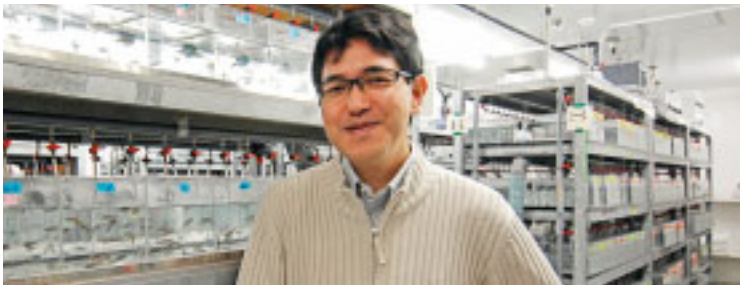
Vertebrate Axis Formation

Masahiko HIBI M.D., Ph. D.

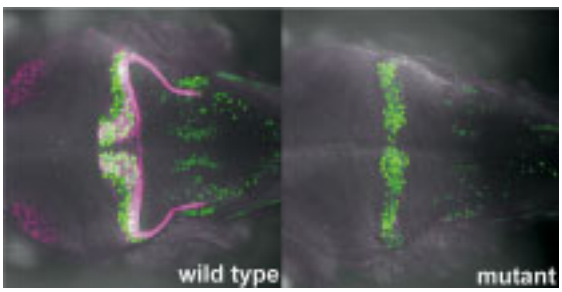
Masahiko Hibi received his M.D. from Hiroshima University School of Medicine in 1988, and his Ph. D. from the Osaka University Institute for Molecular and Cellular Biology in 1992. From 1992 to 1995, he worked as a postdoctoral fellow in Michael Karin's lab in the University of California, San Diego 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 also a question of interest to our team. Neuronal tissues are generated in a stepwise manner in vertebrates; these steps include neural induction, AP patterning and neurogenesis. In amphibian and teleost embryos, neuroectoderm is initially induced by the dorsal organizer proteins. The induced neuroectoderm is anterior in character and is subsequently subjected to posteriorization and regionalization. Accordingly, the central nervous system becomes highly ordered along the AP axis – 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 *cdx1α* and *cdx4* are expressed in the posterior neural tissue and control the formation of posterior spinal cord by repressing anterior fate. We are also extensively studying the mechanisms that establish the complex structure of the cerebellum.



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

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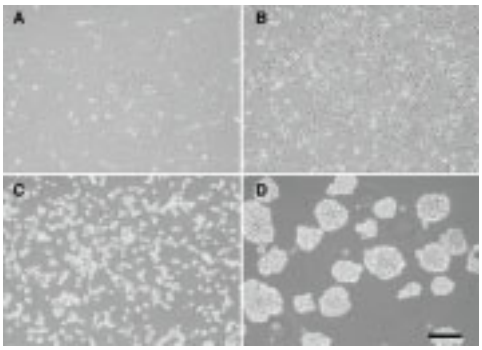
Cell Lineage Modulation

Toru KONDO Ph. D.

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

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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Evolutionary Morphology

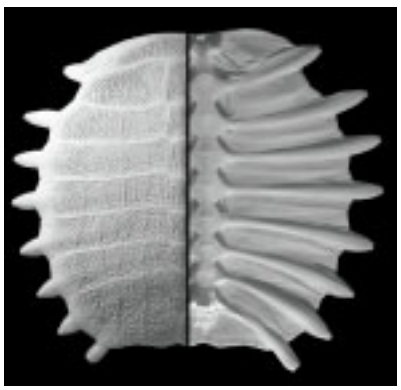
Shigeru KURATANI Ph. D.

Shigeru Kuratani received his masters and Ph. D. from the Kyoto University Department of Zoology. He spent the period from 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 species, I hope to gain a deeper insight into the secrets behind the fabrication of the vertebrate body. 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. 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 by gnathostomes. We aim to resolve the question of what primary factors that 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 chicken and turtle, 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.

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Sensory Development

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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Cold proof: Cadherin-8 in neural circuitry and cold sensation

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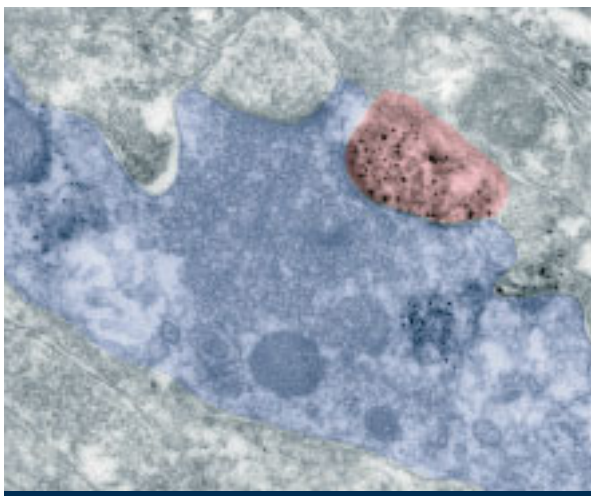
Suzuki S C, et al. Cadherin-8 is required for the first relay synapses to receive functional inputs from primary sensory afferents for cold sensation. *J Neurosci* 27. 3466-76 (2007)

The classic cadherin family of membrane proteins is best known as essential for its mediation of the cell-cell adhesion that enables multicellular organization, which is primarily achieved through homophilic binding between two identical extracellular domains. The role of these cadherins in the maze of neuronal components that comprises neural circuitry, however, is less clear.

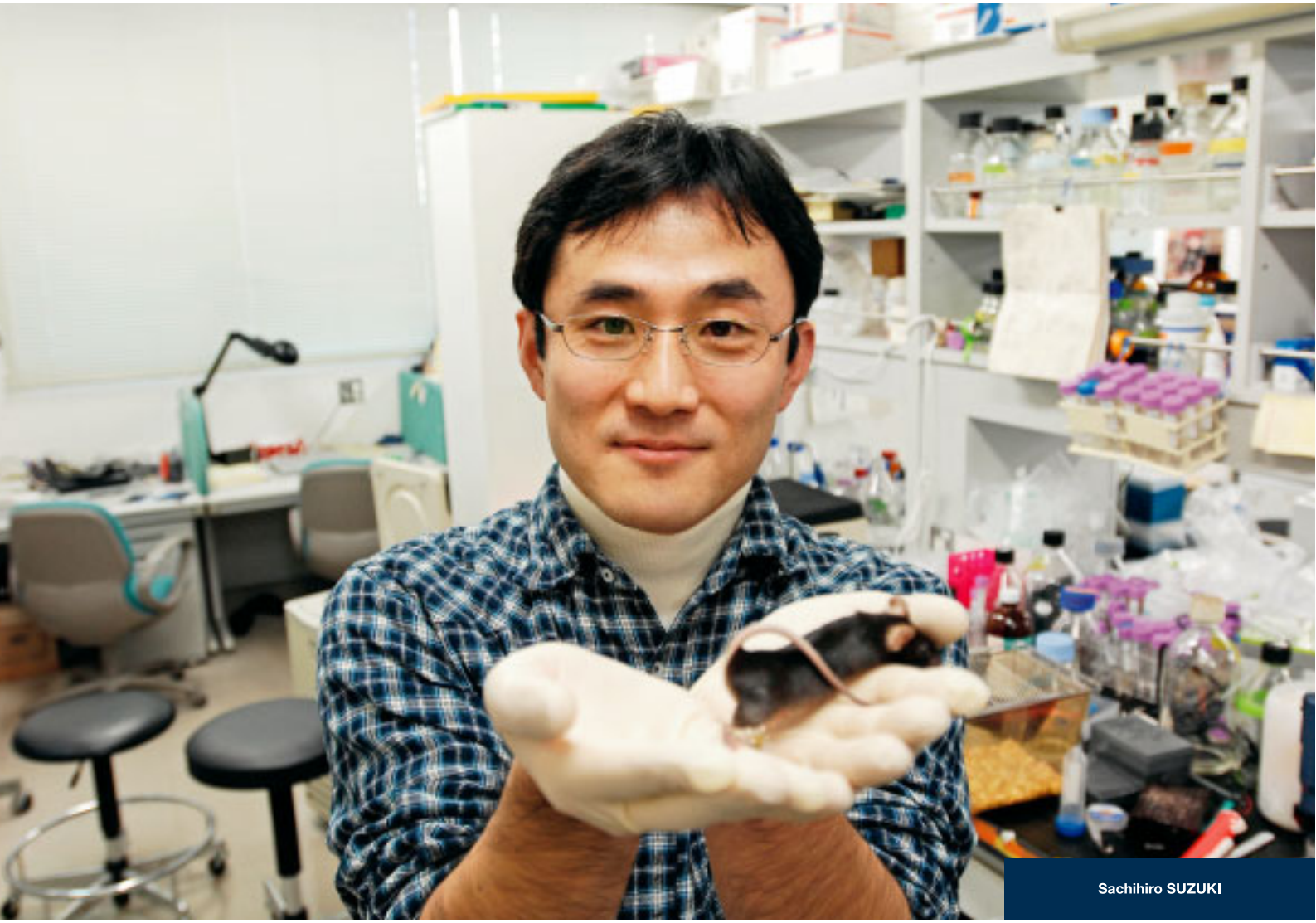
In an article published in *The Journal of Neuroscience*, Sachihiro Suzuki of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) and his collaborators helped shed light into that fog, demonstrating that one of the classic cadherins, cadherin-8 (cad8), is essential for the synaptic transmission of cold sensation, as evidenced by the finding that *cad8* knockout mice (*cad8*$^{-/-}$) are insensitive to cold stimuli.

Each classic cadherin subtype is differentially expressed by functionally connected neurons, a property that Suzuki had been studying for some time. In his efforts to develop a better understanding of the potential roles of different subtypes in the nervous system, he generated *cadherin-8* (*cad8*) knockout mice. "We noticed that knocking out this gene in mice led to the animals exhibiting a tail-raising behavior – a phenotype similar to that observed on morphine administration," says Suzuki. As both the μ-opioid receptor and cad8 are heavily expressed in the dorsal spinal cord, which is important for such somatic sensations as temperature, pain and touch, the group set out to examine cad8's exact role by focusing on neural circuitry in that region of the mouse nervous system.

Suzuki first identified the types of cells that express cad8 to be sensory neurons in the DRG, and examined the relationship between cad8-expressing sensory neurons and cad8-expressing DH neurons. The group found a significant overlap between cad8 sensory neurons and those expressing TRPM8, a cold and menthol receptor in DRG: most of the cad8-expressing neurons also expressed TRPM8, and vice versa. Electron microscopy showed that synapses were formed between sensory neurons and DH neurons expressing cad8. Next they examined effects of *cad8* genetic ablation on these connections and found that, even in the absence of cad8, synapses still formed. The group also performed electrophysiological analyses to examine the effects of loss of cad8 on functions of the synapses. Although cad8-expressing DH neurons received inputs from menthol-activated fibers (that is TRPM8-expressing sensory neurons in the *cad8*$^{+/-}$ population), originally cad8-expressing DH neurons in *cad8*$^{-/-}$ did not receive functional input from TRPM8-expressing sensory neurons. Based on these results, the authors concluded that cad8 is not essential for formation of synapses between cad8-expressing sensory and DH neurons, but is somehow involved in the physiological function of the synapses between them.



Immunoelectron microscopic image of a synapse between an axon of a cad8-expressing sensory neuron (blue) and a dendrite of a cad8-expressing neuron in the dorsal part of the spinal cord (red)



Sachihiro SUZUKI

Suzuki et al. further observed that a single cad8-expressing sensory neuron expresses nearly 10 classic cadherins, and that β-catenin and αN-catenin, which are binding partners of classic cadherins, were still localized at the originally cad8-localized synapses in cad8$^{-/-}$. They speculated that cell-cell adhesion at the synapses between TRPM8-expressing sensory neurons and their target DH neurons were linked by cadherins other than cad8 in cad8$^{-/-}$. Despite the presence of other cadherin subtypes in the synapse, however, cad8-deficient synapses were functionally abnormal. This suggests that this particular cadherin has a unique physiological function that cannot be replaced by other members of the classic cadherins, indicating that these molecular family members cannot be regarded as functionally identical.

Suzuki notes that, “cad8 may facilitate synaptic vesicle release at the axon terminals of TRPM8-expressing sensory neurons. In the future, we hope to clarify how cad8 regulates activities of the synapses between TRPM8-expressing neurons and their target neurons in the spinal cord.”

Retina redux: Wnt signaling promotes regeneration in mammalian retina

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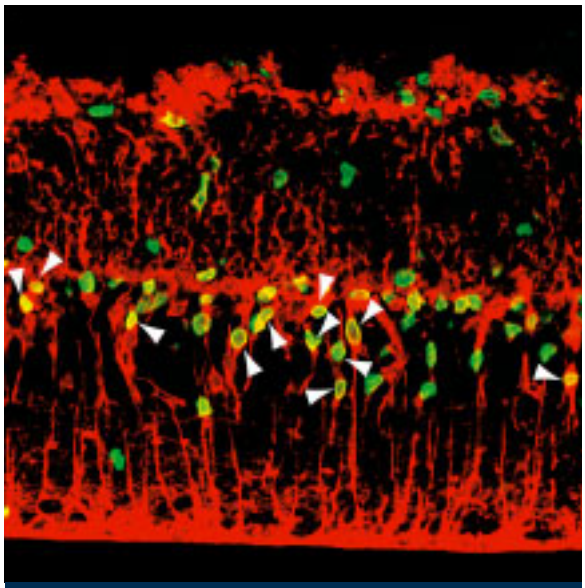
Osakada F, et al. Wnt signaling promotes regeneration in the retina of adult mammals. *J Neurosci* 27. 4210-9 (2007)

That the neurons of the adult mammalian nervous system do not regenerate was an article of conventional wisdom only recently overturned, by studies showing physiological turnover in the hippocampus, as well as post-injury regeneration in the retinas of mice. This retinal regeneration in a mammal is of especial interest, as degeneration of or trauma to retinal neurons are among the commonest causes of vision loss in humans, and the anatomical position of the eye makes it the most readily accessible region of the central nervous system. Previous studies of retinal regeneration, which occurs when support cells known as Müller glia de-differentiate to assume a neuronal fate, represent an important proof of principle, but have also revealed that although regeneration does take place, it does so in only a very few cells.

Fumitaka Osakada, a research scientist in the Laboratory for Retinal Regeneration (Masayo Takahashi; Team Leader), reported a method for spurring the regeneration of damaged retina cells by treatment with a Wnt signaling factor. In an article published in *The Journal of Neuroscience*, Osakada and colleagues from the RIKEN CDB and Kyoto University described how the secreted protein Wnt3a increases the proliferation of de-differentiated Müller glia twenty-fold in an in vitro model of retinal damage. Importantly, this Wnt3a-mediated regeneration also occurs in a mouse model of retinal degeneration, pointing to a possible new avenue for therapeutic enhancement of the body's limited natural ability to replace damaged retinal neurons.



Treatment of damaged retina with Wnt3a induces proliferation of Müller glia-derived progenitor cells (shown by arrowheads)

"It's still in very early days," admits Osakada, "but as my background is in pharmacology, it's interesting to think that it may be possible one day to develop an injectable small molecule of similar function that would boost this in-built regenerative mechanism, rather than relying solely on the more frequently proposed transplantation of cells grown in culture."

The study began as a follow-up on previous findings from the Takahashi lab, which showed for the first time that non-neuronal glial cells from a mammalian (rat) retina are able to backtrack to a less mature stage on their differentiation pathway following retinal damage. This regenerative capacity was extremely limited, however, and so the hunt was on to identify factors able to improve it. After testing of various candidates, Osakada et al. decided to look at the effect of Wnt signaling on retinal neuron regeneration. Wnt factors are prevalent in aspects of development ranging from limb development to axon guidance, and are known to control the self-renewal of certain forms of stem cells as well, but their study of Wnt function in the adult central nervous system has been hampered by the fact that many mutations affecting this signaling pathway result in embryonic lethality.

By developing a system for administering Wnt directly into an *in vitro* model of retinal damage, detecting the number of glial cells that re-entered the cell cycle and tracing their progeny, the Takahashi lab was able to show that Wnt3a treatment significantly increased the level of proliferation of neuronal progenitors from the de-differentiated cells. Tracking the fates of these Müller glia-derived progenitor cells, Osakada et al. determined that these cells migrated into the outer nuclear layer of the retina, where they differentiated into rod photoreceptors, capable of detecting light.

Fumitaka OSAKADA

They further verified the involvement of the canonical Wnt pathway, in which Wnt activation protects cytoplasmic β-catenin from degradation, enabling its accumulation in the nucleus and function as a regulator of gene transcription. In animals in which retinal injury had been induced, they found that trauma caused an increase in the nuclear accumulation of β-catenin. Blockage of Wnt signaling by the inhibitor Dkk1 led to a decrease in the number of post-injury retinal progenitors, and the regenerative effect of Wnt3a treatment could be mimicked by the administration of a GSK-3b inhibitor (GSK-3b is normally inhibited by Wnt activation).

Retinal degeneration, such as occurs in retinitis pigmentosa, is a deteriorative condition in which the rod and cone cells of the eye gradually die, leading to vision impairment and eventual blindness. The lab was understandably interested in finding out whether the Wnt-induced regeneration they had observed in an *in vitro* retinal injury model would be seen in a mouse genetic model of degeneration as well. Using retinal explants from *rd* mutant mice, which exhibit dramatic retinal degeneration from a very early age, they found that, as in the injury model, Wnt3a boosted cell proliferation and that the resultant progenitors could then be steered to differentiate into photoreceptor cells. Osakada notes, “While this work was done in vitro, the eye itself is an attractive target for this type of therapeutic concept, as it is relatively isolated from the rest of the nervous system and you can control the dose and area of delivery.”

This report represents an important step forward in the understanding of the molecular mechanisms that underlie the innate ability of the mammalian retina to respond to damage, and points to one possible approach into the pharmacological remedy of retinal degeneration. “Much remains to be done before we can start thinking about actual therapeutic applications,” cautions Masayo Takahashi, who heads the lab where the study was done. “These findings still need to be confirmed using human cells and, as a protein, Wnt is too unstable to be used as a treatment, but we are nonetheless very excited to have been able to show for the first time that it is possible to promote the regeneration of photoreceptive neurons in a mammalian model of retinal damage.”

Wnt Amp: XTsh3 enhances Wnt signaling in *Xenopus* axis determination

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Onai T, et al. XTsh3 is an essential enhancing factor of canonical Wnt signaling in *Xenopus* axial determination. *Embo J* 26. 2350-60 (2007)

The formation of the dorsal-ventral axis is one of the most important developmental milestones in early embryogenesis. The establishment of this axis distinguishing the back from the belly side is known to rely on the unequal distribution and subsequently the asymmetric action of the canonical Wnt pathway, which is known to be triggered as early as sperm entry. But the initial differences in Wnt intensity along the DV axis, at only about two-fold, appear to be too slight to account for the dramatic polarization of the embryo that they induce, suggesting that other factors may act to boost the Wnt signal.

An important piece was fitted into this puzzle in a study by Takayuki Onai, Mami Matsuo-Takasaki and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) that shows how the factor XTsh3 functions as a molecular adjuvant, amplifying the effects of canonical Wnt signaling in DV axis determination in the embryo of the African clawed frog, *Xenopus laevis*, by increasing the nuclear accumulation of the transcriptional activator, β-catenin.

The XTsh3 protein belongs to a family of zinc-finger gene products whose founding member, Tea-shirt (Tsh), was first identified in the fruit fly *Drosophila* as a homeotic selector specifying thoracic segment identity, and was subsequently shown to bind to Armadillo, the fly homolog of the Wnt downstream factor β-catenin. While the frog genome includes at least three members of the Tsh family, their patterns of expression vary, and it was the distinct strong expression of one of these, *XTsh3*, in the dorsal aspect of the early embryo that caught the Sasai group's attention and prompted their search for a possible role in DV axis determination. In situ hybridization showed XTsh3 transcripts throughout the animal region of cleavage-stage embryos, and that by early gastrulation, its expression was concentrated strongly in the dorsal ectoderm and marginal zone even as it gradually faded in the ventral half of the embryo.

Studying the effect of its loss of function, Onai and Matsuo-Takasaki next interfered with the dorsally-expressed XTsh3 using antisense morpholinos against its mRNA sequence, and found that this resulted in the ventralization of normally dorsal tissues. Looking at the genetic level as well, they found that signature dorsal genes such as *Chordin* and *Goosecoid* were downregulated in the morphant, while the expression of ventral markers encroached into dorsal territory, suggesting that XTsh3 plays an important part in setting up the DV axis by supporting dorsal specification.

The mechanism by which it does this, however, remained unclear. Past work in *Drosophila* had showed possible involvement of Tsh in canonical Wnt signaling through its binding with β-catenin, prompting the group to explore the possibility of a similar scenario in the very different context of amphibian development. They discovered that the activity of Wnt-mediated genes was upregulated on the forced expression of XTsh3, and that this effect was reliant on the β-catenin step of the Wnt pathway, indicating that the two might work together as co-factors.

Injection of antisense morpholinos against XTsh3 interfere with normal axis formation (right)

On entering the nucleus, β-catenin binds with factors such as Tcf and activates the transcription of target genes. Interestingly, however, the β-catenin protein does not exhibit any of the typical localization sequences that serve as entry passes into the nucleus, which led Onai and Matsuo-Takasaki to ask whether this might be XTsh3's role. Using an animal-cap assay as an in vitro model of the early embryo, they determined that XTsh3 accumulates predominantly in the nucleus, and that this preferential localization was unaffected by either overexpressing or knocking down Wnt pathway proteins. Significantly, the converse was not true – injection of XTsh3 dramatically increased the accumulation of β-catenin in the nucleus, while loss of XTsh3 function had the opposite effect.

This set of findings, reported in *The EMBO Journal*, tells the story of how dorsally expressed XTsh3 enhances canonical Wnt signaling by promoting the entry of β-catenin into the nucleus, thus playing a decisive role in the determination of the DV axis. The work by Onai and Matsuo-Takasaki et al is also the first report of a Tsh-family protein's function in a vertebrate model, adding a new dimension to a body of knowledge first developed using *Drosophila* genetics. "We still don't know how XTsh3 is causing β-catenin to collect in the nucleus," admits Sasai, "It could be protecting it from degradation, or promoting its nuclear transport. Its expression pattern also suggests it may be working in the caudal specification of the central nervous system, so we'll be very interested to find out if its enhancement of Wnt signaling is involved in other developmental processes as well."

Takayuki ONAI

Key to quiescence?

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Fujita M, et al. Cyclin E and CDK2 repress the terminal differentiation of quiescent cells after asymmetric division in *C. elegans*. *PLoS ONE* 2. e407 (2007)

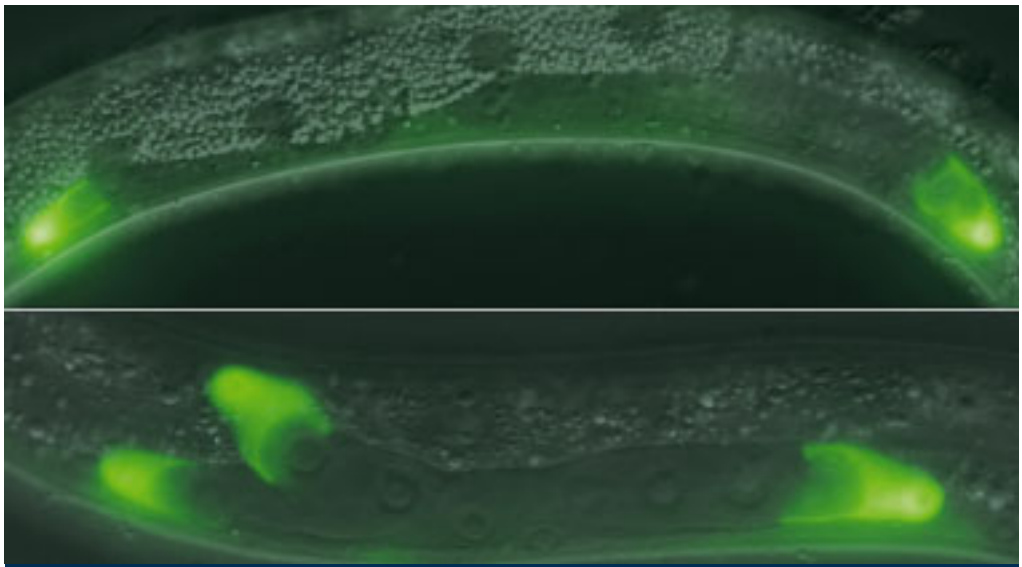
In many biological contexts, proliferation and differentiation stand at odds, as dividing cells tend to be undifferentiated, and terminally differentiated cells tend not to divide. Cell cycle regulators frequently play a part in governing this diametric relationship, but little is known of what roles such factors might play in maintaining the uncommitted state of quiescent (non-proliferating) cells.

This question was addressed in a study by Masaki Fujita and others in the Laboratory for Cell Fate Decision (Hitoshi Sawa; Team Leader), which looked at how a tightly maintained balance of the activity of cell cycle regulators keeps such cells from differentiating in the roundworm, *C. elegans*. In an article published in the open access journal *PLoS One*, the team described how the interplay between the worm homologs of a triad of cell cycle regulators – cyclin E, CDK2 and CKI – simultaneously prevents differentiation and division in uncommitted, quiescent cells.

The *C. elegans* gonad develops from a pair of precursor cells, called Z1 and Z4, which undergo two rounds of division to yield four progeny, the most distal of which (Z1.aa and Z4.pp) differentiate into the distal tip cells, or DTCs, that lead the developing gonad as it follows its U-shaped migratory path through the larval body. Fujita found that worms lacking the gene *cye-1* (the *C. elegans* homolog of *cyclin E*) frequently developed extra DTCs, suggesting a role for this gene in regulating differentiation. By laser-ablating daughter and granddaughter cells in the Z1 and Z4 lineages, individually and in various combinations, the team determined that the supernumerary DTCs in the *cye-1* mutants are generated from the sisters of the normal DTCs (Z1.ap and Z4.pa).

Cyclin E is known to function cooperatively with a second cell-cycle protein, CDK2, in other organisms, so Fujita et al. next examined the effects of its loss of function by inhibiting the roundworm gene using RNAi. As with the *cye-1* mutants, the *cdk-2* knockdown worms showed the extra DTC phenotype, in a manner suggesting that the two factors act in partnership.

Cell-cycle regulators such as cyclins and cyclin-dependent kinases are typically kept in check by factors known as CKIs. On analyzing the patterns of expression of *cye-1* and the roundworm CKI, *cki-1*, in worms in which the protein products of these genes had been engineered to fluoresce green, the Sawa team found that they were expressed asymmetrically, with *cye-1* levels higher in the Z1.ap and Z4.pa cells (which are normally quiescent) and *cki-1* expression stronger in the Z1.aa and Z4.pp



Expression of *lag-2::GFP* (a marker for DTC) in wildtype (upper panel) and a *cye-1* mutant (lower panel). The *cye-1* mutant exhibits supernumerary DTC.

cells that normally differentiate into DTCs. Using a temperature-sensitive mutation of *wrm-1* (roundworm *β-catenin*) to allow them to initiate its loss of activity after the division of the Z1/Z4 cells, they found that this unequal distribution is under the control of the Wnt/MAPK pathway, as is the case in a number of other asymmetric cell divisions in *C. elegans*.

Given the higher levels of *cki-1* in the quiescent sisters of the DTCs, the lab next looked at a possible interaction between this gene and the partnership of *cye-1* and *cdk2*. In worms in which *cki-1* alone had been knocked down, the majority of animals showed extra cell divisions, but when this interference was combined with either *cye-1* mutation or *cdk-2* RNAi, no such phenotype occurred, suggesting that *cki-1* inhibits the proliferation of the Z1.ap and Z4.pa cells, preventing *cye-1/cdk-2* from triggering cell division but leaving their activity high enough to prevent terminal differentiation.

A similar mechanism appears to be at work in at least one other cell lineage exhibiting asymmetric cell divisions, as usually quiescent seam cells in *cye-1* mutant worms adopted an abnormal syncytial fate, indicating that *cye-1* is needed to repress terminal differentiation in multiple quiescent cell types. “As many stem cells are quiescent, the cell cycle regulators we identified as functioning in this context may also serve to maintain the undifferentiated state of stem cells in other organisms,” notes Sawa, highlighting the potential importance of this new role for cyclins, CDKs and their inhibitors in maintaining quiescence.

Masaki FUJITA

Role of Sox2 in the maintenance of pluripotency pinpointed

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Masui S, et al. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat Cell Biol* 9. 625-35 (2007)

Embryonic stem (ES) cells are governed by a small complement of genes that regulate their own and each other's activity. This network of regulatory feedback loops has been believed to rely on the synergistic effect of the factors Oct3/4 and Sox2 on a group of transcriptional activators, which then regulate the expression of genes required for maintaining pluripotency, including both *Sox2* and *Oct3/4*. Under this scheme, both factors were supposed to be necessary to maintaining ES cells via these Oct-Sox enhancers; Oct3/4 is well known as a warden of pluripotency, and ES cells in which *Sox2* function is blocked undergo differentiation, pointing to an essential role for this gene as well.

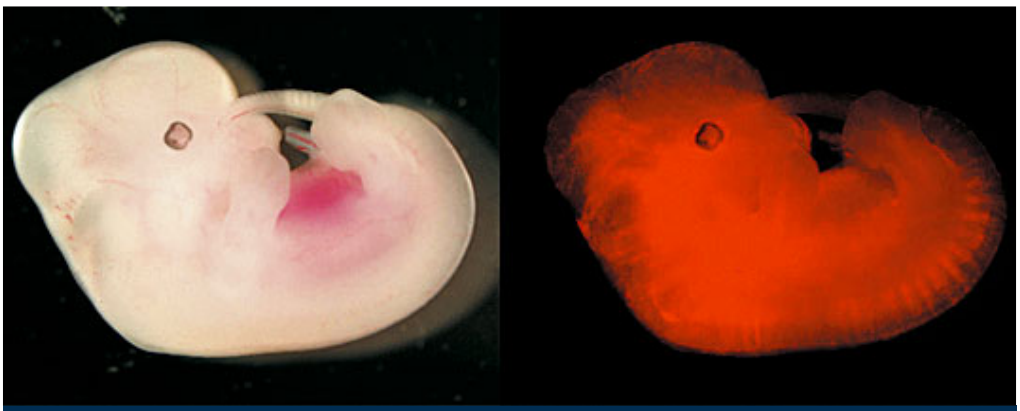
Shinji Masui, working at the Laboratory for Pluripotent Cell Studies (Hitoshi Niwa; Team Leader), showed that *Sox2* is in fact not an absolute requirement for the activation of the so-called Oct-Sox enhancers. Masui, who now heads a lab at the International Medical Center of Japan, determined that *Sox2* is instead indispensable in maintaining the stable expression of Oct3/4 itself, a finding with implications for our understanding of the control of the undifferentiated state that is a hallmark of both embryonic stem cells in culture and transient embryonic cellular populations, such as the blastocyst's inner cell mass.

"A number of recent studies have greatly improved our understanding of the transcriptional network that maintains pluripotency and as a result the model had been getting simpler and simpler," says Masui. "But one of the most important pieces, *Sox2*, still hadn't been fit into place. Now that we have worked out its precise function, for better or worse, the model has gotten a bit more complicated again."

Previous studies had shown that the silencing of *Sox2* by RNA interference in ES cells resulted in their differentiation into multiple lineages and that most pluripotency associated genes contain Oct and Sox binding motifs, which pointed strongly, but not conclusively, to the possibility that these two transcription factors were regulated by a positive feedback loop.

After confirming that *Sox2*-deficient cells do indeed differentiate (into trophectoderm-like cells; an effect similar to that caused by loss of *Oct3/4* function), they looked at enhancer activity by comparing cells transfected with Oct-Sox enhancer constructs carrying mutations in their Sox binding sites. Unexpectedly, there was little effect on enhancer activity, opening up the possibility that *Sox2* might function redundantly in cooperation with other Sox factors, meaning that its loss could be compensated for by the activity of one or more related genes.

This, however, left the team with the puzzle of why *Sox2* null mutant ES cells would fail to maintain their pluripotency and differentiate into trophectoderm. One interesting observation was the number of genes whose expression changed over time in response *Sox2* loss-of-function, particularly the upregulation of genes known either to downregulate Oct3/4 or to cause ES cells to undergo



Chimera mouse generated using *Sox2* KO-Oct3/4-rescued ES cells

Shinji MASUI

differentiation. This pattern suggested that rather than influencing the activity of the Oct-Sox enhancers, Sox2 might instead be promoting the expression of positive regulators Oct3/4, while repressing its inhibitors and inducers of differentiation.

Masui devised a strategy for studying this question by introducing an *Oct3/4* transgene into ES cells with a conditional *Sox2* deletion. On inhibiting *Sox2*, levels of endogenous Oct3/4 dropped precipitously, but the transgene remained unaffected and, tellingly, the ES cells remained pluripotent, as evidenced by their ability to contribute to all three germ layers in chimeric embryos.

This work was published in *Nature Cell Biology*, and stands as a significant revision of the conventional wisdom regarding the Oct-Sox interaction, making a strong case for the unique function of *Sox2* in the maintenance of pluripotency via its regulation of the regulators of Oct3/4.

“The molecular events governing pluripotency remain mysterious, even though the factors to establish pluripotency have been identified and in silico analyses have been used to attempt to develop a simplified model for the transcription factor network,” says Niwa, “but I think the only way to solve this puzzle will be to keep chipping away at the molecular functions one by one.”

ROCK-blocked human ES cells thrive

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Watanabe K, et al. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat Biotechnol* 25. 681-6 (2007)

Human embryonic stem (hES) cells have captured the attention of the scientific and biomedical research communities for their ability to self-renew seemingly indefinitely in culture and to give rise to any type of differentiated cell in the body. Much of the excitement surrounding these cells is the potential of inducing their differentiation into pure populations of cells of a given type, which may then be transplanted into patients in what is known as cell replacement therapy. Much progress has already been made in the mass culture and induced differentiation of mouse ES cells, but research using human ES cells has been problematized by their tendency to undergo cell death in vitro, making it difficult to generate and sustain colonies of the sizes that would be needed for therapeutic applications. The causes of the precarious hold on life that hES cells exhibit, however, have remained unclear.

In a report published in the journal *Nature Biotechnology*, Kiichi Watanabe and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) revealed that this cell death can be blocked simply by pretreatment with a reagent, known as a ROCK inhibitor. Watanabe et al. showed for the first time, a marked reduction in hES cell death by administration of the ROCK inhibitor Y-27632, opening up new avenues for the culture, study and manipulation of hES cells in clinically relevant quantities. This breakthrough by the Sasai lab (in collaboration with the National Center for Geriatrics and Gerontology) is of particular importance in that it enables the growth and differentiation of hES cells under stressful conditions such as dissociation and suspension, which is necessary to take advantage of recent advances in mouse ES cell biology, such as the induction of telencephalic (forebrain) precursors.

The Sasai group has developed a number of techniques for inducing the differentiation of mouse ES cells into specific neuronal fates to date, some of which, such as the generation of dopaminergic neurons (which are deficient in Parkinson's disease) have been reproduced using human ES cells as well. But the susceptibility of hES cells to cell death when subjected to stressful culture conditions has remained a major obstacle to fulfilling their clinical promise. These cells are especially sensitive to dissociation, in which digestive enzymes are used to separate individual cells in order to expand a single population into multiple colonies. While this is standard protocol for mouse ES cells, the dissociation of human ES cells causes 99% attrition due to cell death within two days, which has meant that hES researchers have been forced to resort to other, more finicky methods.

By developing a means of preventing such massive die-offs, Watanabe, now at the California Institute of Technology, has put mouse ES cell culture techniques within reach of the human embryonic stem cell research community. The study began with indications that an atypical form of cell death observed in specific cell types, such as motoneurons, might be linked to the activity of ROCK (Rho-associated coiled coil kinase). On looking at its expression in dissociated hES cells, he found that ROCK became activated soon after trypsinization. On pretreatment with the ROCK inhibitor Y-27632, isolated hES cells showed a nearly thirty-fold increase in the efficiency at which they could be cloned into new colonies. This boost in survival and proliferation was seen in other culture methods as well, and had no detectable effect on the cells' pluripotency or self-renewal.

Figure 1

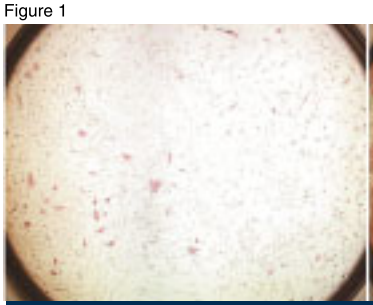


Figure 2

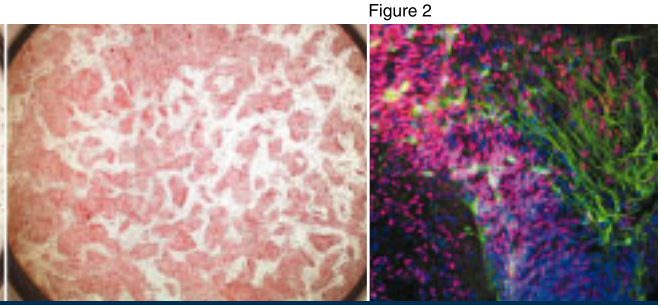
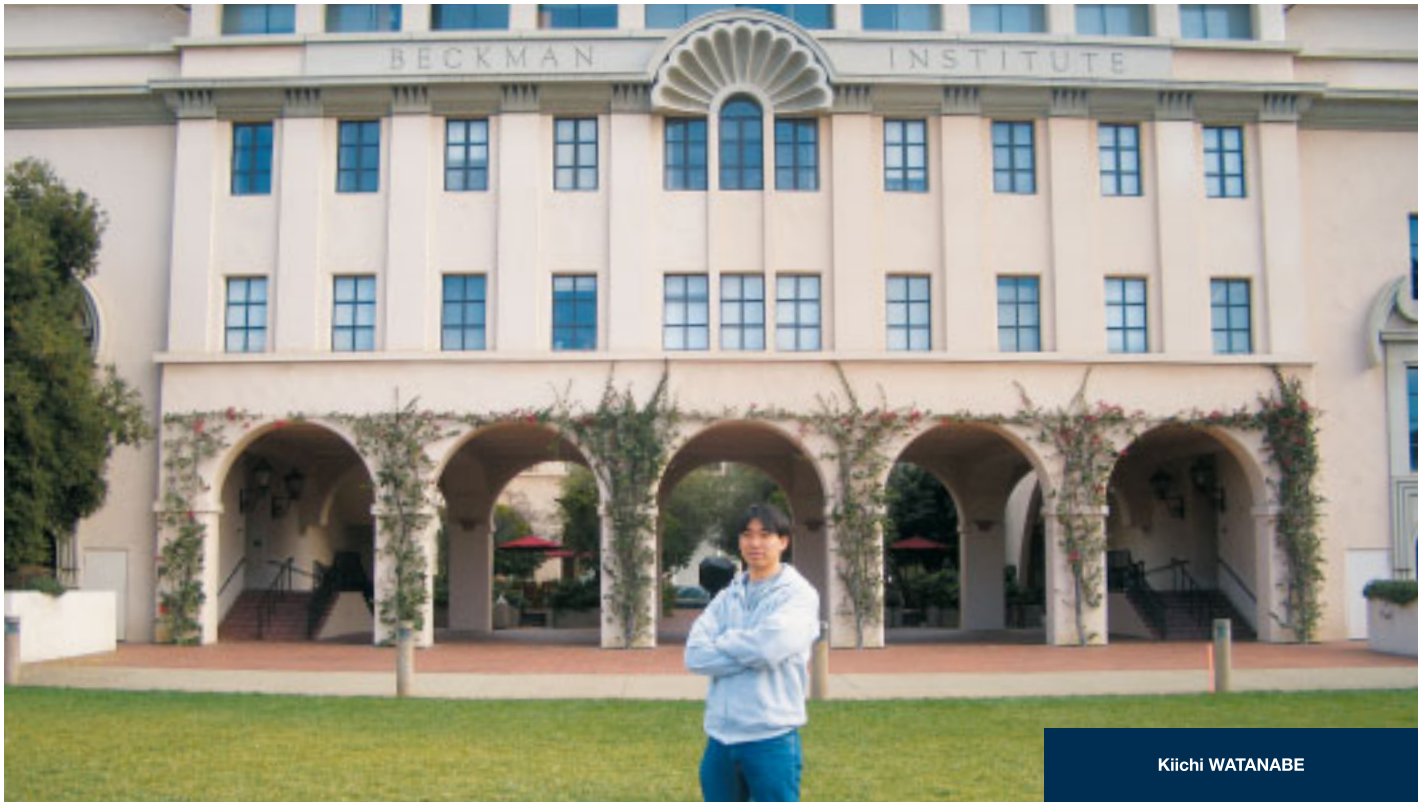


Figure 1
Dramatically increased survival of hES cells treated with ROCK inhibitor (right; control shown on left)

Figure 2
Telencephalic cells differentiated from ROCK-inhibited hES cells



Kiichi WATANABE

ROCK inhibition also considerably facilitated the selective cloning of hES cells after gene transfer. The introduction of exogenous genes into the genome of hES cells is a notoriously difficult procedure, with a success rate of less than one in a thousand tries, meaning that transgenesis experiments performed to date have required enormous quantities of cells. But the dramatic reduction of hES cell death by treatment with Y-27632 or other ROCK inhibitors now makes it possible to conduct such studies on a single 10-cm plate.

In an exciting proof-of-concept demonstration, Watanabe et al. further showed that ROCK inhibition can be used to enable the neural differentiation of hES cells. The Sasai group had previously developed a method known as SFEB (for Serum-free Floating culture of Embryoid Body-like aggregates) for inducing the differentiation of mouse ES cells into cells expressing genes characteristic of embryonic telencephalon, but as this required the dissociation and suspension of the cells, the technique was impracticable for use in human ES cells. But the improved robustness of ROCK-inhibited cells to such unfavorable culture conditions made it possible for even hES cells to proliferate in SFEB culture, and the group found that after 35 days of culture in the presence of inhibitors of neural antagonists a full third of the cells had taken on a telencephalic precursor or neuronal fate.

This relatively simple methodological refinement of ROCK inhibition is a major step towards the realization of the great potential of human embryonic stem cells, as it allows their large-scale culture at levels that had previously only been attainable with mouse ES cells, and enables or facilitates such fundamental lab techniques as gene transfer, induced differentiation, and the establishment of colonies from single hES cells.

“We look forward to sharing this method with collaborating labs and institutes around the country, in the hopes of accelerating the pace of biomedical research,” says Sasai. “And at the same time, we will be looking very closely at why it is only human ES cells that exhibit this peculiar form of cell death and the role that ROCK plays in that process.”

A new route to programmed cell death

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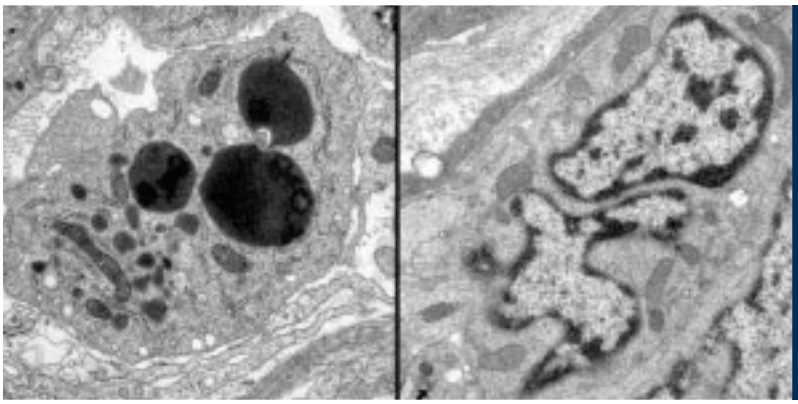
Uesaka T, et al. Conditional ablation of GFRα1 in postmigratory enteric neurons triggers unconventional neuronal death in the colon and causes a Hirschsprung's disease phenotype. *Development* 134. 2171-81 (2007)

Neurons rely on molecular cues, known as neurotrophic factors, for their survival, and the culling of cells deprived of such factors plays an important part in the development of most regions of the nervous system. The enteric nervous system (ENS) that innervates the gut, however, appears to be an exception, as it shows no signs of undergoing the form of physiological programmed cell death known as apoptosis. The ENS does depend on signals, nonetheless, as enteric neurons fail to develop in the distal intestine in mice deficient for either the neurotrophic factor GDNF, or either of its receptor components, RET and GFRα1. Unfortunately, the severity of the phenotype hampers the ability to study the mechanism by which RET/ GFRα1-mediated GDNF signaling enables ENS development, as the embryos of mice carrying loss-of-function mutations in any of these genes exhibit no neurons whatsoever in the small or large intestine.

Scientists interested in the function of GDNF and its receptor complex have tried various workarounds to overcome this obstacle, but none had conclusively demonstrated the physiological role of this signaling trio in the enteric nervous system. By an elaborate feat of genetic engineering, however, Toshihiro Uesaka and others in the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto; Team Leader) have conditionally abolished *GFRα1* late in ENS development, and made the unexpected finding that while these neurons do die en masse in the absence of GDNF signaling, they do so by a different process than canonical apoptosis. These findings were described in detail in an article published in the journal *Development*.



GFRα1 regulates enteric neuron survival in the distal bowel
Distinct morphological characteristics are observed in sympathetic neurons undergoing apoptosis (left) and degenerating enteric neurons in conditional GFRα1 KO colon (right).

"This discovery that an unconventional form of neuronal cell death is triggered by inactivation of GFRα1 in the distal colon is exciting for us," says Uesaka, "as it may provide valuable insights into the pathogenesis of Hirschsprung's disease, a disease in which ganglia fail to form in the distal colon in humans."

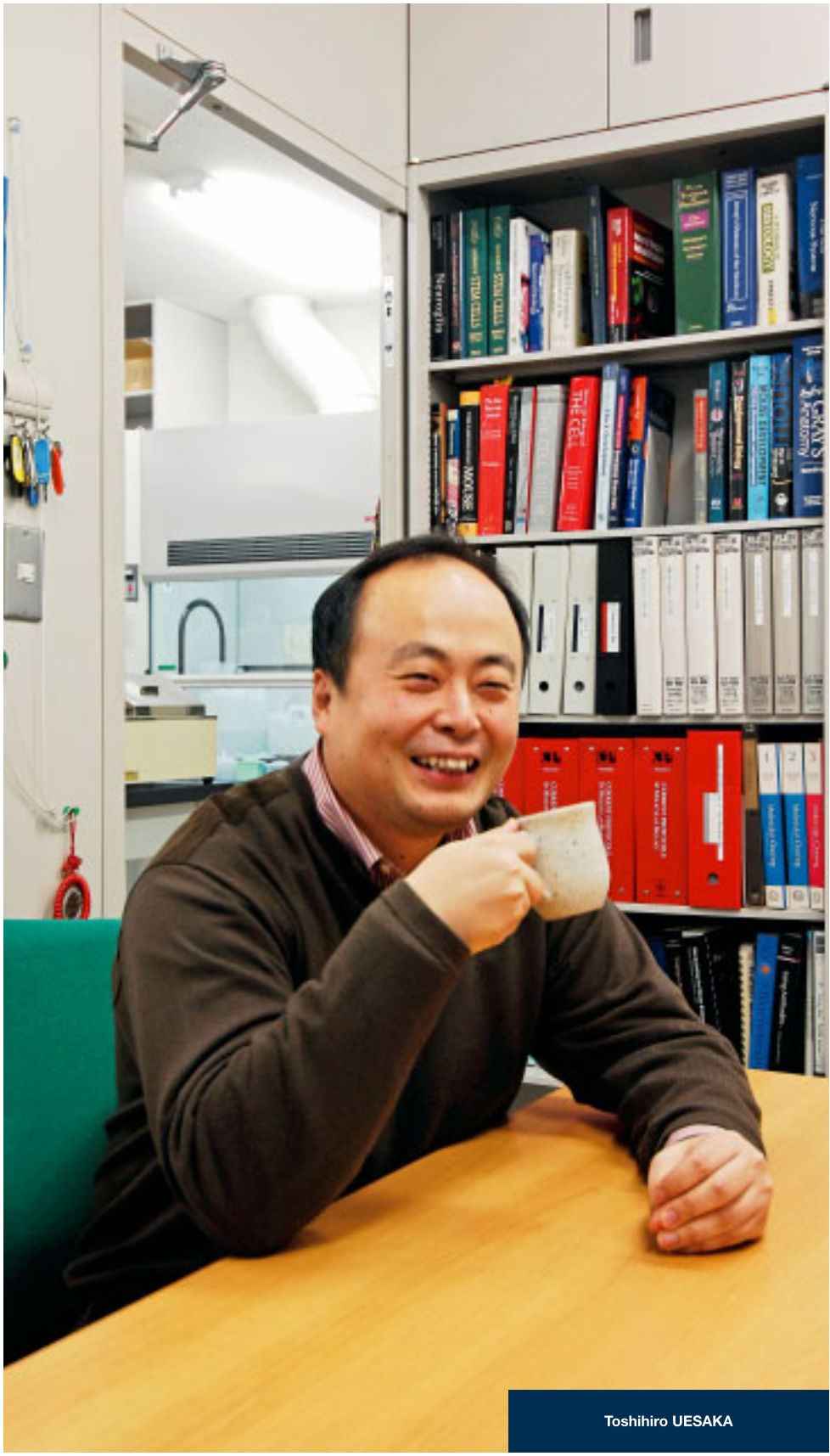
Early in ENS development, the GFRα1 receptor is expressed in the majority of enteric neuronal progenitors throughout the gut, but its distribution gradually shifts over time until it is predominantly expressed in the enteric neurons of the colon. This caught the team's attention, because GDNF is also detected at particularly high levels in the colon and caecum during later stages of embryogenesis. Uesaka was interested in studying the role of GFRα1, so to avoid the total loss of enteric neurons characteristic of the unconditional knockout of the gene, he painstakingly generated a line of mice in which the function of *GFRα1* could be excised contingently and cells carrying an inactivated allele could be traced by the expression of green fluorescent protein.

When he looked at embryos in which *GFRα1* was inactivated at day 15.5 (by which point neuronal *GFRα1* expression is mainly confined to the colon), he found that the normal pattern of lower gut innervation was completely disrupted, with a massive loss of enteric ganglia. Tests of the conditional knockout at other developmental time points showed that this effect was time-dependent; embryos in which

GFRα1 was excised earlier in development showed defects in enteric neuronal proliferation, but not survival.

Curiously, however, the conditional E15.5 knockouts showed none of the telltale signs of apoptosis; tests revealed no significant upregulation of typical cell death markers, such as activated caspase, nor the DNA fragmentation apoptotic cells undergo (as revealed by TUNEL assay). Following up on this startling observation, the Enomoto lab turned next to the proapoptotic factor, Bax, but found that Bax deficiency had little influence on enteric neuronal death induced by GDNF deprivation in culture. And, while a number of abnormal morphological features were seen in the nuclei of cells in colonic neurons of conditional *GFRα1* knockout embryos, they showed none of the nuclear hallmarks of apoptosis, such as globular or crescent-shaped chromatin condensation, nor did dying cells appear to be succumbing to autophagy, as many apoptotic cells do.

In addition to demonstrating that GDNF is a survival factor for enteric neurons, a role it plays in many other arenas of neurodevelopment, Uesaka's study points to an intriguing new form of physiological cell death, which may be important in other developmental contexts and neuronal pathologies as well. Enomoto notes of the study's medical implications, "We think that the possible links to the etiology of Hirschsprung's disease are especially interesting, as we know that RET, the signaling component of the GDNF receptor complex, is most frequently mutated in those patients. It will certainly be worth looking into whether and how this novel cell death mechanism might apply in humans."



Toshihiro UESAKA

Educational Programs

Although RIKEN is not an academic institution, the CDB works with students and educators to help encourage greater understanding of the fields of development, regeneration, stem cell biology and regenerative medicine, as well as to cultivate future generations of scientists and a scientifically literate public.

Summer school for high-school students

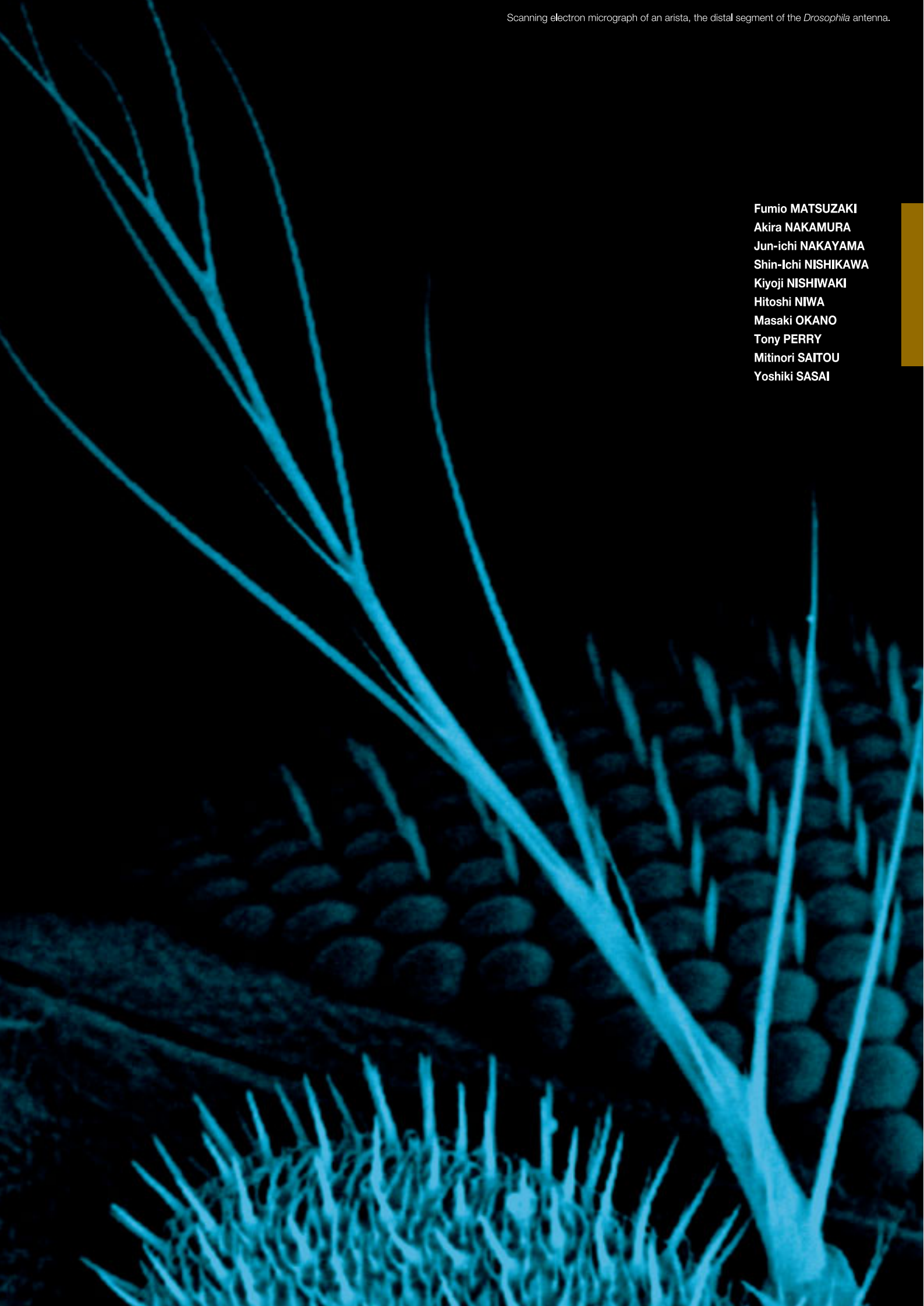
Continuing on from 2006's successful summer school for high school students, the RIKEN Center for Developmental Biology (CDB) once again hosted a number of students from local high schools on August 8 and 10. A total of 28 students in two groups enjoyed comprehensive one-day programs that offered insight into life and research at the CDB, and provided an opportunity for some hands-on laboratory experience. The program included visits to labs, scientific talks and a chance to perform immunofluoresence staining of cultured cells.

Intensive lecture program for grad school affiliates

The CDB maintains close relationships with a number of major graduate and medical schools in the Kansai region, and holds a two-day lecture program every year to introduce students to the Center's labs. The 2007 program saw 160 students visit on July 25 and 26, to participate in lectures by ten PIs, join in demonstrations of research techniques, and visit labs throughout the CDB for discussion of scientific interests and a peek at a day in the life as a research scientist.

Workshop for high-school teachers

In October, the CDB hosted its first-ever practical workshop for local high school teachers, inviting nearly thirty teachers from Kyoto Prefecture to participate in a one-day program on the fundamentals of developmental biology, and techniques for culturing and observing *C. elegans* roundworms in the classroom. Members of the Laboratory for Cell Fate Decision (Hitoshi Sawa; Team Leader) assisted with hands-on instruction, while Asako Sugimoto, head of the Laboratory for Developmental Genomics, gave a talk on basic nematode biology.



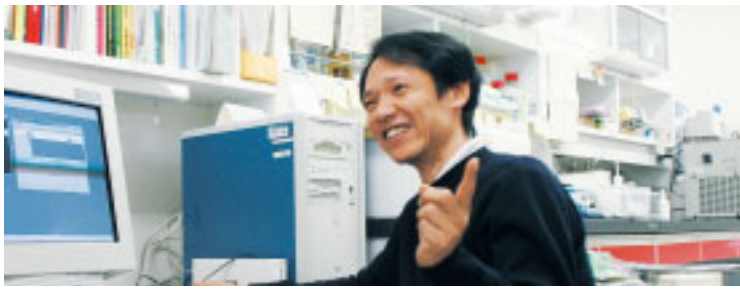
Scanning electron micrograph of an arista, the distal segment of the *Drosophila* antenna.

Fumio MATSUZAKI
Akira NAKAMURA
Jun-ichi NAKAYAMA
Shin-Ichi NISHIKAWA
Kiyoji NISHIWAKI
Hitoshi NIWA
Masaki OKANO
Tony PERRY
Mitinori SAITOU
Yoshiki SASAI

Cell Asymmetry

Fumio MATSUZAKI Ph. D.

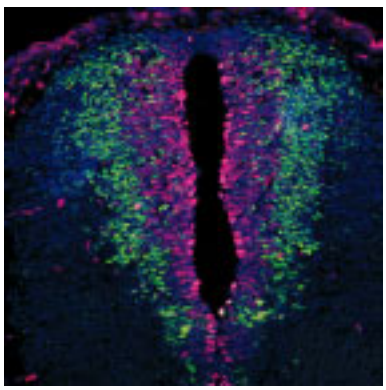
Fumio Matsuzaki received his B. Sc. from the Department of Biochemistry and Biophysics at the University of Tokyo in 1979, and his doctorate from the same institution in 1984, for his work on the characterization of the erythrocyte cytoskeletal structure. He spent the period from 1984 to 1988 as a postdoctoral 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, such as the mechanisms responsible for asymmetrically sorting cellular components to the cortex, maintaining the neuroblast's cell polarity, and producing the smaller daughter GMC. 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*. However, in both vertebrate and *Drosophila*, this huge number of neural cells is generated from a relatively small number of neural stem cells. Previous work has shown that neural progenitor cells divide both asymmetrically and symmetrically to produce descendant neurons. Vertebrate homologues have been found for most of the components acting in the asymmetric division of *Drosophila* neuroblasts, but the modes and roles of asymmetric divisions in vertebrate neurogenesis remain incompletely understood. Furthermore, still little is known about how asymmetric division contributes to neuronal fate determination. We are investigating the problems of how asymmetric division is involved in neuronal fate decisions and in organizing 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).

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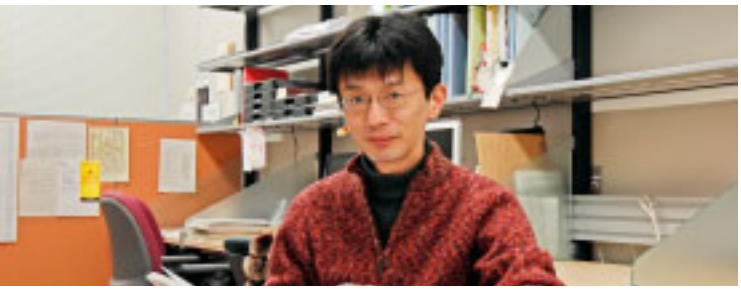
Germline Development



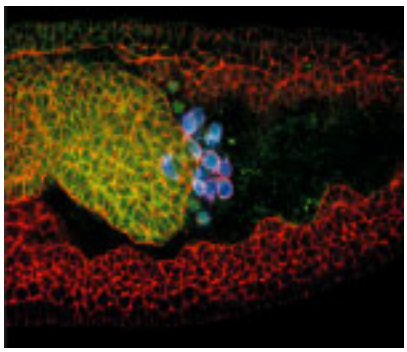
Akira NAKAMURA Ph. D.

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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Chromatin Dynamics

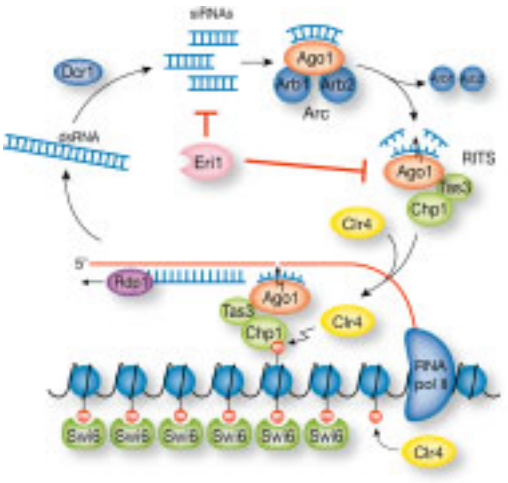
Jun-ichi NAKAYAMA Ph. D.

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. In the future, we plan to perform more detailed analyses of the molecular mechanisms that underlie epigenetic function, as well as studies in higher organisms and epigenetic gene expression in developmental processes.



Model for the RNAi-mediated heterochromatin assembly in fission yeast

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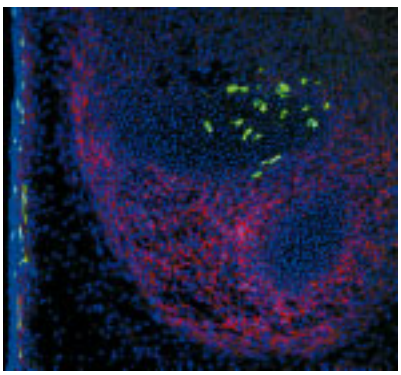
Stem Cell Biology

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 inducing hematopoietic stem cells, endothelial cells, and steering early stages of endoderm differentiation. In addition to these two central themes, other members of the laboratory are studying angiogenesis, the formation of blood vessels, as blood supply is an absolute requirement to the establishment and maintenance of any deep or extensive biological tissue. 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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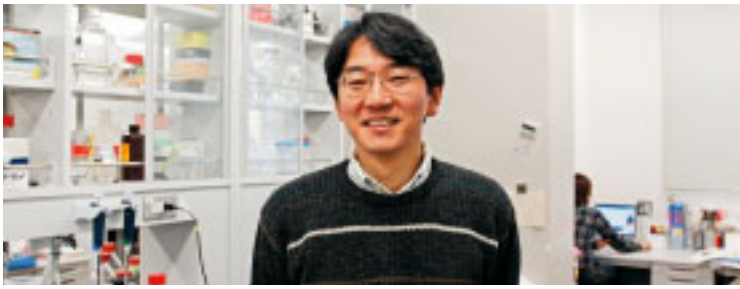
Cell Migration

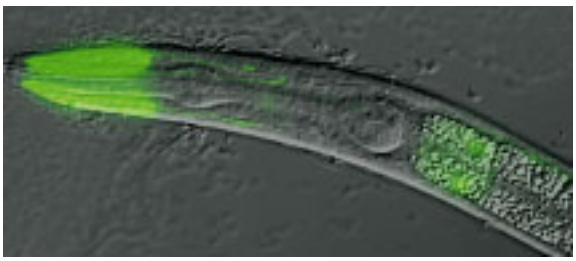
Kiyoji NISHIWAKI Ph. D.

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 species of tiny roundworm 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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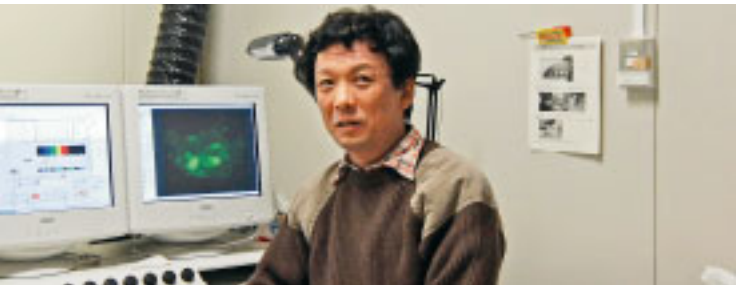
Pluripotent Cell Studies

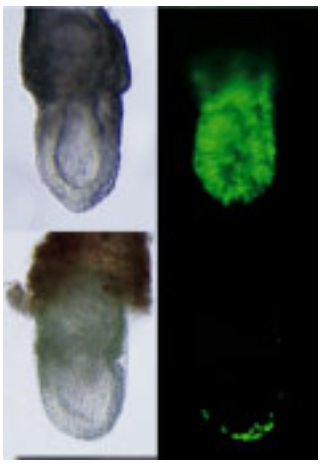


Hitoshi NIWA M.D., Ph. D.

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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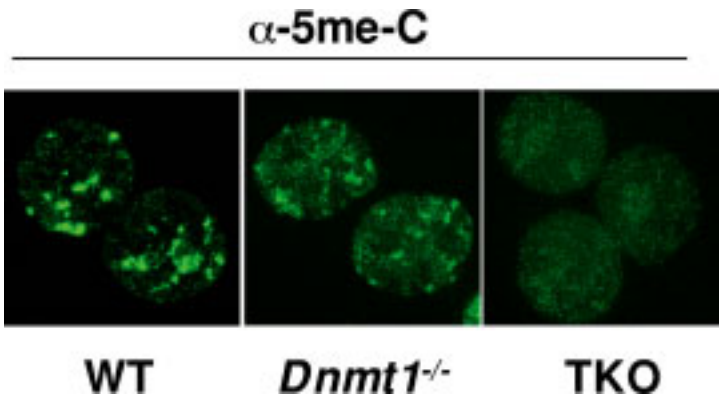
Mammalian Epigenetic Studies

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.



Near absence of DNA methylation in *Dnmt1*$^{-/-}$*Dnmt3a*$^{-/-}$ *Dnmt3b*$^{-/-}$ triple knockout (TKO) ES cells as revealed by immunofluorescence analysis using anti-5-methylcytosine antibody

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Mammalian Molecular Embryology

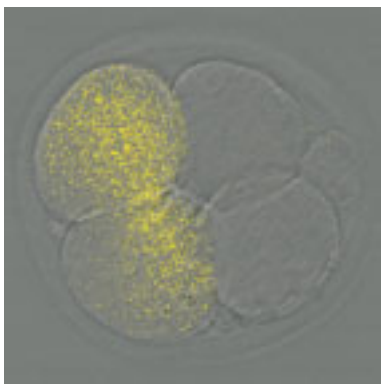
Tony PERRY Ph. D.

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

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

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

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



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

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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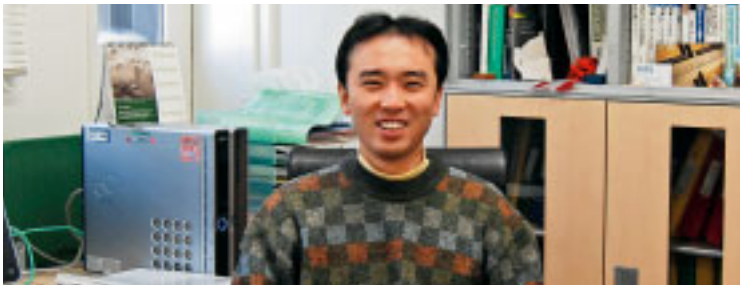
Mammalian Germ Cell Biology

Mitinori SAITOU M.D., Ph. D.

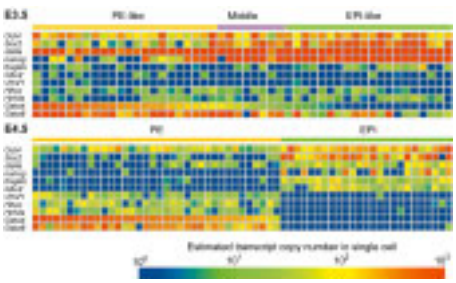
Mitinori Saitou received his M.D. from the Kyoto University Faculty of Medicine in 1995, and received his Ph. D. in 1999 for his study of the structure and function of tight junctions under Shoichiro Tsukita in the Kyoto University Graduate School of Medicine. He then moved to the Wellcome Trust/Cancer 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.



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.

In the mouse, PGCs are first specified during early gastrulation, around day 7 of embryonic development, as a small group of about forty cells in the extraembryonic mesoderm. Our single-cell comparative analyses with cells of somatic fate revealed the molecular mechanisms underpinning germ cells' acquisition and maintenance of totipotency and their escape from somatic differentiation. Epigenetic studies further showed that PGC chromatin structure is extensively remodeled following their formation. These findings suggest that mammalian germ cell development relies on specific inductive signals targeting cells that would otherwise be destined to somatic fates, leading us to propose that the mechanisms of germline development (both totipotency and epigenetic reprogramming) are ultimately determined by genetic programs. A single-cell microarray analysis system developed in our lab will further the study of transcriptional dynamics in this system, while the analysis of mutant phenotypes showing defects in germline development is looked to to provide clues regarding the roles of molecular families of interest. By elucidating the logics underlying the germline system, we hope to be able to reconstitute germ cell development 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

Yoshiki SASAI M.D., Ph. D.

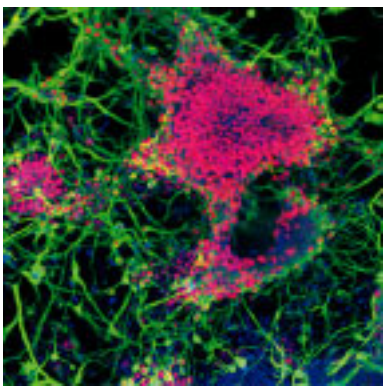
Yoshiki Sasai received his M. D. from the Kyoto University School of Medicine in 1986, subsequently performing internships in general practice and emergency medicine. He completed the Ph. D. course at the same institution in 1992, for work on neural specific transcriptional regulators. In 1993, he took 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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Ridge analysis: Function of the carapacial ridge in the development of the turtle shell

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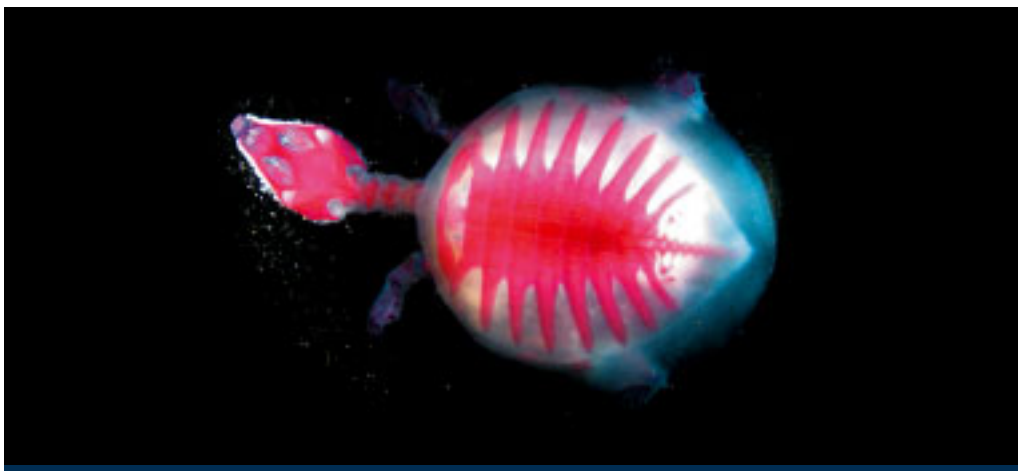
Nagashima H, et al. On the carapacial ridge in turtle embryos: its developmental origin, function and the chelonian body plan. *Development* 134. 2219-26 (2007)

Turtles have what no other vertebrate does, a shell. But the means by which this novel structure arose during the evolution of the chelonian (turtle and tortoise) body plan remains imperfectly understood. Studies of turtle embryogenesis have revealed that, contrary to the common misconception that it is some form of thickened and hardened skin, the carapace in fact develops from the turtle's ribs, which grow externally, splay into a fan-like arrangement and fuse to form the final bony structure. How the turtle's ribs came to overlie the scapula (equivalent to our shoulder blades) in such a brief span of evolutionary time, however, is unclear.

One candidate for the developmental source of the carapace is an embryonic structure known as the carapacial ridge (CR), which appears on the flank of the pharyngula stage embryo. This structure is interesting in that it is apparently unique to the turtle embryo, and bears some resemblance to another tissue, the apical ectodermal ridge, which serves as an inductive center in the development of vertebrate limbs. Embryological and molecular characterization of development in the Chinese soft-shelled turtle, *Pelodiscus sinensis*, performed by Hiroshi Nagashima and colleagues in the Laboratory for Evolutionary Morphology (Shigeru Kuratani; Group Director) have shown, however, that the carapacial ridge is unique to turtles and cannot be directly likened to any other vertebrate embryonic structure.



Skeleton of juvenile *P. sinensis* stained histochemically with alizarin red, showing the carapace being formed from the ribs.

Nagashima et al. started by looking closely at the morphology of the region where the carapacial ridge develops in the turtle and comparing it with equivalent regions in other non-chelonian amniote species, such as chicken. Observation of the period during which the ridge forms, revealed that it is an axial structure derived entirely from the somites (transient mesodermal structures that give rise to trunk skeletal muscle, vertebrae and ribs). Unlike in chicken, in which the ribs invade the lateral body wall, the ribs in *P. sinensis* never do so, suggesting that in turtles the ribs (and so the carapace) are confined solely to the axial domain dorsal to the embryonic flank.

The group next looked at the roles of genes that had previously been shown to be expressed specifically in the carapacial ridge. They found that the introduction of a dominant negative version of the CR-specific gene *LEF-1* resulted in the abnormal development of the carapacial ridge, indicating that this gene plays a role in the formation and maintenance of that structure.

Turning next to classical embryology, Nagashima et al. performed transplantation and ablation experiments to ascertain what role, if any, the carapacial ridge played in determining the turtle's unique pattern of rib growth. Interestingly, the carapacial ridge was found frequently to regenerate and re-

Hiroshi NAGASHIMA, Shigehiro KURAKU, Shigeru KURATANI

tain its axial position in embryos in which the region was cauterized at stage 14. But examination of the subsequent development of the CR-ablated embryos did show that the fan-shaped pattern of the ribs was partially disturbed. This suggests that while the carapacial ridge is not responsible for the axially restricted growth of turtle ribs, it might, rather, be involved in the radiation of the ribs in an arc relative to the midline.

“This study showed that the turtle has not added anything new to the ancestral anatomical components – there are no turtle-specific skeletal elements,” says Kuratani. “Rather, their ribs are arrested dorsally, in their original position of development and simply grow laterally, which does not happen in other amniotes. The CR might in some way function in changing the direction of growth, but we still do not know how this might be achieved.” The lab is continuing to work on anatomical relationships between skeletal and muscle elements in the hopes of identifying the gene or regulatory system that actually altered the direction of rib growth, which will provide a better understanding of 'the making of the turtle.'

How MIG-17 shows the way

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Ihara S and Nishiwaki K. Prodomain-dependent tissue targeting of an ADAMTS protease controls cell migration in *Caenorhabditis elegans*. *Embo J* 26. 2607-20 (2007)

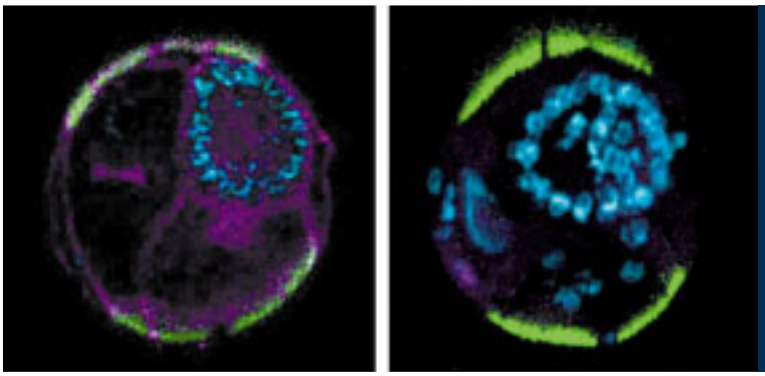
When cells move through a body, they need to be told where to go and when to stop. These directions are often provided by molecular signals that serve to mark the path. In the roundworm *C. elegans*, the gonad normally develops as a pair of U-shaped structures, which follow the route traced by pioneers called the distal tip cells (DTCs). These cells rely on a number of protein signposts to guide them on their way. One of these, MIG-17, is secreted from the muscle in the body wall and is localized to the gonadal basement membrane, keeping the migrating DTCs on course. But it has yet to be fully explained at the molecular level just how MIG-17 shows the way.

Of particular interest is the preliminary step in which this enzyme, a member of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family, is recruited to the basement membrane. New work by Shinji Ihara and Kiyoji Nishiwaki of the Laboratory for Cell Migration (Kiyoji Nishiwaki; Team Leader) identified an unexpected structural element of the MIG-17 protein as critical to its proper localization. In an article published in *The EMBO Journal*, the authors described how a subunit of the full-length protein, known as the prodomain, which must be post-translationally modified in order for the protein to localize correctly, must then be removed in what appears to be an autocatalytic fashion in order for MIG-17 to be activated.

Ihara and Nishiwaki began their study by addressing the question of whether MIG-17 localization to the DTC surface is truly a requirement for normal migration. (This had been assumed to be the case, but had never been directly shown.) Using a membrane-bound version of the protein, they showed that the DTC surface is indeed its primary site of action. They next followed up on a lead that had emerged from previous work by the Nishiwaki lab, which had showed that MIG-17 localization relied on a form of protein modification called glycosylation mediated by a second protein, MIG-23. By comparing DTC migration in wildtype and mutant worms carrying defects in MIG-17 glycosylation sites, they developed a model for the role of this modification by MIG-23 in which MIG-17 produced in the body wall muscle cells is glycosylated in the secretory pathway, enabling its homing to the DTC surface.

Focusing on the details of MIG-17 protein structure, they analyzed the nine candidate glycosylation sites (six in a region known as the prodomain, and three in the MP domain) to determine which were involved in gonadal localization, and found that a subset of the prodomain sites were essential. Extending their analysis, they studied worms with mutations in each of the known MIG-17 domains, and concluded that the prodomain is necessary, but not sufficient, for normal DTC migration.

Most other members of the ADAMTS family undergo a cleavage between the prodomain and the MP domain during the process of secretion; this cleavage, however, is dependent on other processing enzymes. Interested to find out if this was the case for MIG-17 as well, Ihara and Nishiwaki created an in vitro assay to study prodomain processing, and found that the cleavage of the prodomain was dependent on autocatalytic cleavage mediated by MIG-17's own protease activity. By engineering mutant constructs of the protein to lack this function, they determined that prodomain processing is essential for gonadal migration in vivo as well.



Worm cross sections stained with anti-MIG-17 prodomain antibody (pink), fluorescein-phalloidin (green; muscle) and DAPI (blue; nuclei). Pro-MIG-17 localizes to gonadal and other basement membranes in wild-type (left), but is not detectable in *mig-17* mutants (right).

Kiyoji NISHIWAKI, Shinji IHARA

These latest findings from the Nishiwaki lab point to an unanticipated role for the prodomain in ADAMTS recruitment and function. Although previous reports had indicated a role for the prodomain in maintaining enzymatic latency, protein folding or secretion, the Ihara and Nishiwaki study showed that the MIG-17 prodomain is also crucial in the targeting of that protein to the gonad. Whether this is true in other ADAMTS proteins remains to be seen, but the fact that some of them are also secreted into the extracellular space as pro-enzymes having prodomains makes this an intriguing possibility. As ADAMTS is a protein family of recognized medical importance, these new insights are sure to be of interest to basic and clinical researchers alike.

The *C. elegans* MIG-17 system is the only available platform in which the spatial-temporal action of an ADAMTS can be monitored during organ morphogenesis in living animals, which the Nishiwaki team has deftly exploited in demonstrating a novel function of the prodomain required for targeting MIG-17 to the gonad. “Because ADAMTS enzymes are secreted proteins that must be brought to their target tissues during development, it is possible that prodomain targeting is one of the key strategies employed by ADAMTS proteins to localize to specific tissues where they function in organogenesis,” notes Nishiwaki, highlighting the potentially broad impact of this work.

clockwork orange keeps flies on time

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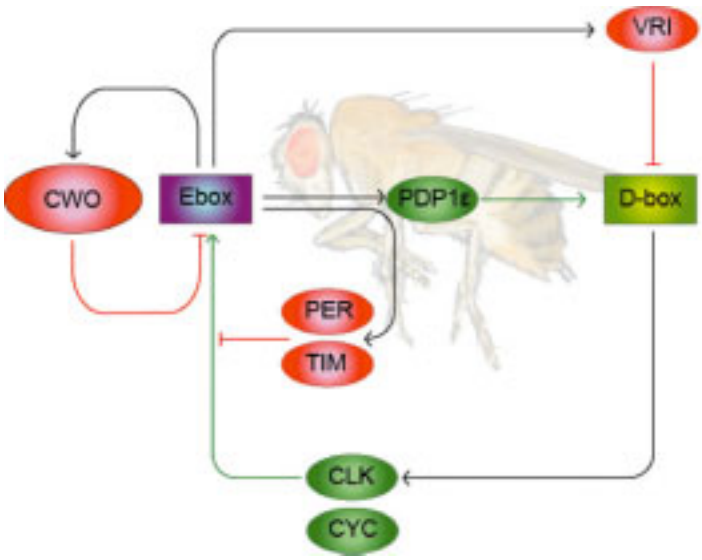
Matsumoto A, et al. A functional genomics strategy reveals *clockwork orange* as a transcriptional regulator in the *Drosophila* circadian clock. *Genes Dev* 21. 1687-700 (2007)

Many of the body's biological activities are controlled by a well-conserved network of genes that ensures that they follow an approximately 24-hour cycle tuned to the earth's own pattern of day and night. Sleep and wakefulness, changes in blood pressure, body temperature and the secretion of hormones have all been shown to be regulated by such circadian genes, which show a high degree of homology in a wide range of species, from human to fly. Previous research from the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader) revealed a large number of previously unreported elements of the complex network of transcriptional regulators that controls the mouse biological clock, including 20 novel clock genes and 3 transcriptional inhibitor sequences.

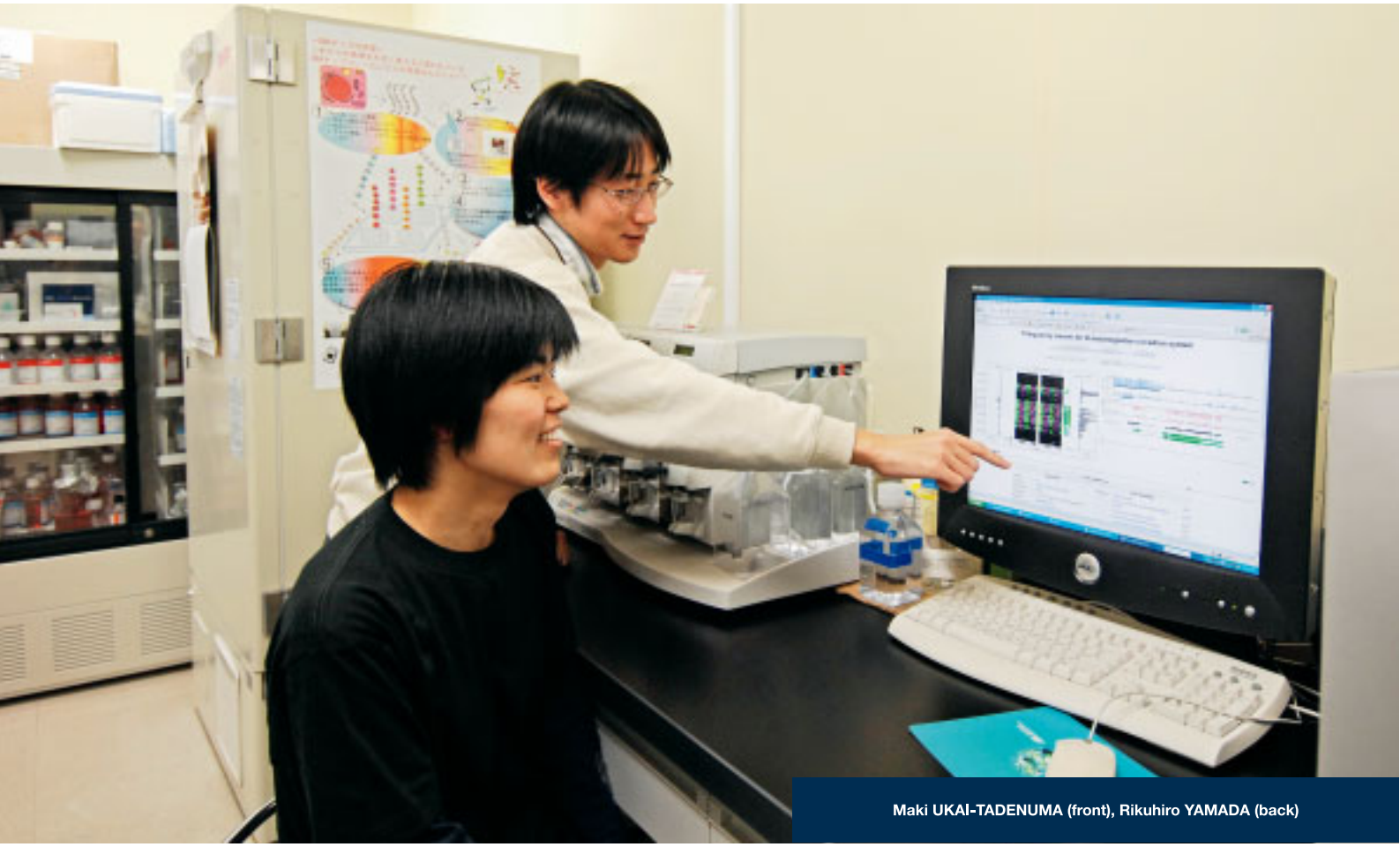
In a study conducted in collaboration with researchers from Kyushu University, the National Institute of Genetics and Texas A&M University (USA), the Ueda lab added yet another member to the growing list of circadian regulatory genes. The work, published in *Genes & Development*, describes the identification of a new clock gene, *clockwork orange* (*cwo*), in a genome-wide functional screen.

The comparatively simple *Drosophila* genome provides an excellent model for studying the elaborate network of circadian controls in human, as it is more comprehensively characterized, more amenable to experimentation, and many circadian genes are known to be conserved between these two apparently dissimilar species.



Schematic diagram of the relationships of the proteins now thought to be involved in the *Drosophila* circadian clock. Activators are in green, repressors in red.

A genome-wide analysis of the *Drosophila* genome by DNA microarray revealed on the order of 200 genes whose expression in head regions exhibited 24-hour cyclical patterns of expression. To tease out the central players from this set of candidates, the lab next screened for phenotypes using RNA interference (RNAi), which allowed them to study the effects of the tissue-specific loss of function of individual genes in vivo. Watching for disturbances in the normal behavioral patterns of flies, the team observed five RNAi phenotypes marked by dramatic changes in circadian rhythmicity. Of these, the gene with the most pronounced effect on biological clock activity was found to code a transcriptional factor containing a basic helix-loop-helix and an ORANGE domain, leading to its colorful name, *clockwork orange*, in reference to the Anthony Burgess novel and Stanley Kubrick film of the same title.



Maki UKAI-TADENUMA (front), Rikuhiro YAMADA (back)

The Ueda team then looked for possible targets of CWO using a combination of microarray and chromatin immunoprecipitation technologies, also known as ChIP-on-chip, to isolate and identify DNA-binding proteins with an affinity for *clockwork orange*. An assay of the full *Drosophila* genome showed that CWO binds to a number of E-box genes, which are known to play a key role in the transcriptional network that regulates the body's internal clock. They also determined that CWO negatively regulates the expression of numerous clock genes, including its own, and that loss of *cwo* function results in the halving of the daily amplitude of oscillatory clock gene expression in comparison to wildtype. This may be attributable to a loss of the normal inhibition of direct CWO targets, resulting in a reduction of the thresholds in their transcriptional control and, as a consequence, an elevation of their trough expression levels.

These latest findings from the Ueda team indicate that the novel gene *clockwork orange* interacts closely with other known clock genes and plays an important role in the maintenance of circadian rhythmicity, highlighting the power of such advanced methodologies as DNA microarrays, RNAi and ChIP-on-chip in addressing complex biological networks. "The work is still far from complete," says Ueda, "but I feel the discovery of *clockwork orange*, which has a homolog in the human genome as well, represents an important step in deciphering biological clocks at the systems level."

Tracing the steps toward germline reprogramming

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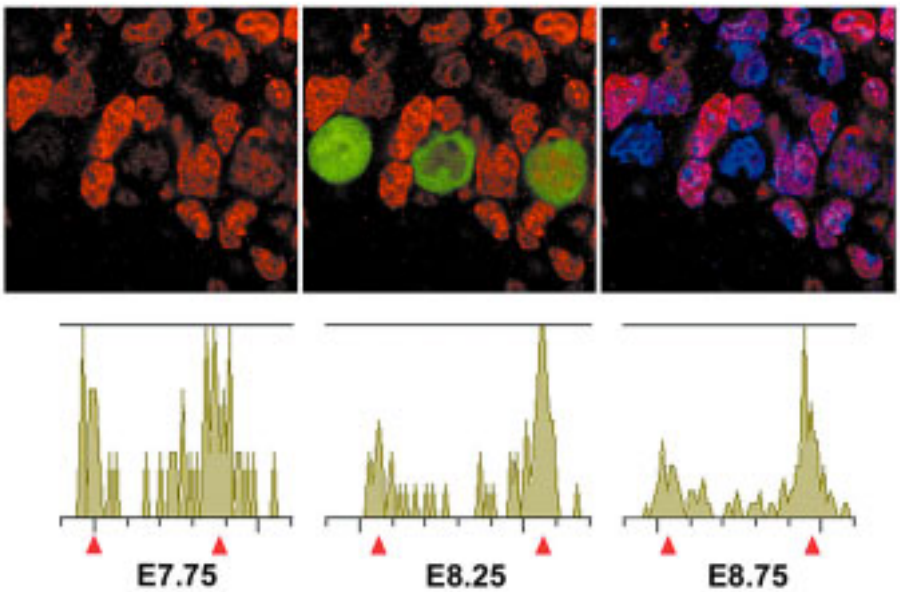
Seki Y, et al. Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. *Development* 134. 2627-38 (2007)

The cells of the germline are unique in their ability to revert to and maintain a state of developmental totipotency, which requires extensive reprogramming of the epigenetic markers that determine the transcriptional state of their genome. In the short window of embryonic time between the specification of primordial germ cells (PGCs) and their full commitment to that lineage, these cells shed DNA methylation and the di-methylation of histone H3 proteins at lysine 9 (H3K9me2), while gaining high levels of tri-methylation of H3 lysine 27 (H3K27me3). The manner in which they achieve this comprehensive reworking of their epigenetic state, however, remains poorly understood.

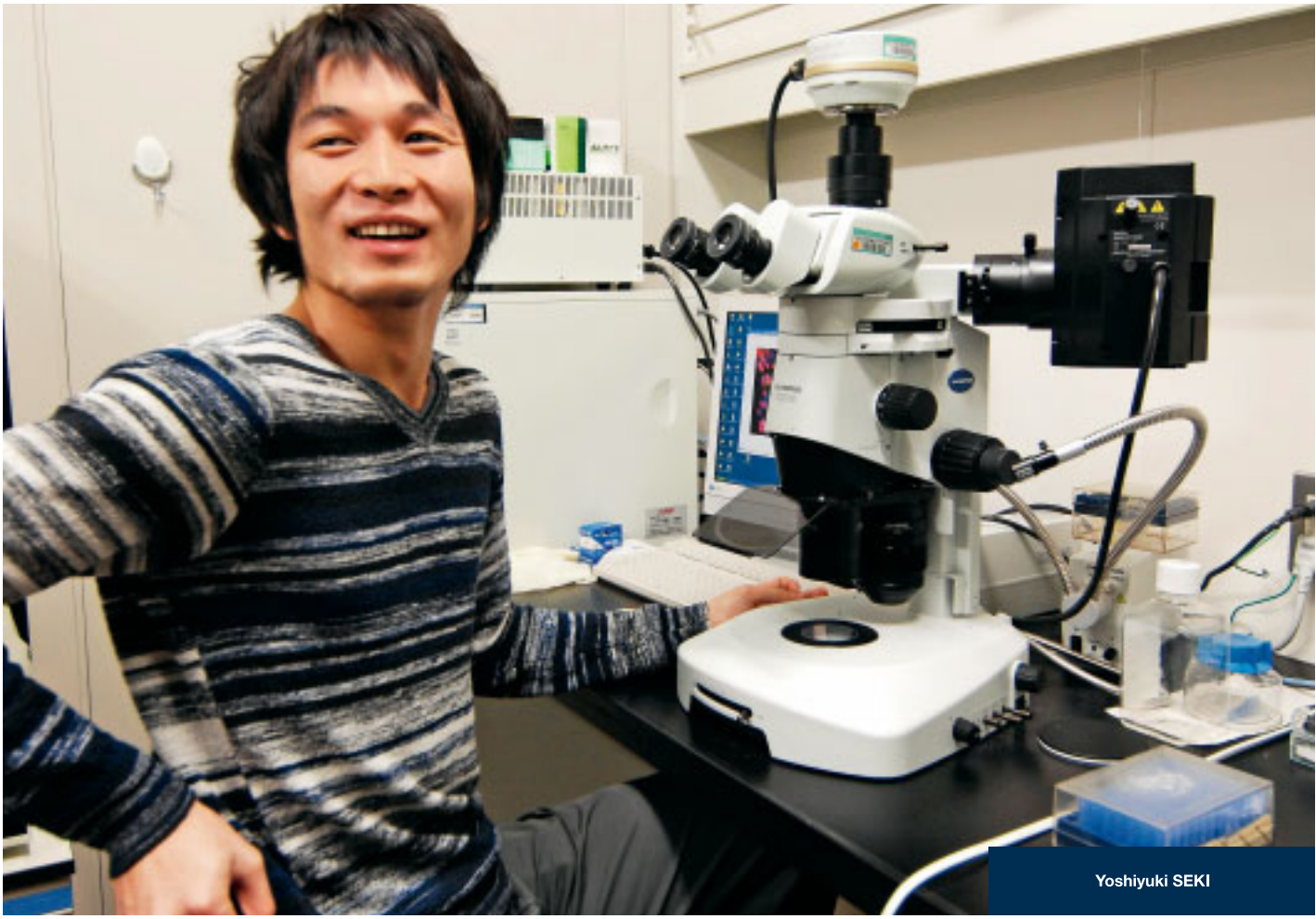
Working with colleagues from the Laboratory of Mammalian Germ Cell Biology (Mitinori Saitou; Team Leader) and collaborators in labs across Japan, Yoshiyuki Seki worked out the details of the stepwise mechanisms whereby PGCs reprogram the "cellular memory" held by the germline precursors. This work, published in the journal *Development*, revealed that the changes in histone methylation follow a progressive, cell-by-cell routine in an initially small population of migratory PGCs.

The team began by comparing chromatin modifications in PGC precursors (which express the germ cell specifying gene *Blimp1*) with somatic neighbors and found that there were still no significant differences in epigenetic marking at this early stage before the PGCs begin their migration. Immunohistochemistry using antibodies against histone modifications performed slightly later (starting at about embryonic day 7.75), however, showed that H3K9me2 levels progressively declined in PGCs, dropping to very low levels by E8.75. The team speculates that this may be due to the downregulation of the histone methyltransferase GLP in migrating PGCs. A second histone modification, H3K27me3, conversely became upregulated, starting about a half-day later at E8.25 and continuing until E9.5, at which point about 85% of the cells stained strongly for H3K27 trimethylation.

Methylation of histones frequently represses the transcriptional activity of the genes included in the affected chromatin region, so Seki et al. next asked whether transcription in PGCs is affected by this massive reprogramming. When they looked at levels of RNA polymerase II (RNAP II) phosphorylation, which serves as a good index of global transcriptional activity, they were surprised to find only low levels of this modification. The repression of RNAP II-dependent transcription appears to begin in



Top: stella-positive PGCs (green, images from E8.25 embryo) erase genome-wide H3K9me2 (red) in a progressive, cell-by-cell manner. Hoechst staining is shown on the right.
Bottom: Cell cycle state of PGCs from E7.75 to E8.75 measured by FACS analysis.
G1 (left) and G2 (right) peaks are indicated by red arrowheads.



Yoshiyuki SEKI

migrating PGCs at about E8.0 and to occur by a yet-unknown mechanism that interestingly does not rely on chromatin-based silencing. Although questions about the mechanistic details remain, the Saitou team now has a timeline of the events in PGC reprogramming, in which H3K9me2 first starts to decline, followed by the transient loss of RNAP II phosphorylation, the upregulation of H3K27me3, and finally the resumption of RNAP II-dependent transcription.

Noting that the PGC population appears to undergo a slowdown in cell proliferation between E8.0 and about E9.25, the team next turned to analyze the mitotic activity of these cells. BrdU labeling showed that the majority of PGCs during this period failed to enter the S-phase of mitosis. Expression levels of cyclin B1, which accumulates in the cytoplasm of G2-phase cells, suggested that PGCs are arrested at the G2 phase of the cell cycle during their migration. They finally confirmed this by directly measuring the DNA contents of migrating PGCs by FACS analysis, shoring up the case that the epigenetic reprogramming most likely occurs in the G2-phase of the cell cycle.

“The mammalian germline system may be the best in vivo system with which one can study the precise mechanisms of genome-wide epigenetic reprogramming,” says Saitou, “since germ cells continuously reprogram their epigenome by genetically tractable pathways throughout their development. We hope that these studies will provide essential insights into the reprogramming of somatic cell nuclei in general.”

First MSC pathway revealed

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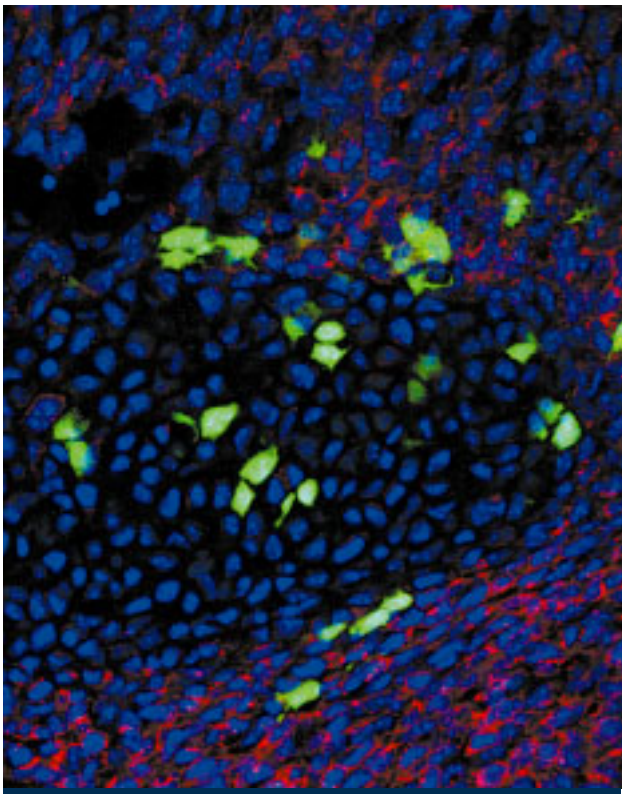
Takashima Y, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. *Cell* 129. 1377-88 (2007)

Mesenchymal stem cells (MSCs) have attracted considerable attention in the scientific community due to their availability in both embryonic and adult tissues, their therapeutic potential, and a number of recent reports of a related type of cell, the multipotent adult progenitor cell, which has been shown to give rise to cells from all three germ layers, an ability that had previously been thought to be limited to embryonic stem cells. Despite this widespread interest, however, little is known of how these cells behave in vivo, or indeed of their origins during embryonic development.

In a study published in the journal *Cell*, Yasuhiro Takashima, Takumi Era and colleagues in the Laboratory for Stem Cell Biology (Shin-Ichi Nishikawa; Group Director) revealed that mesenchymal stem cells first arise from a formative tissue known as neuroepithelium in the trunk region of the embryonic mouse. While their work showed that this initial wave is not the sole source of these stem cells, its identification provided the first solid insights into one of the pathways leading to MSC differentiation.

"It is somewhat surprising that almost nothing has been known about the developmental origin of MSCs," says Nishikawa, "as they are widely used in clinical practice. Now I'm happy to say we have unequivocally determined one of the multiple developmental pathways leading to these stem cells as well as developed a method for enriching MSC progenitors."

The group began by comparing a pair of methods for inducing mesenchymal lineages from embryonic stem (ES) cells, one involving culture on a collagen-coated dish and another in which the ES cells are exposed to pulses of retinoic acid and then incubated under adipocyte-inducing conditions. They found that under both conditions, cells would begin to express PDGFRα, a mesenchymal marker, but that the cells cultured under the second condition (Condition B) were capable of sustained growth and showed ten times the adipogenic activity of Condition A, suggesting that only cell populations cultured using this method contained mesenchymal progenitors. As Condition A is known to favor mesodermal differentiation, the group concluded that MSCs must arise from a non-mesodermal source.



Neuroepithelium-derived MSCs in embryonic trunk

They next examined expression of lineage-specific molecular markers in cell lines cultured under Conditions A and B, and found that under Condition A, markers for mesoderm and endoderm were expressed, as expected, but that neural markers were detected only in lines cultured under Condition B. Using Sox1 as a definitive neural marker, the group used GFP labeling to visualize $Sox1^+$ cells and found that Condition B does indeed support preferential differentiation toward the neural lineage. On maintaining these cells for longer periods, they discovered that a subset of the $Sox1^+$ cells eventually stopped expressing this and other neural genes, and began to express PDGFRα, indicating a lineage shift toward mesenchyme.

Having dissected the multistep MSC differentiation in vitro, Takashima and Era sought to confirm it in vivo as well. Taking cells from the embryonic trunk at E9.5,

the group sorted them into three subpopulations of Sox1^{+} PDGFRα^{-}, Sox1^{-} PDGFRα^{+}, and Sox1^{-} PDGFRα^{-} cells, and found that their expression profiles agreed with the conclusions of the in vitro work: Sox1^{+} PDGFRα^{-} cells corresponded to neuroepithelium, while Sox1^{-} PDGFRα^{+} cells were mesodermal, not neural, in character. Only the Sox1^{+} group was capable of sustained growth in culture and of giving rise to multipotent MSC lines. Using a persistent labeling system to trace the developmental origins of mesenchymal cells, they found that these are derived both from trunk neural crest and a second, still unidentified origin that appears to be the major source of PDGFRα^{+} cells. Importantly, the MSCs within this PDGFRα^{-} population are distinct from the oligodendrocyte precursor cells that arise from the neural tube, suggesting that mes-enchymal stem cells are a unique developmental entity.

“As a research group that has long been involved in research using ES cell differentiation culture, we wanted to show that this can be a versatile tool for defining the developmental origins of specific cell lineages,” adds Nishikawa. “We hope that the same methodology can be applied to clarify the developmental origins of other important cell lineages such as pericytes and microglial cells, which remain obscure to this day.”

Takumi ERA

Globins coordinate: ß globin co-transcription in primitive hematopoiesis

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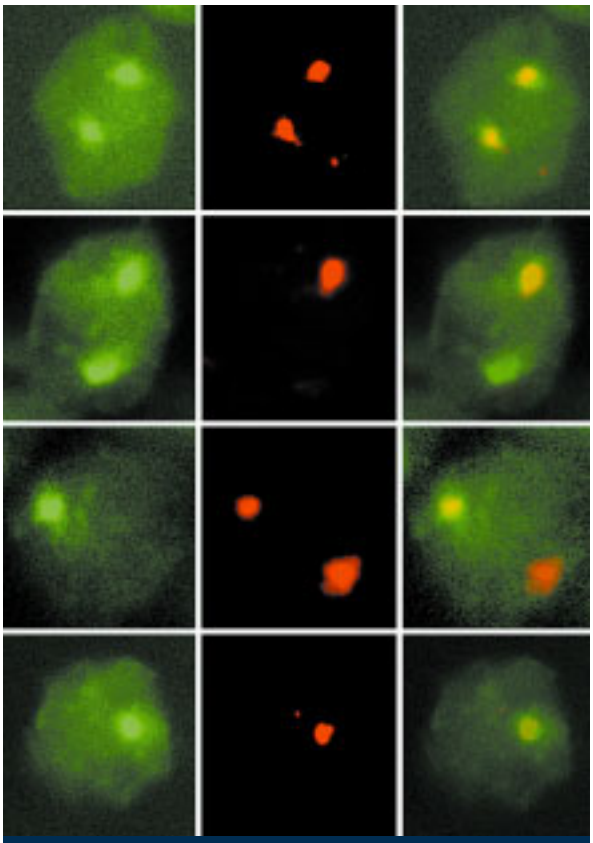
Nagai H and Sheng G. Cis-cotranscription of two β globin genes during chicken primitive hematopoiesis. *PLoS ONE* 2. e703 (2007)

The globins are a group of complex, oxygen-carrying proteins encoded by a highly conserved family of genes. In vertebrates, the hemoglobin molecule, which allows red blood to serve as a transporter of oxygen, consists of two kinds of globins, α and β, which form a complex of interlinked chains each encasing a heme capable of binding and releasing oxygen with great efficiency. In humans, there are five β globin genes, which are expressed differentially during the embryonic, fetal and adult stages of the life cycle and are arranged sequentially on a single chromosome locus in the developmental order of their expression. In the chicken, although there are (also deleted) four β globin-coding genes – ρ(rho),β^H,β^A, and ε(epsilon) – on a single chromosome locus, their order on the chromosome appears to have no relationship to their order of expression during development; the ρ and ε genes that flank the β hatching and adult stage genes are expressed earlier in development, during the first wave of blood development, known as primitive hematopoiesis. It is not known, however, whether the ρ and ε globins are expressed following a similar stage-dependent convention.

Hiroki Nagai and Guojun Sheng of the Laboratory for Early Embryogenesis reported that the ρ and ε globins not only co-transcribe, but do so in an apparently coordinated manner. These findings, published in *PLoS One*, point to an alternative to one extant model for β globin transcription in the mouse, which proposes that the transcription of these genes is sequential and mutually exclusive.

Nagai and Sheng began by using in situ hybridization to assess whether both, one or neither of the copies of the ρ and ε globins were transcriptionally active in the primitive blood cells of a stage 10 chicken embryo, and found that both loci of a given β globin gene in any given nucleus tended to make similar decisions regarding transcription. This then raised the question of how the two genes are transcribed in relation to each other at this stage of development. The results of double FISH analyses



Double staining with rho (green) and epsilon (red) probes reveals significant colocalization (merge).

Guojun SHENG, Hiroki NAGAI

using $\rho^{(+/-)}$ and $\varepsilon^{(+/-)}$ nuclei revealed not only that these genes overwhelmingly tend to be co-transcribed, but that this co-transcription is cooperative: of all loci in which at least one signal was detected for either ρ or ε, more than 80% were positive for both. “We think this shows quite clearly that the majority of loci are concurrently transcribing both ρ and ε in the embryo,” says Sheng.

The evidence for simultaneous transcription of two β globin genes in a single locus challenges some long-held views about the nature of beta globin expression, which plays out sequentially with different genes in the same locus preferentially transcribed at different stages in development to meet the changing oxygen demands of the organism. And, as the β globins have served for decades as a workhorse system for studies of molecular biology and genetics, the implications for our understanding of transcriptional activation may be far-reaching.

“For me, this study leads to some interesting questions about when we can truly say a gene has been 'switched on,'” says Sheng. “From what we're seeing here, what people have been calling the on state for a β globin is really more of a cumulative effect of multiple factors influencing the abundance of transcript and any given time. Of course, that leaves open the question of what is happening when you have an open locus and all the necessary transcription factors to activate a gene? There is probably some key additional step that we don't understand well.”

Protocadherin in neural pathways

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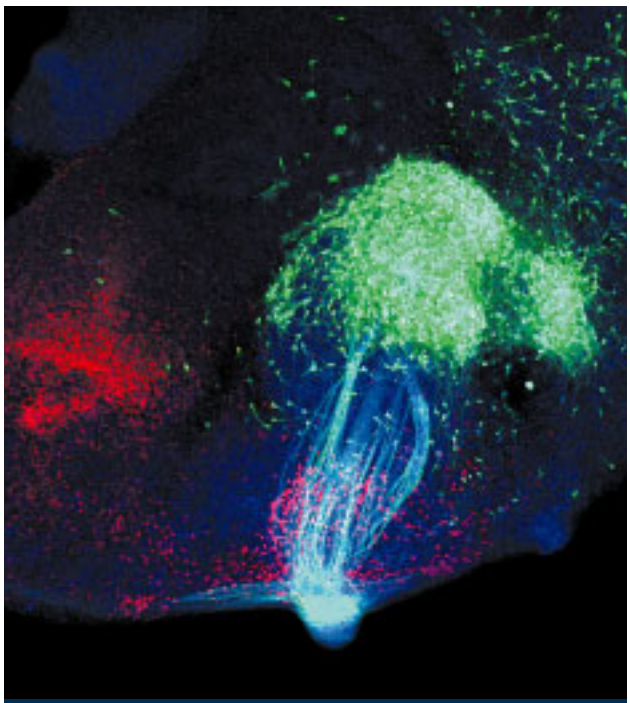
Uemura M, et al. OL-Protocadherin is essential for growth of striatal axons and thalamocortical projections. *Nat Neurosci* 10. 1151-9 (2007)

For the brain to fulfill its role as the seat of learning, emotion, motor control and cognition, it needs to establish the requisite circuitry early during embryogenesis. But the mechanisms by which migrating axons identify and home to their destinations and form synapses with other neurons remain imperfectly understood. One thing that is becoming increasingly clear is that the molecules mediating cellular adhesion play important roles in determining interneuronal affinities. Members of one family of adhesion molecules, the cadherins, have already been implicated in regulating the process of synaptogenesis.

Studies of a different cadherin family member, known as OL-protocadherin (OL-pc), by Masato Uemura, Shinji Hirano and colleagues in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) revealed a new function for these adhesion proteins in the development of the neural network. The group found that mice lacking the gene encoding OL-pc experienced failures in axon extension in striatal neurons and disoriented patterning of the ventral telencephalon, which they attributed to its hitherto unsuspected role in the regulation of guidance cues used in axon migration during neurodevelopment. Their findings were published in the journal *Nature Neuroscience*.

During the development of the mouse brain, axons from various brain regions enter the forebrain region known as ventral telencephalon, where they then must decide where to turn next. This decision-making process is informed by guidance cues, molecular signposts that steer the migrating axons on their way. The ventral telencephalon is also a site of abundant OL-pc expression, which led Uemura and Hirano to test the function of the molecule within that context. To do so, they engineered a line of mice in which this gene was disabled and made a close examination of the wiring of the brain at relevant developmental stages. The homozygous mutants (which were viable through embryogenesis, but died within a few weeks of birth) exhibited a range of neuronal circuit defects, the most pronounced of which involved the cortex and thalamus. Axons in these regions showed a range of targeting problems, although neuronal differentiation itself appeared unaffected. The disappearance of barrel structures in the region suggested that the majority of thalamocortical axons had not reached their normal destinations in the cortex.



Axons from the striatum (green), grafted onto a ventral telencephalic slice, extend toward the globus pallidus (red) suggesting this region serves as a guidepost for axon migration.

Looking within the ventral telencephalon, Uemura and Hirano found no abnormalities in transcription factors involved in patterning, specification and axon guidance, but did note that Nkx2.1, a marker of the brain region known as the globus pallidus, was absent from the tail end of this region. This was telling, as it has been speculated that the globus pallidus is important for the pathfinding of thalamocortical axons. Using a cell labeling strategy, the group determined that putative guidepost cells in this region were either absent or mislocalized in the homozygous mutant.

When they next studied a second region, the striatum, they found that axons emanating from the mutant striatum became entangled and failed to project normally to the globus pallidus. Seeking to explore this more closely in vitro, they isolated striatal tissue from transgenic mice expressing GFP and grafted them into slice from the ventral telencephalon. Interestingly, the graft projected axons to the Nkx2.1^{+} region and immediately formed an axon tangle in wildtype, but failed to extend axons in the OL-pc mutant, suggesting that this protein is essential for sustaining the migration of striatal axons toward the globus pallidus.

“In this work, we were able to demonstrate a previously unknown function for OL-protocadherin in the formation of multiple neural pathways in the ventral telencephalic network,” says Hirano. “And, given our findings, we now also believe that striatal axons may play an important role in patterning of ventral telencephalon and subsequent neural projections in this region, which we’ll certainly be interested in learning more about.”

Masato UEMURA, Shinji HIRANO

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)

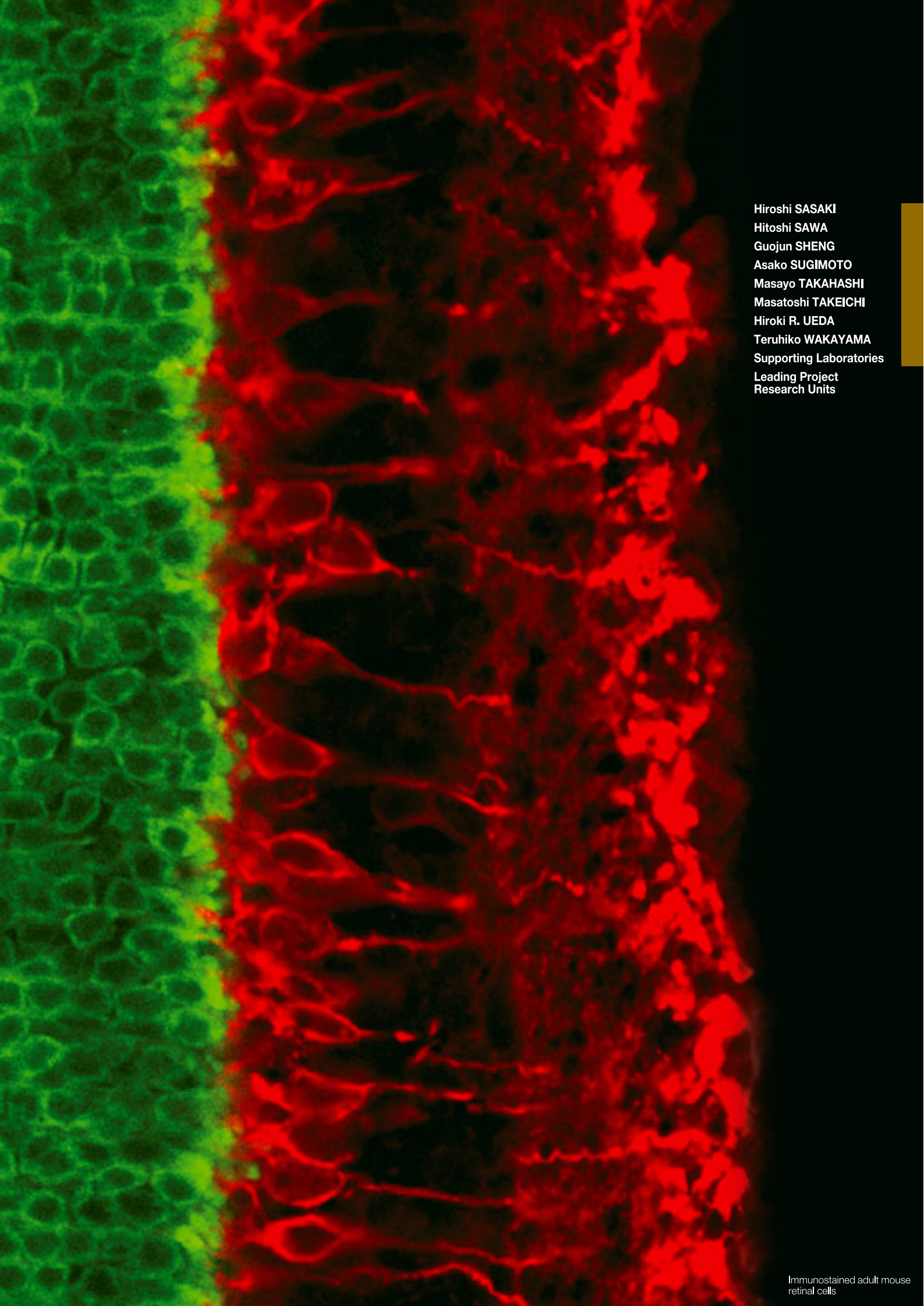
http://www.cdb.riken.jp/arb/

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 2008 annual meeting of the ARBS will be held in Yunnan, China. 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 will hold its first scientific meetings and launch its website in 2008. CDB Deputy Director Shin-Ichi Nishikawa serves as chair of the SNP steering committee.



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Supporting Laboratories
Leading Project Research Units

Immunostained adult mouse retinal cells

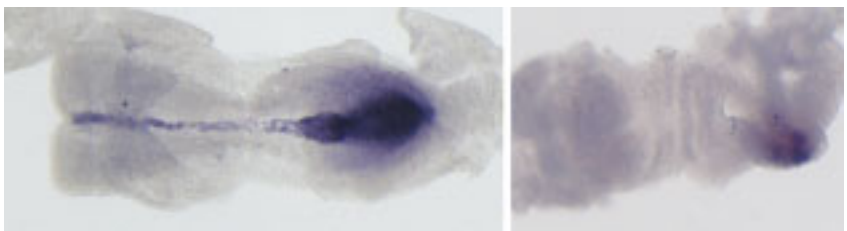
Embryonic Induction

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 regulation, and differentiation in the preimplantation embryo. Our lab is now analyzing Tead family function and regulation 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; the remaining staining is in the primitive streak).

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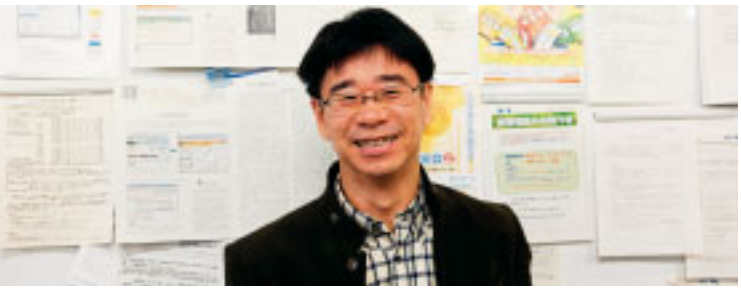
Cell Fate Decision



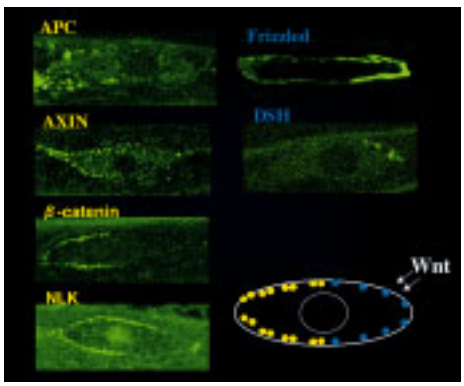
Hitoshi SAWA Ph. D.

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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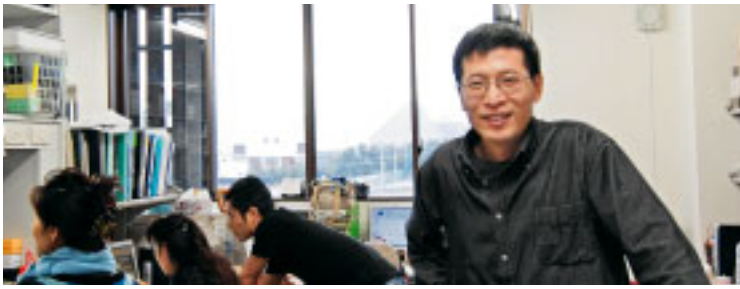
Early Embryogenesis

Guojun SHENG Ph. D.

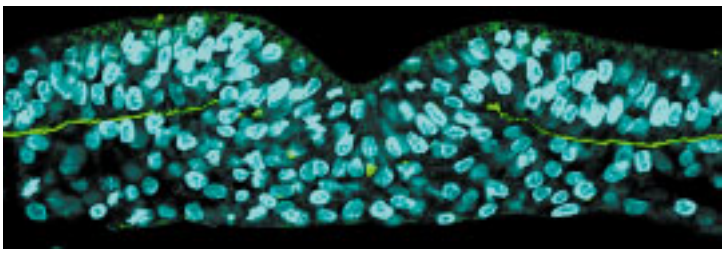
Guojun Sheng received his B.S. in microbiology from Fudan University in Shanghai, China in 1990. He entered the Rockefeller University the next year, attending the summer course in embryology at the Woods Hole Marine Biological Laboratory in 1996 and receiving his Ph. D. under Claude Desplan in 1997. 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.



We study the molecular and cellular mechanisms of gastrulation, an early developmental process conserved throughout the animal kingdom. During gastrulation, three germ layers (ectoderm, mesoderm and endoderm) are formed from a uniform layer of epiblast cells. Each of these germ layers undergoes further regional specification to form the functional tissues and organs of a developing embryo. Using chick as our main model, we are interested in developing a more complete understanding of how signaling molecules affect cellular behavior (morphology, migration and cell-cell interaction) during initial germ layer patterning, and how they act together with local molecular cues in specifying cell fates. Our research currently focuses on two aspects: the initial formation and regionalization of the mesoderm layer, and the differentiation of ventral mesoderm into several early functional cell types.

Mesoderm cells form from a sheet of epithelial-shaped epiblast cells by a process called “epithelial to mesenchymal transition” (EMT). One site of EMT in amniotes is the primitive streak. We are interested in understanding the molecular and cellular mechanisms governing the EMT process. We are also interested in understanding how the initial regional identities in the dorso-ventral mesodermal axis (axial, paraxial, intermediate, lateral plate and extraembryonic) are conferred along the anterio-posterior axis of the primitive streak during this process.

The extraembryonic mesoderm comes from the ventral-most primitive streak and gives rise to the earliest functioning cell types in the developing embryo. It serves as a simple model for the study of how diverse cell types are generated from the newly formed mesoderm populations. Our team is investigating how three major extraembryonic mesoderm cell lineages (blood, blood vessel and vessel wall muscle) are specified by extrinsic and intrinsic signaling inputs during their differentiation.



A section through gastrulation stage chicken primitive streak, stained with DAPI (marking nuclei) and 6G7 antibody (marking a special modified beta-tubulin). Mesoderm cells are generated through a process called epithelial-mesenchymal transition, during which basal microtubule dynamics regulate the interaction between the epithelial cells and the basement membrane.

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Publications

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Developmental Genomics

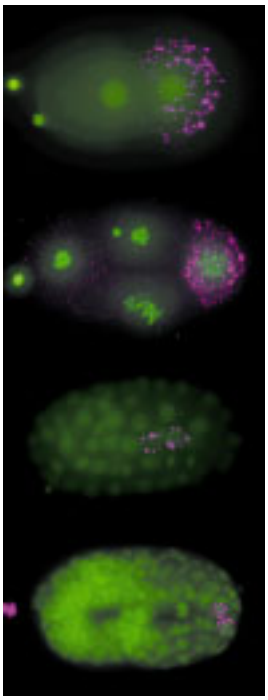
Asako SUGIMOTO Ph. D.

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.

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

The cooperation between groups of genes encoded in an organism's genome is essential to the development of complex structures from a single cell, the fertilized egg. Our laboratory has adopted the nematode *Caenorhabditis elegans* as an experimental model. This organism provides an extremely useful system for studying developmental programs at the whole genome level, as its genome has been fully sequenced, and the lineage of each cell in its body is known, features that we seek to exploit in identifying the means by which sets of genes working in combination help to establish and direct developmental processes.

Although the complete genome sequence is characterized, when, where, and how each gene works is still largely unknown. To obtain such information, we performed systematic RNAi to interfere with gene function and profiled phenotypes resulting from their loss of function. We now hope to take findings from these studies as a base for advancing the understanding of developmentally important mechanisms.



Our current research focuses on understanding the mechanisms to give rise to diverse cell types in the development of multicellular organisms. To produce a diversity of cell types, cells have to be polarized and segregate distinct sets of cell fate determinants to their daughter cells. In these processes, actin and microtubule cytoskeletons play crucial roles, and they have to be strictly controlled both spatially and temporally. Using the high-resolution microscopy to trace dynamic behaviors of proteins in combination of gene knockdown by RNAi, we are investigating the gene networks that control these cytoskeletons in early embryogenesis of *C. elegans*.

We are also analyzing the structure and assembly mechanism of P granules, which have been long believed to be the "germ cell determinants" in *C. elegans*. P granules are large complexes containing mRNAs and many RNA binding proteins, and specifically segregated into germline. They are thought to confer the specific characteristics of germ cells, but their exact role is still unclear. We aim to understand the function of P granules by identifying their new components and their assembly and segregation mechanisms.

In a concurrent project, we are developing new techniques (e.g., 4D microscopy) and tools (e.g., cell-type- or subcellular-structure-specific monoclonal antibodies and GFP markers) to improve the temporal and spatial resolution of phenome analysis. By studies such as these, we hope to develop a more detailed picture of the regulation of dynamic processes by networks of genes.

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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Retinal Regeneration

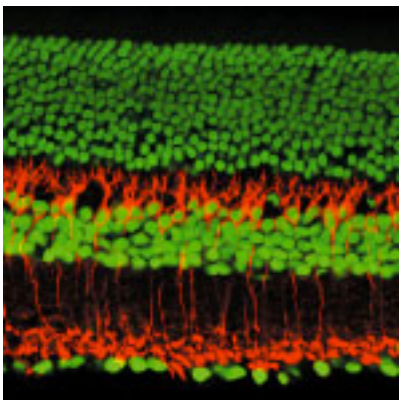
Masayo TAKAHASHI M.D., Ph. D.

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, and to ensure that such cells establish viable grafts on transplantation and induce the reconstitution of functional neural networks. We also hope to develop means of promoting true regeneration by activating endogenous stem cells to replace cells lost to trauma or disease and thus repair damaged tissues. Access to a broad spectrum of developmental biological research information will be key to the achievement of these goals, and we appreciate the opportunities for exchange that working in the environment provided by the RIKEN CDB 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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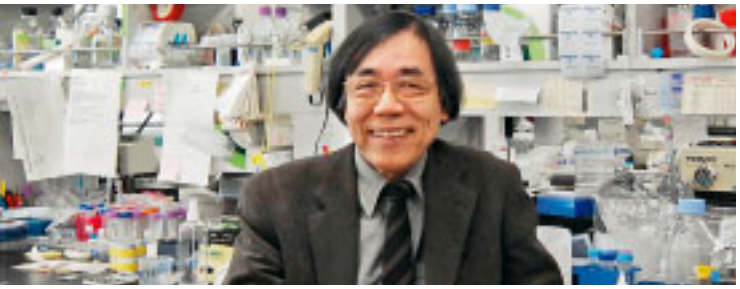
Cell Adhesion and Tissue Patterning



Masatoshi TAKEICHI Ph. D.

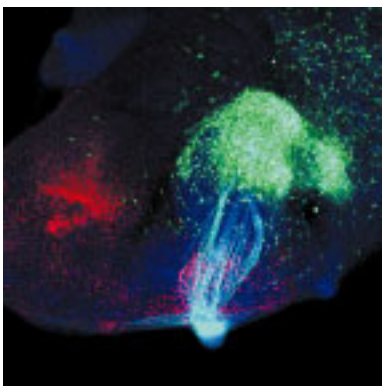
Masatoshi Takeichi is director of the RIKEN Center for Developmental Biology as well as director of the Cell Adhesion and Tissue Patterning research group. He completed the B. Sc. and M. S. programs in biology at Nagoya University before receiving a doctorate in biophysics from Kyoto University in 1973. 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 thought to be essential for the construction of tissues; this adhesion machinery is the main subject of our research.

Cell-cell adhesion is dynamic and reversible; 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 or microtubules, which 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 uncovering novel regulatory mechanisms specific to cell-cell adhesion.

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 tissues levels, using the brain cortices as model systems, our team is attempting to determine how cell migration and positioning are controlled by cadherin or catenins during the formation of laminar structures in these tissues. We are also investigating the roles of protocadherins and Fat cadherins, members of the cadherin superfamily, in the regulation of tissue organization.

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 now 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.



Axons from the striatum (green), grafted onto a ventral telencephalic slice, extend toward the globus pallidus (red), suggesting this region serves as a guidepost for axon migration.

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Systems Biology

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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Matsumoto A, et al. A functional genomics strategy reveals clockwork orange as a transcriptional regulator in the *Drosophila* circadian clock. *Genes Dev* 21. 1687-700 (2007)

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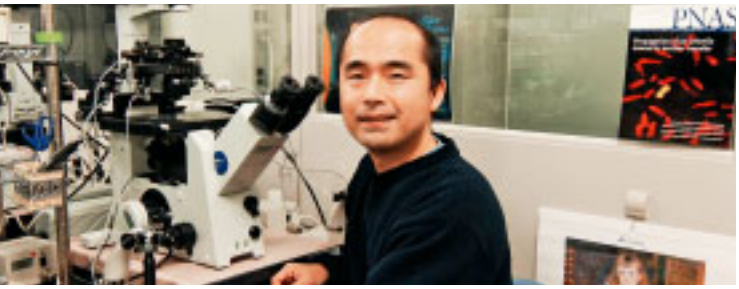
Genomic Reprogramming



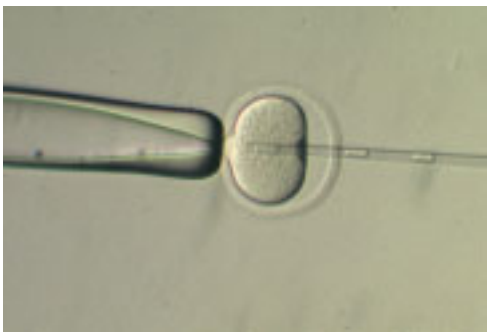
Teruhiko WAKAYAMA Ph. D.

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 reprogramming, the process in which a cell's developmental potential is reset to a totipotent state (such as is exhibited by a naturally fertilized egg). However, the specific details of the technical flaws, the ways in which they lead to reprogramming failure, and possible solutions to the problem all remain completely unknown. Despite these challenges, cloning continues to be a biotechnology of great promise, given its potential applications in the field of regenerative medicine, such as the generation of embryonic stem (ES) cells by nuclear transfer, which may one day allow us to grow replacement cells that perfectly match a patient's own genetic and immune profile, thereby eliminating the risk of rejection.

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



Transfer of a somatic nucleus into an enucleated egg

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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, as well as handling the shipping and receiving of mutant mice both within the CDB and with other domestic and international institutions. In addition, the Unit provides support services for the IVF breeding of animals, including cleaning, colony development, and cryopreservation by vitrification. We also provide pregnant females, mouse embryos, and surrogates to support the CDB animal experimentation community, and develop technologies for the study of rodent reproductive biology.

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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 analyze over 100,000 genes a year, and 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.

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Functional Genomics Unit

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

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

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

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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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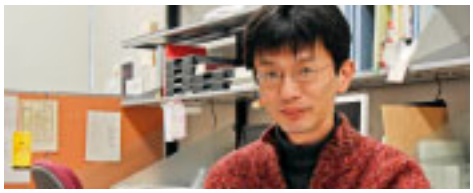
Proteomics Laboratory

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

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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Technical Staff
Michiru MATSUMURA-IMOTOI
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Satoshi ANDO

Leading Project Research Units

The implementation of the national Leading Project in Regenerative Medicine has been consigned to universities and research organizations across Japan, and a number of CDB labs have received funding to conduct research into key areas including the experimental manipulation and maintenance of stem cells, the reprogramming and targeted differentiation of somatic stem cells, and the ex vivo reconstitution of physiological structures with potential clinical uses in regenerative medicine. Shin-Ichi Nishikawa, Group Director of the CDB Laboratory for Stem Cell Biology, was appointed to head this Leading Project.

Two Research Unit laboratories were recruited in 2003 to conduct mission-oriented, fixed-term research projects as part of this program. These Research Units are funded by Leading Project grants and are financially independent of RIKEN, but operate, in whole or in part, in laboratories located within the RIKEN CDB campus, enabling them to take advantage of the extensive shared-use equipment and facilities at the Center.

Research Unit for Cell Plasticity

Mitsuko KOSAKA Ph. D.

Our research studies the possibility of achieving the regeneration of ocular tissue, such as neural retina, by using iris pigmented epithelial (IPE) cells. We have recently found that postnatal avian or mammalian IPE cells can transdifferentiate into lens or neuron under appropriate conditions. Furthermore, we could identify putative stem cells and showed cellular heterogeneity within adult IPE tissue. Multipotent stem cells in the IPE may provide a source for autologous retinal transplantation. We hope this project will open new avenues in the field of stem cell biology and ocular regenerative medicine.

Staff

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Visiting Scientist
Guangwei SUN

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Yuka NAKATANI
Eri YUGAMI

Research Unit for Organ Regeneration

Hideki TANIGUCHI M.D., Ph. D.

With its goal of exploiting the self-renewal potential of living cells, the emerging field of regenerative medicine stands poised to play an essential role in providing innovations toward the treatment of many human disorders. Although remarkable advances in this field have been achieved, to date these have been limited to certain tissues, such as vessels or bone/cartilage. The regeneration of solid organs, i.e. liver or pancreas, remains as a wide-open research frontier.

Using a combination of flow-cytometry and fluorescence-labeled monoclonal antibodies, we have established a novel and utile methodology for the isolation of individual cells, which has opened up one avenue to this frontier of science. This approach ultimately allowed us to identify and characterize stem cells in such organs. We now seek to achieve a better understanding of the underlying stem cell biology and the development of basic technologies that will enable us to realize the promise of regenerative medicine for patients suffering from health conditions involving these organs.

Staff

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Shedding light on singularity

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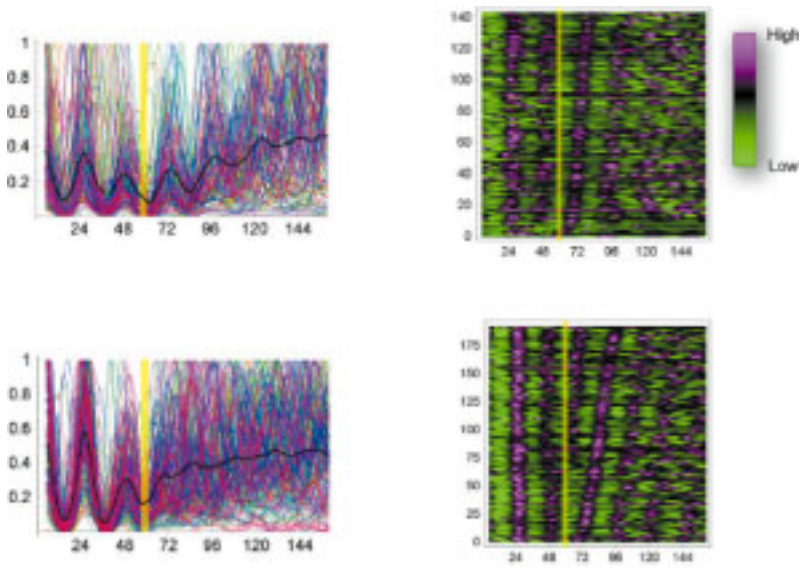
Ukai H, et al. Melanopsin-dependent photo-perturbation reveals desynchronization underlying the singularity of mammalian circadian clocks. *Nat Cell Biol* 9. 1327-34 (2007)

Many of the mammalian body's cyclical activities, from sleep to the secretion of hormones, follow schedules set by biological clocks. Such clocks are subject to regulation by a conbination of environmental cues and intrinsic signaling determined by a complex network of genes. Under normal daylight conditions, biological clocks provide a reliable timetable for a wide spectrum bodily functions, but they are also susceptible to what is known as "singularity behavior," the loss of robust periodicity when challenged by critical stimuli, such as a pulse of bright light during the night hours. The mechanisms underlying such singularities, however, have remained unclear despite decades of intensive study.

A study by Hideki Ukai and Tetsuya J. Kobayashi of the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader) revealed the likely means by which singularity is triggered in the mouse. Using exogenous melanopsin, a photosensitive molecular receptor, to render cultured cells responsive to light stimuli, the team determined that singularity involves the desynchronization of individual cellular clocks, challenging one popular model, which suggested that it is the result of the arrhythmicity or suppression of clocks. This work, conducted in collaboration with scientists from Kinki and Nagoya Universities, was published in *Nature Cell Biology*.

Reckoning that a photoinducible system in vitro would allow them to study singularity in a simpler, more controlled fashion, Ukai transfected melanopsin, which is normally only expressed in retinal cells, into mouse fibroblasts, exposed them to a 6-hour pulse of light and measured the effect on biological clock activity by monitoring changes in the expression of *PER-2*, a clock gene intrinsic to these cells. Melanopsin-positive cells exposed to a light pulse showed changes in both the phase and amplitude of *PER-2* expression, while untransfected cells did not, indicating that the melanopsin protein had made the fibroblasts photo-responsive. A series of tests using light pulses delivered at different time-points yielded one critical pulse capable of inducing singularity in vitro.

They looked next at the individual cells to decipher how singularity manifests itself, and found that although each cell generally maintained their intrinsic rhythmicity, they cycled out of step with other cells. This finding stands in contrast to those of several previous studies, which had suggested that



In non-photo-responsive cells, oscillations gradually degrade over time independent of exposure to a light pulse (top left) and rhythmicity of individual cells is not lost; while in photo-responsive cells, the amplitude of the oscillations in the multi-cell sample (bottom left) is reduced dramatically although the individual cell maintains its periodicity.

multi-cell singularity is the result of loss of rhythmicity in individual cells. So whether desynchronization would be observed in vivo remained an important question.

The team entrained rats in a laboratory under conditions known to induce singularity in humans, and studied a section of their brains, the suprachiasmatic nucleus (SCN), a brain region known to be the master of the body's biological clocks. The expression of clock genes *Per1* and *Per2* showed a dramatic decrease in total amplitude, as well as a pattern of expression that correlated to the desynchronization seen in vitro, suggesting that a similar mechanism may be at work in vivo as well. The model is not without its caveats, though, as the SCN cells appear to lack cell-cell coupling and the induction of singularity in vivo occurs indirectly, via the effect of light pulses on retinal neurons, while the effect is direct on the melanopsin-transduced fibroblasts *in vitro*.

Nonetheless, this work by Ukai, Kobayashi et al., represents an important new insight into a question now more than 30 years old. The phenomenon of singularity is of fundamental interest not only for its biological significance, but also for the role it plays in some forms of sleep disturbance inked with late-night exposure to artificial light, an increasingly common occurrence in the modern world. "It's interesting what this has revealed about how well-balanced a system the biological clock is," says Ueda. "The individual cells keep each other in a steady cycle that is robust in the face of most environmental stimuli."

Tetsuya KOBAYASHI, Hideki UKAI

Link between fertilization and tumor formation in phospholipase C zeta

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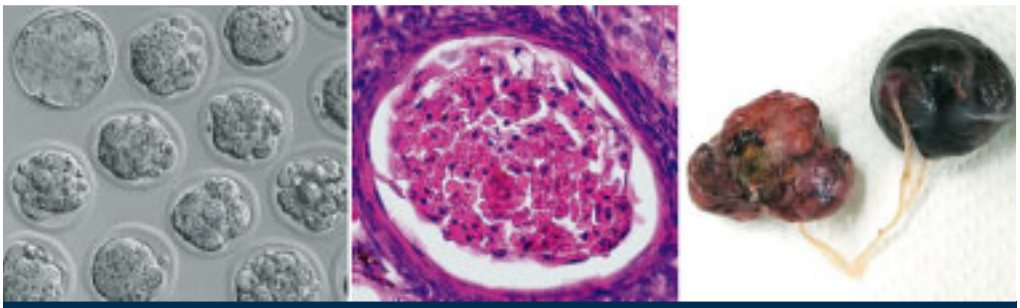
Yoshida N, et al. Broad, ectopic expression of the sperm protein PLCZ1 induces parthenogenesis and ovarian tumours in mice. *Development* 134. 3941-52 (2007)

Mammalian oocytes arrest at a point of the cell cycle called metaphase II (mII). The purpose of this arrest is to prevent the initiation of embryonic development until the arrival of a fertilizing sperm, which sends a signal to the oocyte to stop behaving like an egg and start behaving like an embryo. This transformation includes release from mII arrest, resumption of the cell cycle and the initiation of the developmental program. But the nature of the sperm-borne signal and the means by which it triggers fertilization have proven to be elusive quarry. The Laboratory of Mammalian Molecular Embryology (Tony Perry; Team Leader) previously showed that one component of the sperm signal corresponds to the phospholipase C (PLC) isoform PLC-zeta, PLCZ1. This jibed nicely with the view that sperm prompt meiotic resumption by generating waves of calcium ions, because PLCs in general are able to induce ionic calcium release by hydrolyzing membrane phospholipids. One problem with this idea is that oocytes have PLCs of their own, albeit different isoforms than PLCZ1, begging the question of why PLCZ1 should specifically have the signaling effect it is proposed to have.

In a report published in the journal *Development*, Naoko Yoshida and colleagues extended their studies to address these questions. In particular, they wanted to get a better idea of whether PLCZ1 is able to induce cell cycle progression in oocytes in the absence of other sperm proteins, and if so, whether this activity really was specific to oocytes. Using a transgenic approach, they first determined that *Plcz1* mRNA transcripts are found in the brains of both sexes and in the testis, allowing them to devise experiments in which its expression could be driven ectopically, in sites from which the mRNAs (and presumably protein) are typically absent, including oocytes. To do this, the team focused on two mouse lines with different transgenes in which *Plcz1* expression was driven by a broadly active promoter.



Three stages of tumor formation. Oocytes (eggs) from transgenic, PLCZ1-expressing mice initiate development by themselves (parthenogenesis, left panel), which may seed early ovarian tumors (center) that grow into large masses.

The transgenic lines behaved similarly to each other. Pups were born and developed normally at first. However, females exhibited very low fertility. Their oocytes underwent normal maturation, arriving at mII just like those of their non-transgenic littermates. But unlike normal oocytes, the ones from transgenic mice then immediately progressed to anaphase II and beyond, as if they had been fertilized, often developing to the blastocyst stage in vitro. These findings indicate the exquisite specificity of PLCZ1, which enables it to induce parthenogenetic exit from meiosis II (it is even specific to a particular stage of a particular cell-cycle) in otherwise healthy mice. “This is the first demonstration of ovarian teratoma formation in mice whose oocytes complete meiotic maturation,” says Perry.

The work didn't categorically address whether additional sperm proteins are normally required to lower the threshold of the PLCZ1 signal, because the amount of transgenic PLCZ1 in the oocytes was too small to measure. But the group was able to show that the PLCZ1 does act directly. When they transferred *Plcz1* transgenic cumulus cell nuclei into wildtype oocytes, the oocytes frequently became activated and could develop to the blastocyst stage. It remains to be seen whether the PLCZ1-expressing cells of these mice support full development as if they were 'somatic sperm.'

Although *Plcz1* transgenic mice initially appeared normal and healthy, females developed abdominal swellings caused by ovarian tumors. The frequency of tumor development in females was high (~70% after 6 months) but tumors were never found in transgenic males, corroborating the specificity of the phenotype. The simplest explanation for the tumors is that PLCZ1-induced parthenogenesis occasionally occurred in mature oocytes that failed to be ovulated, and that the resultant trapped parthenogenotes subsequently underwent unchecked ovarian growth to yield the tumors. Questions, however, remain. Most tumors are apparently hemizygous, yet the 'tumor-from-parthenogenote' model doesn't readily explain how. In addition, the parthenogenotes often underwent uterine implantation, but never induced uterine tumorigenesis. It is unclear why ovarian, but not uterine, sites should foster tumor formation. The tumors in some cases accounted for a large proportion of the total body mass, so given their embryonic etiology it is unclear why metastasis was never observed.

Several of the PLCZ1-expressing transgenic mice developed ataxia, a presentation for a subset of clinical ovarian cancer patients. However, the group did not find evidence for mutations in the *PLCZ1* gene in human breast epithelial, ovarian epithelial or benign ovarian germline tumors. Nevertheless, PLCZ1-expressing transgenic mice provide a tractable model for the study of ovarian tumor development, and indicate that PLCZ1 provides an intriguing link between fertilization and tumorigenesis.

Tony PERRY, Naoko YOSHIDA

Np95, enabler of epigenetic inheritance

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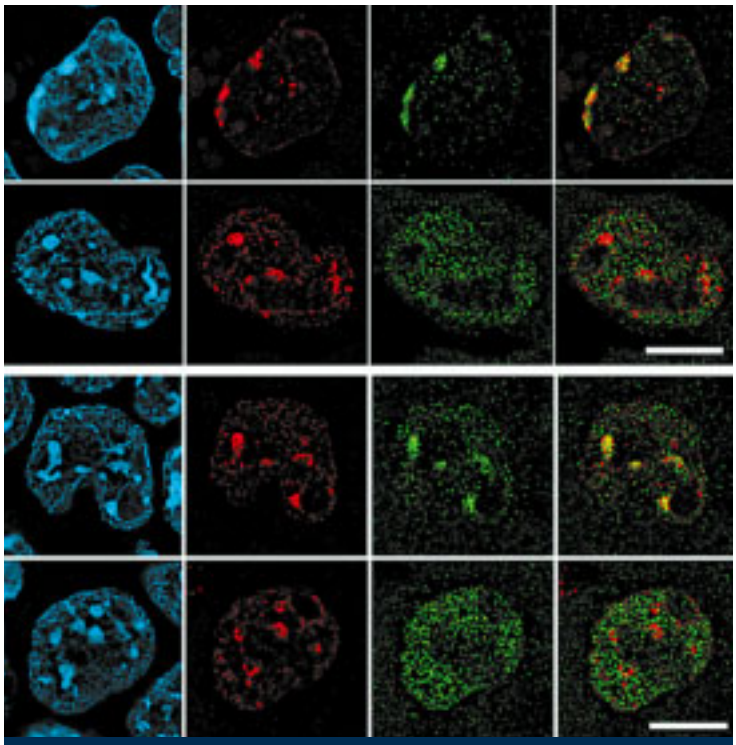
Sharif J, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450. 908-12 (2007)

Methylation is a form of DNA modification that generally mediates stable repression of chromatin and the inhibition of gene expression in methylated regions. While the methylation status of the genome remains relatively stable for most of the mammalian life cycle, the level drops precipitously in the first stages of embryogenesis immediately following fertilization, only to be re-established in the body's many cell lineages as ontogeny moves forward. It is thought that this role in controlling which genes are switched on and off makes DNA methylation an important mechanism in lineage commitment. But it has remained something of a puzzle how the methylation state of a double-strand of DNA can be inherited by both progeny of a cell when the DNA is replicated during cell division.

A joint study conducted by researchers at the RIKEN Research Center for Allergy and Immunology, Tohoku University, and the CDB Laboratory for Mammalian Epigenetic Studies (Masaki Okano, Team Leader), revealed a molecular mechanism by which DNMT1, an enzyme that helps to maintain the heritability of DNA methylation, is recruited to appropriate sites in the genome during replication. Shinichiro Takebayashi, a research scientist in the Okano lab, performed cytological analyses that enabled the collaboration to show the involvement of the protein Np95 in Dnmt1 recruitment. The team's work was published in the journal *Nature*.

DNA methylation takes the form of *de novo* establishment and subsequent inheritance, processes that are dependent on different members of the DNA methyltransferase (Dnmt) family of enzymes: Dnmt3a and -3b work in *de novo* methylation, while Dnmt1 functions in inheritance. This occurs in stretches of DNA known as CpG islands, which are abundant in otherwise relatively uncommon cytosine-guanine di-nucleotide pairings. Methyl groups selectively bind to cytosine in these two-base sequences.

Takebayashi and colleagues were interested specifically in the potential role of Np95, a mouse protein that functions in cell cycle progression, DNA repair and chromatin replication, and which is closely related to a factor known to be involved in the recognition of methylated CpG islands and DNA



Immunofluorescence showing Np95 localization to methylated DNA during DNA replication in ES cells. See Sharif et al. for details.

Shin-ichiro TAKEBAYASHI

methylation in plants. When they looked at wildtype embryonic stem cells, they found that Np95 co-localized with Dnmt1 in replicating nuclei. In cells engineered to lack all three known DNA methyltransferases, Dnmt1, Dnmt3a and Dnmt3b, however, not only was DNA methylation activity lost, but Np95 failed to localize and remained diffusely expressed throughout the triple knockout ES cells as well. Intriguingly, when they induced the methylation of only a single strand of the replicating DNA in these mutant cells, the Np95 localization resumed, suggesting that Np95 must be capable of recognizing and binding to "hemi-methylated" sites in which only one of the two strands of replicating DNA is modified by a methyl group. Mice carrying mutations in the Np95 gene showed a failure of DNA methylation and embryonic lethality, pointing to a critical role for this protein, while in Np95- cells, Dnmt1 failed to accumulate at heterochromatic domains.

This set of findings suggests that Np95 is somehow capable of recognizing hemi-methylated DNA during replication, and recruiting Dnmt1, thereby ensuring that both sets of DNA will bear the same methyl modifications. While the mechanistic details behind this activity are still unknown, the model that emerges from this study goes a long way toward explaining one of the great mysteries of epigenetic inheritance.

"These results extended our understanding of epigenetic inheritance by DNA methylation," says Okano. "Further mechanistic analyses may provide clues for a better understanding of DNA methylation-reducing process during epigenetic reprogramming or aberrant DNA methylation in cancer."

Getting inside invagination

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Nishimura M, et al. A wave of EGFR signaling determines cell alignment and intercalation in the *Drosophila* tracheal placode. *Development* 134. 4273-82 (2007)

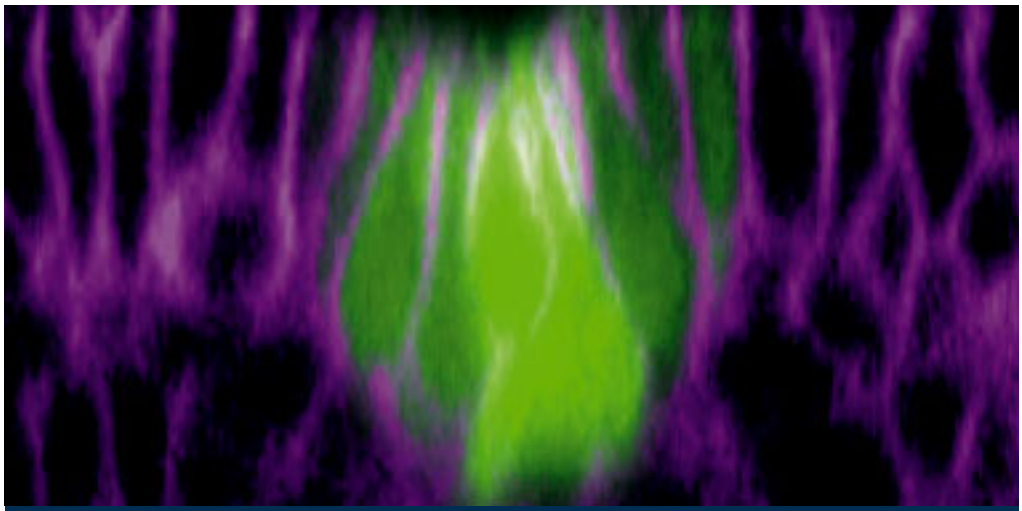
Many organ systems in animals are derived from a sheet-like layer of cells, the epithelium, that encloses the early embryo. Each organ is segregated from distinct primordial regions consisting of thickened epithelium, known as placodes. Orderly internalization of the placode segregates and shapes organ primordia into unique morphologies in the process called invagination. During invagination, the epithelial cells coordinate their movements such that they internalize through a clearly defined pore or furrow in a precise time course. The molecular machinery that enables cells at the placode to communicate with each other, however, is unclear. It has also remained a mystery how the cells in a flat sheet of epithelium could be squeezed through a narrow pore to the inside of the body cavity.

Image analyses by Mayuko Nishimura and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi; Group Director) performed on the *Drosophila* embryo have shown that the invagination of the *Drosophila* tracheal placode involves rearrangements of cells in which the distribution of the non-muscle myosin motor protein is controlled by the EGFR pathway.

The Hayashi group used time-lapse imaging of the shape changes, movement and proliferation of GFP-tagged primordial tracheal cells, allowing them to compare changes in the distribution of cytoskeletal F-actin and myosin. Approximately one hour prior to invagination, the cells stopped dividing and about 10 of them began to narrow at their apical surfaces and shift basally, leaving a depression in the center of the circle (the tracheal pit). Tracing the cells in the crucial minutes before invagination showed that the intercalation process by which migrating cells displace others proceeds in a polarized fashion, with cells moving toward the center of the prospective placode. This intercalation event explains, at least in part, why cells in the flat sheet of epithelium can be squeezed into a narrow tracheal pit.

Nishimura's image analyses also revealed that groups of four to six cells immediately surrounding the placode often arrayed themselves in such a way cells which had once had zigzag contours began to form smoother, arc-like boundaries (a process the group has dubbed "boundary smoothing"). By tracing the accumulation of myosin, a protein linked to contractile activity at cell junctions, they found that although myosin expression started at generally low levels, it was upregulated in cells undergoing boundary smoothing and particulary in those with shrinking junctions.

Suspecting that the signaling pathway mediated by the tyrosine kinase receptor epidermal growth factor (EGFR), which has previously been implicated in tracheal invagination, might be involved in boundary smoothing and intercalation, Nishimura et al. used an antibody against double



An optical cross section of the *Drosophila* tracheal placode (green) in an early stage of invagination; cells showing apical constriction. Cell boundaries marked with magenta.

Mayuko NISHIMURA, Shigeo HAYASHI

phosphorylated ERK (dp-ERK), the downstream factor of EGFR, to visualize the activity of EGFR signaling during the formation of the tracheal primordium. In the 30-minute period before invagination, they saw the dp-ERK move from nuclear and cytoplasmic sites to the apical cortex near the surface of the cell, before fading at the onset of invagination. Interestingly, this pattern of dp-ERK distribution under the control of EGFR appears as a ring expanding outwards from a center that spatially coincides with the tracheal pit where invagination later takes place.

Studies of flies bearing mutations for various genes associated with EGFR signaling showed that this pathway is required for both the apical constriction and for tracheal invagination to occur at the proper timing. In *Egfr* mutants, the onset of invagination was delayed and the apical constriction significantly impaired, and similar, though less pronounced, phenotypes were seen in mutants for an EGFR-activating factor and a target factor that functions downstream of the EGF receptor. These mutants also exhibited defects in the cell arrangements responsible for controlling the location of the tracheal pit, where invagination occurs; in contrast to wildtype, mutants frequently developed ectopic or supernumerary tracheal pits.

Looking next to a possible regulatory relationship between EGFR signaling and myosin, the group examined cells labeled for both myosin and dp-ERK. They found that the boundaries of cells with high ERK expression correlated strongly with the arcs of high-myosin cells, and that such arc-like rows were absent in *Egfr* mutants. Misexpression of an EGFR-activating ligand in normally flat epithelium produced an interesting mimic of the invagination phenotype of epithelial depression and myosin accumulation at the borders of neighboring cells in which one has high, and the other, low, ERK activity.

“In this study, we were able to show a functional link between EGFR signaling and myosin, a major motor protein causing cell contractility,” says Hayashi. “Although the myosin-dependent force in each of the cells is small, its coordination in arc-like cell rows is likely to generate a directed force that drives net movement of the placode. This wave-like propagation of EGFR activity explains how cell contractility in a large field of epithelia remains coordinated. And, as myosin activity is a general requirement for placode invagination and body axis elongation in both insects and vertebrates, we anticipate that a similar wave-like mechanism of signal propagation will be found in other organ systems.”

A mystery of the cadherin-actin interaction resolved

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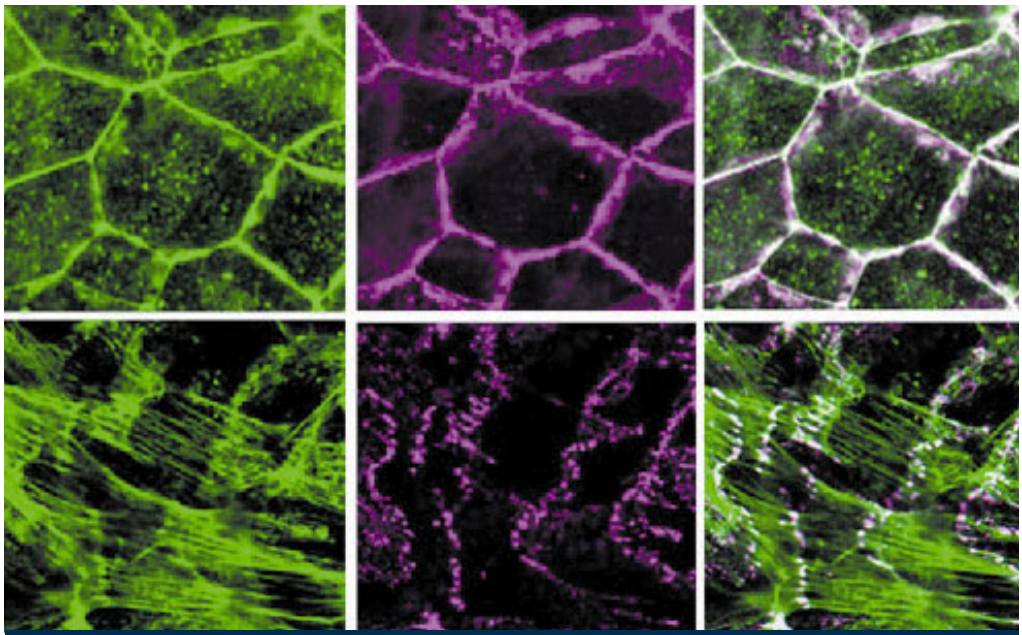
Abe K and Takeichi M. EPLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. *Proc Natl Acad Sci U S A* 105. 13-9 (2008)

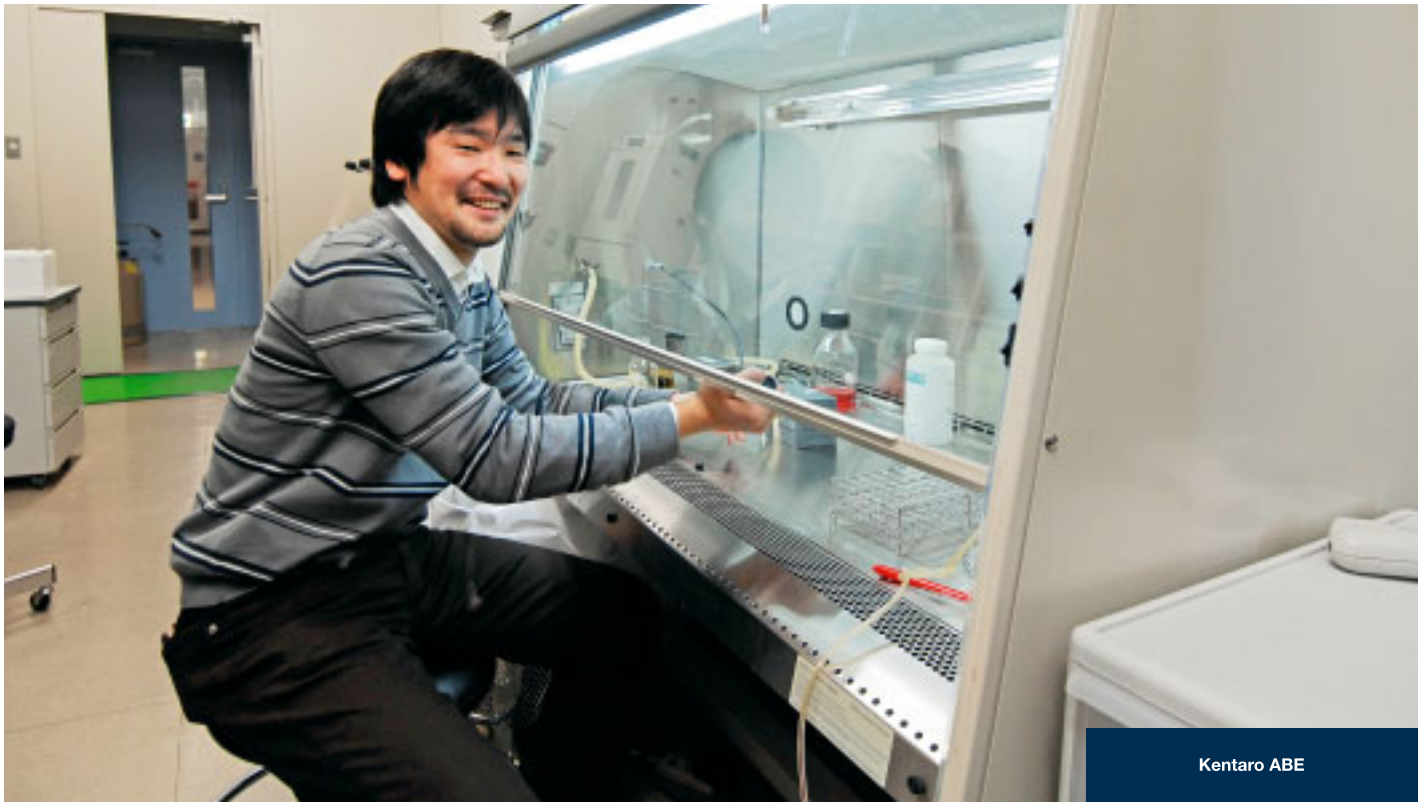
Epithelial cells have well-defined top (apical), bottom (basal) and side (lateral) surfaces. Cadherin-based cell-cell adhesions can form between the lateral aspects of neighboring cells, enabling large groups of cells to aggregate into sheets and tubes, as well as more complex structures. But such adherens junctions are not limited solely to the intercellular space; they extend to into the cytoplasm of the adhering cells as well, through bonds between the cadherin-catenin machinery and the actin cytoskeleton. In addition to serving as a crucial anchor for the cell adhesion molecules, the cytoskeleton is also vitally important for its function in cell morphology and movement. But the molecular mechanism by which the actin cytoskeleton, which extends around the inner surface of the cell membrane in what is called the "circumferential actin belt," is linked to the cell adhesion complex is still imperfectly understood.

Work by Kentaro Abe of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) points to a role for the actin-binding protein EPLIN (Epithelial Protein Lost In Neoplasm) in bringing these adhesion and cytoskeletal apparatuses together. His findings were reported in the *Proceedings of the National Academies of Sciences*.



EPLIN knockdown epithelial (lower panel) and control cells (upper panel) stained to visualize F-actin (green) and E-cadherin (magenta). In control cells, honeycomb-like junctional organization is seen, in which cadherin linearly accumulates at the cell junction and co-localizes with F-actin. In the EPLIN knockdown cells, E-cadherin accumulates only at points, and F-actin runs perpendicular to the cell junction.

Spurred by a 2005 study that showed no direct binding between the catenin-cadherin complex and actin, Abe and Takeichi initiated a screen for binding partners of α-catenin, one of the three catenin proteins (the others are β- and p120-catenin) that bind to cadherin's cytoplasmic tail, in the hopes of identifying candidates for the missing link. Co-immunoprecipitation, a technique used to discover protein interactions, showed that EPLIN bound to α-catenin, and subsequent studies using mutants in which various domains of the EPLIN protein were deleted revealed that this interaction requires both the N- and C-terminal domains of EPLIN, and a protein-binding region known as VH3-C in α-catenin. The case for EPLIN association was further shored up by its localization within epithelial cells, which overlapped closely with cadherin, catenin and F-actin along lateral cell-cell contact sites.



Kentaro ABE

To test for EPLIN's role in intercellular junctions, Abe next knocked down EPLIN expression by siRNA and found this had a dramatic effect; adhesion belt organization was disrupted, and cadherin showed up only in isolated points, rather than its usual uninterrupted pattern of expression. Interestingly, junctional F-actin became radially organized, terminating at points of cadherin expression, while non-junctional actin was apparently unaffected.

EPLIN is known to suppress actin depolymerization (the loss of actin molecular units from the ends of long, strand-like actin fibers known as F-actin), so Abe next tested whether this is important to the role played by EPLIN at the adhesion belt as well, and determined that it does stabilize F-actin, but that it does so independent of α-catenin. The association of catenin with F-actin, however, is highly dependent on EPLIN, as was shown by experiments in which beads coated with α-catenin that had either been treated or untreated with EPLIN were incubated with F-actins: only the treated beads pulled down F-actin. And, by adding EPLIN to the cadherin-catenin complex coated beads, they were able to reconstruct the cadherin-F-actin association, which had been called into doubt by the 2005 study.

Abe's series of experiments has revealed a new function for EPLIN in linking the cadherin-catenin machinery to the actin cytoskeleton, but the fact that F-actin radiates to bind cadherin at punctate cell contact points in EPLIN deletion mutants suggests that other molecules mediating actin-cadherin complex association still await discovery. The roles of the different protein binding VH domains in α-catenin may hold clues to this mystery.

"Although the classic view of the cadherin–F-actin association was recently challenged, it was clear that cadherin always co-localizes with F-actin, which made the new story difficult for some to accept," says Takeichi. "Our results could settle at least part of the controversy, by revealing a new mediator between cadherin and F-actin, which suggests that the old understanding was not so wrong after all. And of course there may yet be new stories to be found, and so we will continue to dissect this mysterious molecular machinery."

RMD-1, ploidy protector

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Oishi K, et al. RMD-1, a novel microtubule-associated protein, functions in chromosome segregation in *Caenorhabditis elegans*. *J Cell Biol* 179. 1149-62 (2007)

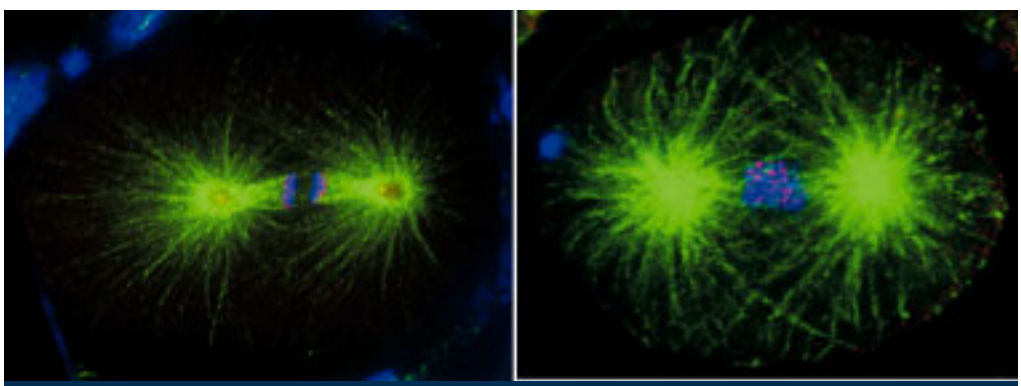
When cells divide, they must first make faithful replicas of the full set of genetic material, then segregate the sister chromosomes in such a way that each daughter cell will receive an equal complement as the halves of the dividing cell pull apart. This involves intricate and tightly controlled coordination between the mitotic nucleus and the cytoskeleton, which provides both the anchorage and traction needed to complete cytokinesis (the division of the cytoplasm). Failures in chromosome segregation, which can result in aneuploidy (an abnormal number of chromosomes), are generally corrected by the anaphase stage of mitosis, thanks to the safeguard action of a mechanism known as the "spindle checkpoint," which prevents cell division from progressing until all chromosomes are properly attached to microtubules. Despite the central importance of this mechanism, however, the molecular workings of the spindle checkpoint have yet to be fully determined.

Kumiko Oishi, a research scientist working in the Laboratory of Cell Fate Decision (Hitoshi Sawa; Team Leader) added an important new piece to the puzzle, with the identification of *rmd-1*, a novel *C. elegans* gene that localizes to spindle microtubules and attachment sites at spindle poles. Working in collaboration with a team at the Keio University School of Medicine, Oishi showed that this microtubule-associated protein is required for chromosome segregation, possibly through an interaction with aurora B kinase, another factor believed to be involved in the recognition and correction of abnormal chromosome-cytoskeleton attachments.

The Sawa team first identified the *rmd-1* phenotype in a screen for mutants lacking a specific cell type known as phasmid socket cells, and pinpointed the genetic defect to a single point mutation. The gene encodes a hitherto unknown protein, but one which, interestingly, has possible homologs in frog, zebrafish, mouse and human. Its pattern of expression shows a high level of co-localization with tubulin (a microtubule marker) during cytokinesis.

She next studied the loss of function phenotype of *rmd-1*, using RNAi to knock the gene down. The *rmd-1* -inhibited embryos showed a high incidence of defects in chromosome segregation, and other aspects of cytokinesis, such as spindle orientation. A subsequent series of experiments showed that these defects were caused by the failure of the microtubules to attach properly to the chromosome, at sites called kinetochores. Specifically, chromosomes in *rmd-1* (*RNAi*) embryos tended to form merotelic attachments, in which a single kinetochore becomes attached to microtubules from both spindle poles, rather than just one.

The defects in chromosome segregation in *rmd-1* (*RNAi*) embryos bore some resemblance to those seen in other organisms in mutants for aurora B, a kinase that destabilizes microtubules that have attached incorrectly to chromosomes. When Oishi conducted a pull-down assay to test for protein-protein associations, she found that RMD-1 indeed co-precipitates with the *C. elegans* homolog of aurora B.



Wildtype (left) and rmd-1(RNAi) (right) embryos at anaphase at one-cell stage. Microtubules are shown in green, kinetochore protein, MCAK is red, DNA is blue, and microtubules that overlap with chromosomes are represented in light blue.

While the Sawa team further determined that RMD-1 plays an additional role in orienting the spindles at opposite poles, they were able to rule out the possibility that the *rmd-1* (RNAi) causes ploidy problems by slowing microtubule growth, strengthening the case for a role in the aurora B-mediated pathway, which serves an important editorial function in maintaining proper chromosome segregation. This is of particular interest, as the evolutionarily conserved nature of *rmd-1* suggests that its human counterpart may play a role in preventing aneuploidy, which is implicated in cancer and various congenital anomalies, in humans as well.

Commenting on the discovery, Oishi says, "We hope that our findings will contribute to a fuller understanding of the evolutionarily conserved mechanism that maintains the fidelity of chromosome segregation. The discovery of the RMD protein family may also deepen our knowledge of microtubule-based processes."

Kumiko (and Momoko) OISHI

Awards and Prizes

A number of CDB scientists were recognized in 2007 by academic societies and research institutions for their recent contributions or lifetime achievements.

Sangmo KWON	The 5th Young Investigator's Award for International Students	Japanese Circulation Society
Takahiro SUZUKI	ARVO/Alcon Early Carrer Clinician-Scientist Research Award	The Association for Research in Vision and Ophthalmology (ARVO) Foundation for Eye Research
Yasuhiro TAKASHIMA	Technical Achievement Award	Japanese Society of Molecular Medicine
Shigeru KURATANI	Society of Evolutionary Studies Prize	Society of Evolutionary Studies, Japan
Shigeru KURATANI	Motoo Kimura Memorial Prize	Motoo Kimura Memorial Fund
Shigehiro KURAKU	Zoological Science Award	The Zoological Society of Japan
Shigeru KURATANI	Zoological Science Award	The Zoological Society of Japan
Kinya OTA	Recognition of Merit Prize	The Zoological Society of Japan
Sugako OGUSHI	Prize for Best Oral Presentation	Japanese Society of Animal Reproduction
Hiroki UEDA	5th Annual Chronobiology Prize	Japanese Society for Chronobiology
Masayo TAKAHASHI	Pfizer Ophthalmics Award, Japan	Pfizer Inc.
Fumitaka OSAKADA	2007 ISSCR Poster Presentation Award	International Society for Stem Cell Research
Shin-Ichi NISHIKAWA	Kobe Industry Achievement Award	City of Kobe

CDB Director elected to the National Academy of Sciences

On May 1, the National Academy of Sciences (USA) announced the election of Masatoshi Takeichi (Director, RIKEN CDB) as a foreign associate member in recognition of his distinguished and continuing achievements in original research. The election inducted a total of 72 new members and 18 foreign associates from 12 countries into the NAS, bringing the total number of active members to 2,025 and the total number of foreign associates to 387. Takeichi was recognized for his significant contributions to science, which include the discovery of the cadherin superfamily of cell-cell adhesion molecules, and the functional characterization of cadherins in systems ranging from embryonic epithelium to the nervous system.

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). The MIRP focuses on the research into the visualization of molecular function and dynamics in living bodies.

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.

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.

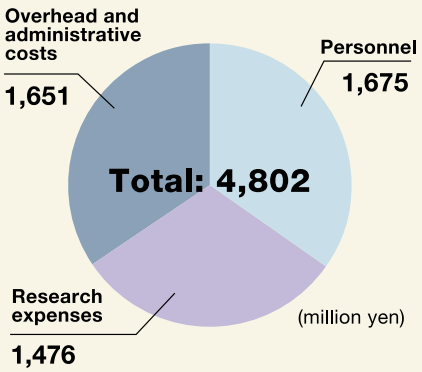
Kobe Institute Administrative Structure

- 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

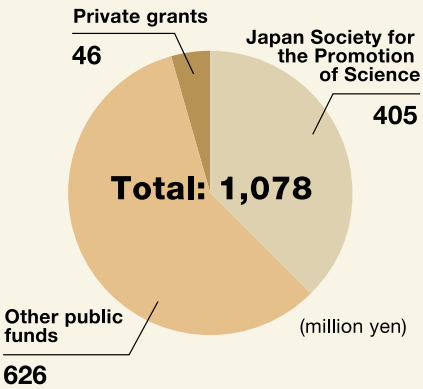
Molecular Imaging Research Program

In the spring of 2007, the CDB was joined by a second research program when the Molecular Imaging Research Program (MIRP) officially joined the RIKEN Kobe Institute. The MIRP was opened in autumn 2006 as a branch of the Discovery Research Institute headquartered in Wako. It seeks to integrate physics, chemistry, engineering, computer sciences, biology and medicine toward the development of more advanced molecular imaging technologies, with a focus on positron imaging tomography. The MIRP will seek to contribute to drug discovery and the development of diagnostic and therapeutic indicators by creating high-performance molecular probes and imaging technologies and techniques.

2007 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 2007.



2007 CDB Staff

Laboratory heads	33
Research scientists	126
Research associates	9
Technical staff	115
Assistants	29
Visiting scientists	64
Student trainees	49
Part-time staff	31
Research Promotion Division	50
Other	51
Total	**557**

2007 CDB Symposium

Germ Line versus Soma: Towards Generating Totipotency

March 26-28, 2007

The fifth CDB symposium focused on the theme, "Germline versus Soma: Towards Generating Totipotency", a dichotomy that has attracted intense scientific interest for more than 100 years. In metazoans, the cells of the germ line are unique in their ability to transmit genetic information across generations, and are characterized by the expression of numerous molecular factors involved in the maintenance of genomic continuity, diversity and cellular totipotency. The advent of germ line cells during embryogenesis involves processes that are believed to be linked with the differentiative potency and nuclear reprogramming exhibited by many types of somatic and embryonic stem cells, as well as distinctive genomic structures and gene regulatory mechanisms. It is also hoped that by developing a better understanding of the genetic and epigenetic control of these processes, it will be possible to recapitulate them in vitro and make real contributions to the advancement of regenerative medicine.

Session 1

Ruth LEHMANN (Skirball Institute, NYU School of Medicine, USA)
Azim SURANI (University of Cambridge, UK)

Session 2

Hiroshi HANDA (Tokyo Institute of Technology, Japan)
Yi ZHANG
(Howard Hughes Medical Institute and University of North Carolina, USA)

Session 3

Susan STROME (Indiana University, USA)
Edith HEARD (CNRS, France)
Joern WALTER (Saarland University, Germany)

Session 4

William G. KELLY (Emory University, USA)
Akira NAKAMURA
(RIKEN Center for Developmental Biology, Japan)
Mitinori SAITOU (RIKEN Center for Developmental Biology, Japan)

Session 5

Margaret T. FULLER (Stanford University School of Medicine, USA)
Patrick WESTERN
(Murdoch Childrens Research Institute / ARC Centre for Biotechnology and Development, Australia)
Shosei YOSHIDA (Kyoto University, Japan)

Session 6

Mark VAN DOREN (Johns Hopkins University, USA)
Satoru KOBAYASHI (National Institute for Basic Biology, Japan)
Goro YOSHIZAKI
(Tokyo University of Marine Science and Technology, Japan)

2008 CDB Symposium

Turning Neurons into a Nervous System

March 24-26, 2008

The sixth CDB symposium, entitled,"Turning a Neuron into a Nervous System," will focus on a developmental neurobiology approach to neurons as units of a functional hierarchical organization that allow the nervous system to interpret sensation, and produce responses, behaviors and higher order mental processes. The father of neuroscience, Ramon y Cajal expressed the importance of the developmental approach eloquently, saying, "since the full grown forest turns out to be impenetrable and indefinable, why not revert to the study of the young wood, in the nursery stage?" This symposium seeks to explore the hierarchical organization of neurons and how this organization comes about, addressing such questions as how connections between nerve cells form, how the developing nervous system prunes redundant neurons, the role of neural activity in modifying the neural circuits established during development, and how the mature nervous system generates new neurons.

Session 7

Thomas JENUWEIN (IMP, Austria)
Tom MISTELI (NIH, USA)
Kazuo YAMAGATA (University of Tsukuba, Japan)

Session 8

Wolf REIK (The Babraham Institute, UK)
Toru NAKANO (Osaka University, Japan)
Gary RUVKUN (Harvard Medical School, USA)

Session 9

Geraldine SEYDOUX
(Johns Hopkins School of Medicine, USA)
Rafal CIOSK
(Friedrich Miescher Institute for Biomedical Research, Switzerland)
Atsushi SUZUKI
(National Institute of Genetics, SOKENDAI, Japan)

Session 10

Minoru S. H. KO (NIH, USA)
Hitoshi NIWA
(RIKEN Center for Developmental Biology, Japan)
Ryuichi NISHINAKAMURA
(Kumamoto University, Japan)

Session 11

John J. EPPIG (The Jackson Laboratory, USA)
Hung-Chih KUO (Academia Sinica, Taiwan)
Shinya YAMANAKA (Kyoto University, Japan)

Arturo ALVAREZ-BUYLLA (UCSF School of Medicine, USA)
Silvia ARBER (University of Basel, Switzerland)
Herwig BAIER (University of California, San Francisco, USA)
Tobias BONHOEFFER
(Max-Planck-Institut für Neurobiologie, Germany)
Mario R. CAPECCHI
(University of Utah School of Medicine, USA)
Aaron DIANTONIO
(Washington University School of Medicine, USA)
Barry J. DICKSON
(IMP-Research Institute of Molecular Pathology, Austria)
Hideki ENOMOTO (RIKEN CDB, Japan)
Joseph G. GLEESON (University of California, San Diego, USA)
Elizabeth GROVE (University of Chicago, USA)
Barbara HEMPSTEAD
(Weill Medical College of Cornell University, USA)
Takao K. HENSCH (Harvard University, USA)
Kozo KAIBUCHI (Nagoya University, Japan)
Matthew KELLEY (NIDCD/NIH, USA)
Raj LADHER (RIKEN CDB, Japan)
Ikue MORI (Nagoya University, Japan)
Hitoshi OKAMOTO (RIKEN Brain Science Institute, Japan)
Hideyuki OKANO (Keio University School of Medicine, Japan)
Edwin W. RUBEL (University of Washington, USA)
Joshua R. SANES (Harvard University, USA)
Yoshiki SASAI (RIKEN CDB, Japan)
Dietmar SCHMUCKER (Harvard Medical School, USA)
William SNIDER (UNC Neuroscience Center, USA)
Thomas C. SÜDHOF (Howard Hughes Medical Institute, USA)
Masatoshi TAKEICHI (RIKEN CDB, Japan)
Li-Huei TSAI (Picower Institute for Learning and Memory, MIT, USA)
Christopher A. WALSH (Howard Hughes Medical Institute, USA)
Mei ZHEN (Samuel Lunenfeld Research Institute, Canada)
Larry ZIPURSKY (Howard Hughes Medical Institute / UCLA, 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 nearly 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, 2007:

date	title	speaker
01-11	Lineage decisions in the early mammalian embryo	Janet ROSSANT
01-11	Polarized guidance of microtubules at the migrating edges of cells by +TIPs	Yuko MIMORI-KIYOSUE
01-15	Genome-wide profiling of histone H3.3 replacement patterns	Yoshiko MITO
01-24	Lymphoid organ development in the human fetus	Tom CUPEDO
02-01	Ran-GTP dependent microtubule stabilization which is involved in spindle assembly	Hideki YOKOYAMA
02-26	Probing intracellular cholesterol trafficking: Lessons from *Drosophila* models of Niemann Pick type C disease	Xun HUANG
02-26	Genetic approach to dissect the mechanisms of Fragile X mental retardation	Yong Q. ZHANG
03-01	NG2 glia (polydendrocytes): their function and lineage	Akiko NISHIYAMA
03-06	Genetic origins of cortical interneuron subtypes	Goichi MIYOSHI
03-08	Nucleocytoplasmic protein transport, its diversity and cell differentiation	Yoshihiro YONEDA
03-20	Centriole function in development	Cayetano GONZALEZ
03-22	Exploration of mechanotransduction in cells: Mechano-sensing by physical extension of the Src family kinase substrate p130Cas	Yasuhiro SAWADA
03-23	Molecular mechanism of anti-cancer therapies	Hideyuki SAYA
03-29	Developmental plasticity of cone-photoreceptor precursor cells in postnatal mouse retina	Yasuyuki WATANABE
04-11	Transcriptional network regulating limb development	Hiroshi ASAHARA
04-19	Downstream components of Wnt signaling that control the maintenance of retinal stem cells in the ciliary marginal zone	Shinichi NAKAGAWA
04-24	Mysteries in growth control	Georg HALDER
04-25	Spermatogonial stem cell renewal in the *Drosophila* testis	Erika MATUNIS
04-25	Transcription factors, miRNAs and neuronal diversity in the nematode *C. elegans*	Oliver HOBERT
05-17	Transcriptional and posttranscriptional gene silencing at heterochromatic domains in fission yeast	Tomoyasu SUGIYAMA
05-21	The role of "*Time*" in mammalian neurogenesis	Federico CALEGARI
05-31	Integration of mechanical and chemical signals in cell protrusion	Gaudenz DANUSER
06-04	Evolution in the vertebrate body plan: Perspective from the lateral plate	Ann CAMPBELL BURKE
06-21	The role of cellular senescence in cancer and aging.	Eiji HARA
07-20	Signaling pathways that regulate growth and development in the *Drosophila* wing imaginal disc	Thomas B. KORNBERG
07-27	TRP channels and magnesium homeostasis	Kouichi IWASAKI
07-31	Roles of the Store-operated Ca2+ entry in T cell development and function	Masatsugu OH-HORA
08-01	Foxc transcription factors in arterial endothelial cell specification	Tsutomu KUME

date	title	speaker
08-02	The FlyMine and InterMine projects	Gos MICKLEM
08-03	Nuclear transport and spindle microtubule formation in fission yeast: Ran GTPase and microtubule-associated protein TACC/Alp7	Masamitsu SATO
08-10	Identifying microRNAs and their targets	Nikolaus RAJEWSKY
08-15	BMP signaling in eye development: from induction to visual function	Yasuhide FURUTA
09-14	Beyond the genome: Automated, continuous analysis of embryonic gene expression with cellular resolution in *C. elegans*	Robert H. WATERSTON
09-18	Development and immunity in the nematode *C. elegans*	Jonathan HODGKIN
09-26	Cis-regulatory perspective of *Hox* complex genes	Takashi KONDO
10-01	Systematic studies of gene expression patterns in *C. elegans.*	Ian HOPE
10-05	Control of neural crest cell migration in the developing gut	Heather YOUNG
10-05	The development of sympathetic neurons and satellite glia	Colin ANDERSON
10-09	Developmental ability of individual 2-cell blastomeres in mice	Mika KATAYAMA
10-09	Physical constraints and limits to precision in early embryonic development	Thomas GREGOR
10-09	The current paradigm of mammalian oocyte cryopreservation, and a proposed alternative.	Steven F. MULLEN
10-25	Epigenetic programming of mesenchymal stem cells	Philippe COLLAS
10-29	Normal and leukaemic stem cells: What's new?	Dominique BONNET
10-30	Role of Rho-kinase in cell polarization and directional migration	Masanori NAKAYAMA
11-01	High throughput gene targeting in the mouse	William C. SKARNES
11-05	Zic2 is required for prechordal plate development in the mouse	Ruth ARKELL
11-12	Embryonic stem cells: the changing genetic landscape	Andras NAGY
11-15	Embryonic skeletal muscle development: Novel aspects in the formation of avian perineal complex and lymph hearts	Ketan PATEL
11-19	Molecular pathways of cardiac progenitor proliferation and survival	Frank CONLON
11-20	Hedgehog signaling in development and disease	Philip A. BEACHY
11-20	Engineering cellular behavior: The polarity machinery of chemotactic cells	Takanari INOUE
11-20	Post-transcriptional regulation during early *Drosophila* development	Howard D.LIPSHITZ
11-21	SOX2 function in retinogenesis	Larysa PEVNY
11-22	Functional variation and evolution of human odorant	Hiroaki MATSUNAMI
11-27	The Wnt/beta-catenin signaling in development and metastasis	Walter BIRCHMEIER
12-07	The first family of eukaryotic cell-cell fusion proteins	Benjamin PODBILEWICZ
12-07	Alternative pathways to polarize the anterior-posterior axis and epithelia in *Drosophila*	Daniel St JOHNSTON
12-17	Novel roles for mouse Polycomb Group proteins in pericentric heterochromatin	Antoine H.F.M. PETERS

RIKEN Activities

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.

RIKEN Website

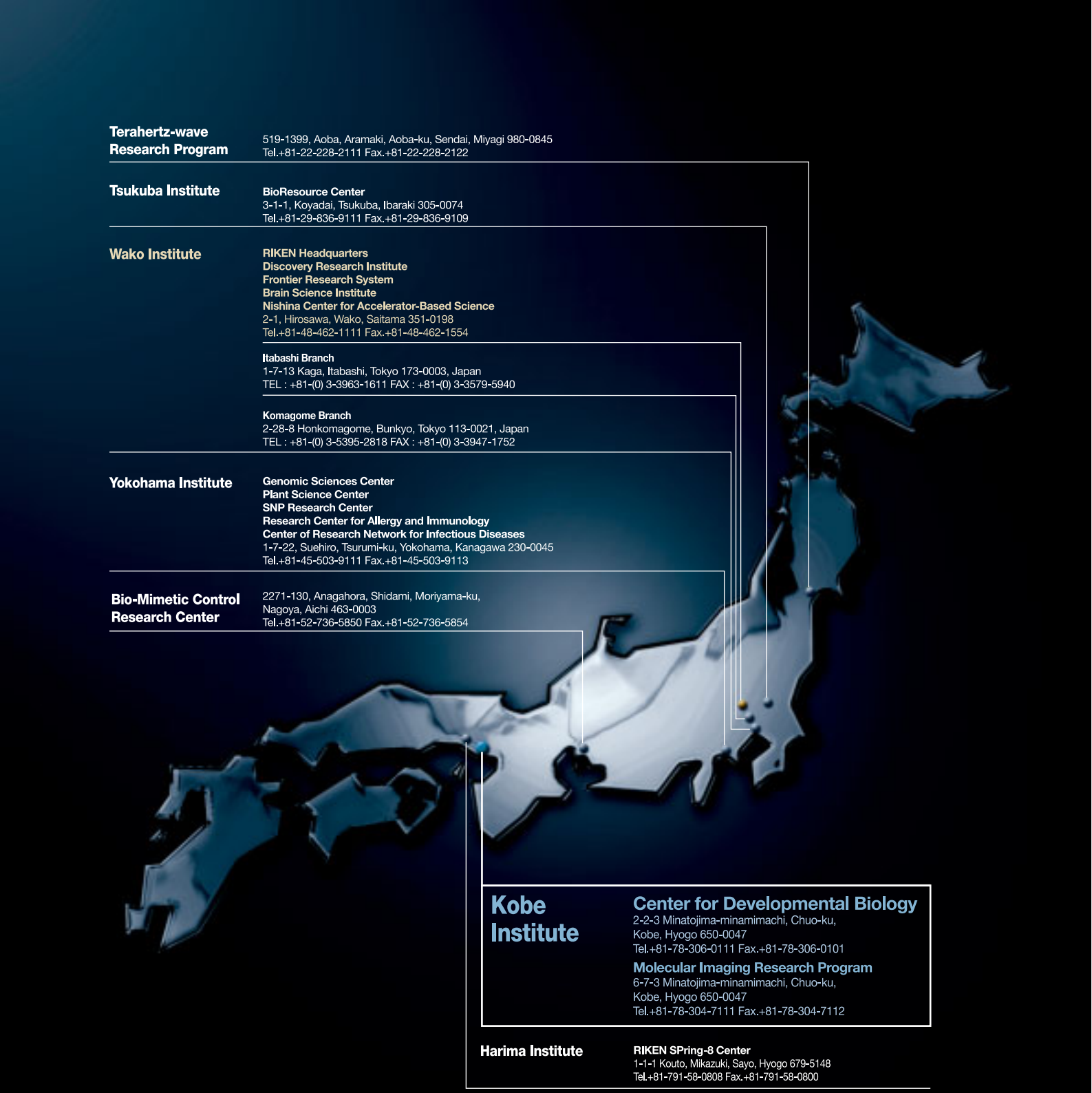
RIKEN has recently re-designed its website to provide a fuller and richer experience for online visitors to the institute. The new site contains important links to all RIKEN institutes nationwide, a full range of public information and materials, as well as databases and other electronic resources developed 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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On The Cover

Scanning electron micrograph of the neural tube of a 7-somite stage chick embryo. The edges of the precursor to the CNS are now touching and will soon fuse to form the hollowed neural tube.

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