RIKEN Center for Developmental Biology

2004 Annual Report

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Director
Masatoshi Takeichi

Deputy Directors
Shin-ichi Nishikawa
Shinichi Aizawa

Institutional Review Board

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Core Program

The Core Program aims to promote highly innovative developmental and regeneration studies in a strategic and multi-disciplinary manner. This program constitutes the core research framework to achieve the aims of the Millennium Project, and 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 laboratories offer technical support, develop new technology, and conduct independent research projects. Their services are available to all CDB research groups and teams.

RIKEN Center for Developmental Biology

It is gratifying to be able to say that, by any standard, 2004 has been a banner year for the RIKEN Center for Developmental Biology. Established less than five years ago under the auspices of the Japanese government's Millennium Project research funding initiative, the CDB has blossomed into one of the world's foremost sites for the study of animal development and regeneration. It feels a bit unusual for me as the director of the Center to make such an unqualified statement of success, but the assessment is not mine alone. In June of this year, the CDB Advisory Council, a panel of ten eminent developmental biologists from around the world, met for its second plenary session to review the Center's performance to date and to make recommendations for the future. The process was intensive, with reviews of individual labs as well as a critical survey at the organizational level. Happily, the Council's findings were positive and encouraging and served to reinforce my own feelings that our young institute has gotten off to a remarkable start.

Of course the true measure of any scientific organization can be found in its research achievements; that, after all, is our *raison d'être*. In this sense, again, 2004 has been a fine year, with CDB labs publishing pioneering studies with far-reaching implications, from investigations of translational repression in *Drosophila* germline development to the explication of a timetable of gene expression useful for measuring the activity of biological clocks in mice. The diversity and depth of the year's achievements is reflected in the organization of this year's annual report, which has been redesigned to feature research highlights from the Center's labs in detail, while continuing to provide an overview of all active research and ancillary programs.

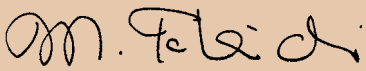
Publication in scientific journals, however, is only one aspect of the research endeavor. The opportunities that scientific seminars and meetings provide for the discussion of research with peers is at least as important to ensuring the quality of experiments and the rigor of conclusions, and provides an immediate forum for disseminating one's findings and receiving informed feedback. The CDB recognizes the value of scientific meetings and has strengthened the support mechanisms that allow our research staff to host individual speakers or organize more extensive programs of multiple talks. This led to a dramatic increase in the number of events hosted at the Center in 2004 that had an international focus, particularly meetings involving scientists from the Asia-Pacific region. We look forward to the continuation of this trend in future years as a means of encouraging new relationships and fostering existing collaborations throughout the global developmental biology community.

RIKEN is one of Japan's largest taxpayer-funded research organizations, and as such is not charged with conducting educational activities as part of its mission. But science does not (and cannot) take place in a vacuum, and we view engagement with students and the lay public as an important role for the Center to fulfill. Affiliations with a number of local graduate programs in basic biology and regenerative medicine keep our labs open to grad students, many of whom joined us in September for a two-day intensive lecture program of talks and practicums conducted by heads of CDB labs. Our yearly Open House also drew a record of more than 1,600 visitors to the Center to take part in hands-on demonstrations, tour laboratories, and attend talks and exhibitions on developmental phenomena given by research staff with non-specialists in mind.

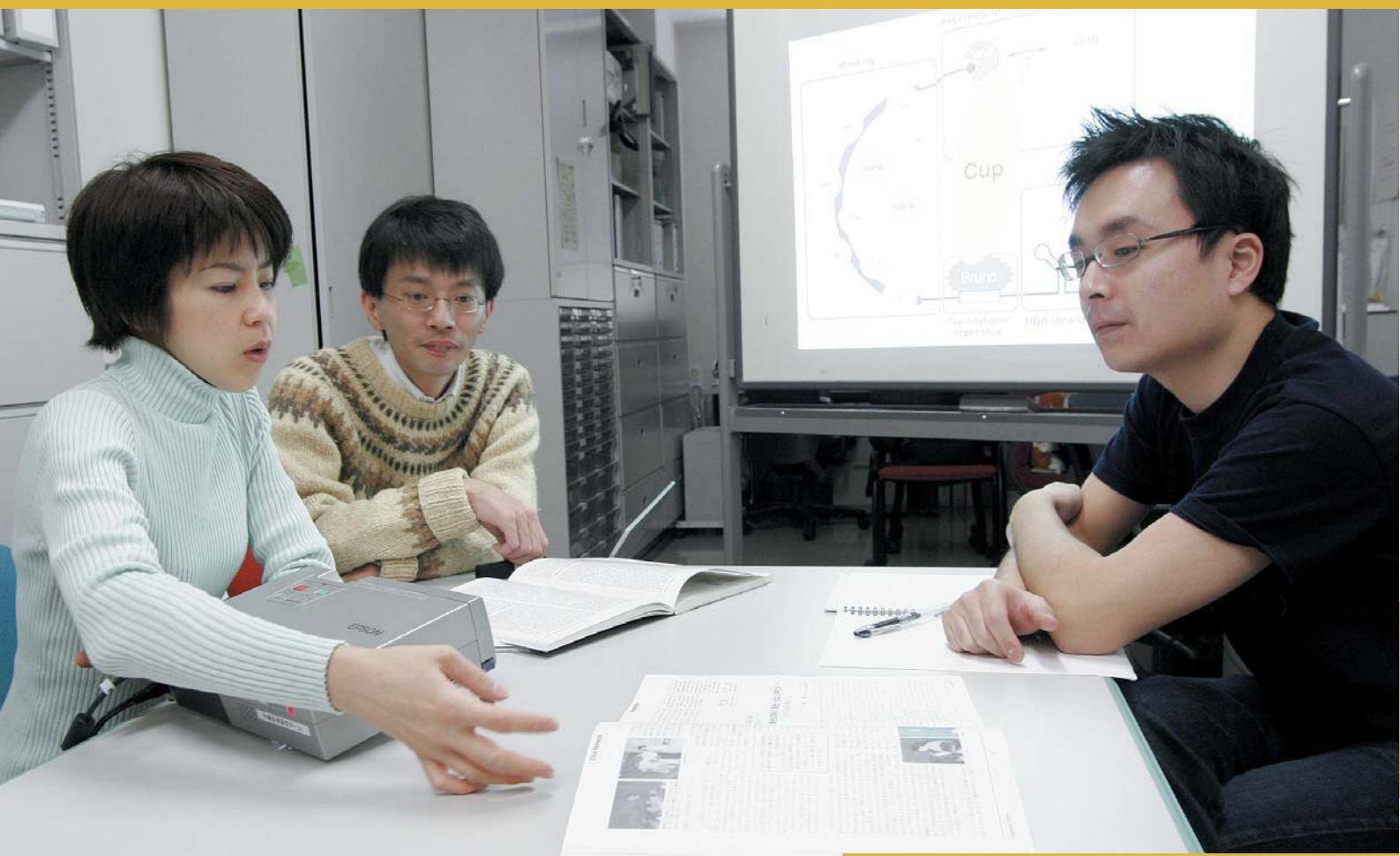
As we approach the end of the Millennium Project's five year term, we also enter a new phase in the CDB's history as an institute. Since the end of the last century, RIKEN has begun to move away from the tenure model of lifetime employment for many of its research positions, a policy conceived in the interests of encouraging mobility and keeping motivation levels high. The coming months will see the research programs appointed in the year 2000, the first year of the Center's existence, complete their initial five-year terms. Transitions will inevitably be made, in line with our vision of making the CDB an incubator for talent at all levels in the fields of development, regeneration and the science underlying regenerative medicine, which we hope will contribute to a larger virtuous cycle of opening up opportunities to gifted young scientists from throughout Japan and around the world.

The closing year has been one of many changes, the great majority of which, I believe, represented real progress. To those involved in the study of development, where constant change is the rule and evolution the ultimate outcome, perhaps that should come as no surprise. I hope you find this year's issue of the CDB annual report to be informative and wish you all the best for the year ahead.

Masatoshi TAKEICHI
Director, CDB



Damping down translation in the germline



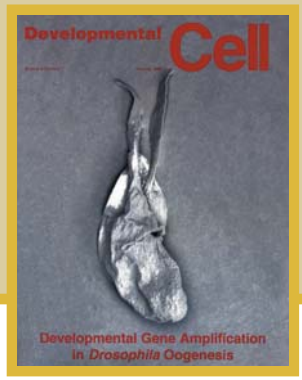
(from left) Kazuko HANYU-NAKAMURA, Akira NAKAMURA, Kenji SATO

In many species, the reproductive cells of the germline can only form properly if certain mRNAs are prevented from translating into proteins before they have been transported to precise target locations in the egg and the appropriate developmental stage has been reached. In a study published in *Developmental Cell*, members of the Laboratory for Germline Development (Akira Nakamura, Team Leader) reported that, in the fruit fly *Drosophila*, this translational repression is achieved by a newly identified complex formed by three associating proteins.

RNA activity during *Drosophila* oogenesis involves a number of sequential processes. The *Drosophila* oocyte shares cytoplasm with neighboring nurse cells via an incomplete cell membrane, allowing mRNAs and proteins from the nurse cells to be transported to the oocyte in the form of ribonucleoproteins. Following their export from the nurse cell nuclei, mRNAs are translationally repressed, or 'masked,' and transported to specified regions of the oocyte, where they establish fixed and precise localizations and regain their ability to undergo translation. In one example of this critically important regulation, the

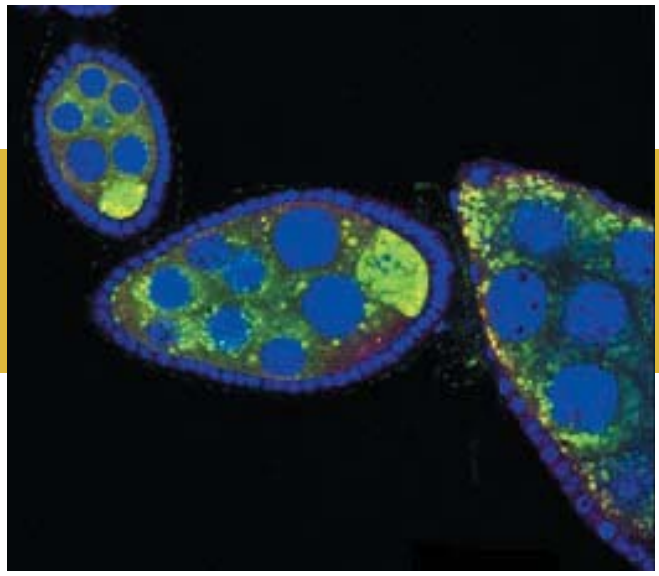
Nakamura A, Sato K and Hanyu-Nakamura K. Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. *Dev Cell* 7:95-106 (2004).

Reprinted from *Dev Cell* 7:95-106 (2004), with permission from Elsevier



translation of the RNA for the maternal gene *oskar*, which has critical functions in embryonic patterning and the formation of germline cells, is repressed during its transport to the posterior pole of the oocyte. This transcript-specific repression is known to be mediated by the protein Bruno, which binds to the 3' UTR of *oskar* mRNA, but the underlying mechanisms for this have remained obscure.

In the *Developmental Cell* study, the Nakamura lab demonstrated that an ovarian protein, Cup, is also required to inhibit the premature translation of *oskar* mRNA, and that Cup achieves this by binding to a second protein, eIF4E, a 5' cap-binding general translation initiation factor. Association with Cup prevents eIF-4E from binding with a different partnering molecule, eIF4G, and thereby inhibits the initiation of translation.



Maternal RNP particles, showing localization of DNA, Me31B and Cup proteins

Findings that a mutant form of Cup lacking the sequence with which it binds eIF4E failed to repress *oskar* translation in vivo, that Cup interacts with Bruno in a yeast two-hybrid assay, and that the Cup-eIF4E complex associates with Bruno in an immunoprecipitation assay, suggest that these three proteins form a complex that achieves translational repression by interactions with both the 3' and 5' ends of the *oskar* mRNA. A similar model of protein interactions is observed in the translational repression of the *cyclin-B1* mRNA in the *Xenopus* African clawed frog, indicating that the paradigm of translational repression through the 5'/3' interactions is conserved across species.

New insights into hindbrain evolution

The advent of the hindbrain was a pivotal and evolutionarily important event in the organization of the central nervous system. In work published in the journal *Development*, Shigeru Kuratani and colleagues in the Laboratory for Evolutionary Morphology revealed discrete mechanisms for segmentation and neural cell specification in one of the most primitive species known to possess a segmented hindbrain.

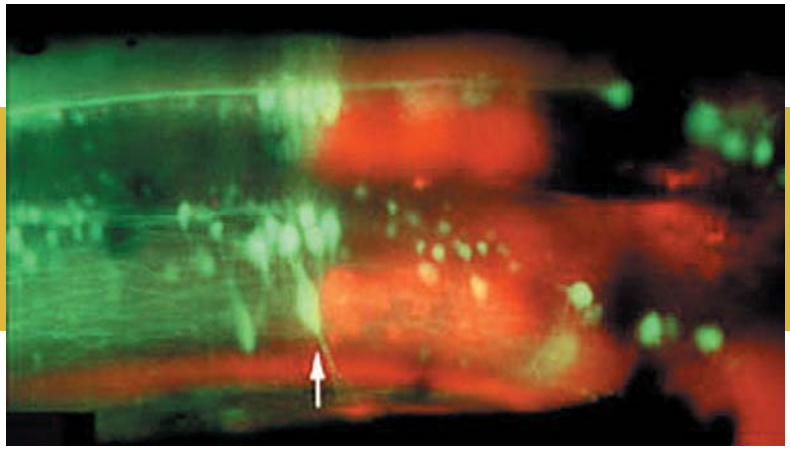
In all vertebrates, the hindbrain is a segmented structure, subdivided into clearly demarcated units called rhombomeres, which generate specific sets of neurons. The lancelet *Amphioxus*, a more primitive chordate, however, lacks this hindbrain segmentation. The lamprey, a jawless fish that arose in the interval between non-vertebrate chordates (such as *Amphioxus*) and gnathostomes (jawed animals), provides a useful model for studying the emergence of the hindbrain developmental plan.

Shigeru KURATANI

Murakami Y, Pasqualetti M, Takio Y, Hirano S, Rijli F M and Kuratani S. Segmental development of reticulospinal and branchiomotor neurons in lamprey: insights into the evolution of the vertebrate hindbrain. *Development* 131:983-95 (2004).

Yasunori Murakami in the Kuratani lab labeled reticulospinal and branchial motor neurons (which derive from rhombomeres) to reveal the neuronal organization of the hindbrain of the Japanese lamprey, *Lethenteron japonicum*, and studied the expression patterns of rhombomere-specific genes. He found that lamprey reticular neurons develop in conserved rhombomere-specific positions, similar to those observed in the gnathostome zebrafish. Interestingly, in lamprey, the positions of other sets of hindbrain neurons – the trigeminal and facial motor nuclei – do not map neatly to rhombomeric borderlines, as they do in gnathostomes. Rather, the trigeminal–facial nerve originates in the middle of rhombomere 4, in the region of expression of the lamprey *Hox* gene *LjHox3*. Murakami found that when retinoic acid (which is known to alter *Hox* gene expression and associated developmental programs) was introduced to the developing hindbrain region, it caused positional shifts of both *LjHox3* expression and branchiomotor nuclei, but no apparent changes in segmentation or the positions of reticular neurons.

These findings indicate that, in *L. japonicum*, hindbrain neural identities and rhombomeric segmentation are governed by independent mechanisms, providing strong counter-evidence to one prevailing model that suggests that the establishment of neuronal identity is a *Hox*-dependent process. Based on their discoveries, Kuratani et al proposed an alternate model in which the positional concurrence between *Hox* expression, rhombomere identity and specific subsets of hindbrain neurons is the result of a convergent process in which originally independent mechanisms became linked secondarily during gnathostome evolution.



Mauthner neuron (arrow), which develops in rhombomere 4 in all vertebrate species

Studies such as these underscore a pair of evo-devo precepts: that molecular designs capable of supporting viable ontogenies tend to act as magnets for convergent evolution, and that Nature is parsimonious with her creations, preferring to repurpose or tinker with existing genes rather than to introduce perfect novelties.

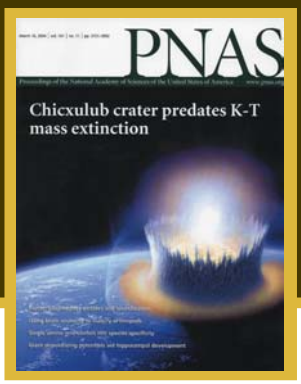
Rich genes travel more

Hiroki UEDA

In a study of changes in gene expression covering taxa from bacteria to human published in the *Proceedings of the National Academy* of Sciences, Hiroki R. Ueda and colleagues reported their discovery of a fundamental governing principle to the dynamics capable of producing heterogeneous distributions of gene expression in species from microbes to plants to man.

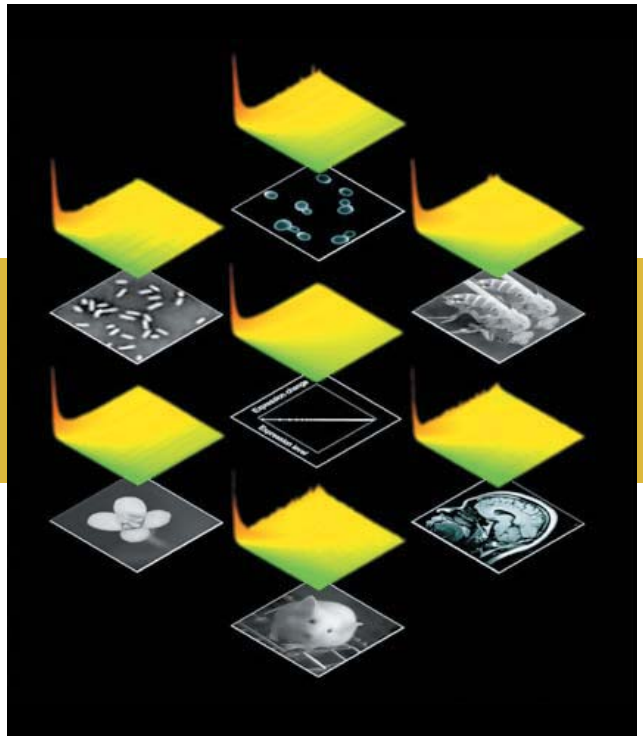
Ueda, who heads the Laboratory for Systems Biology, found that changes in gene expression scaled closely with initial expression levels in every organism studied; highly expressed genes tend to change in a highly dynamic way, while genes with lower expression levels are less likely to show such variability. Such proportionality governs many forms of changes in expression level, such as temporal oscillations, responses to environmental stimuli, and developmentally determined or tissue-specific gene regulation, and underlies the heterogeneous distribution of gene expression. The distribution of gene expression can be described using what is known as a “power law” distribution. In such a system, it can be shown that the number of genes having expression level X is 100 times larger than that of the population having a tenfold greater expression level (10X).

Ueda H R, Hayashi S, Matsuyama S, Yomo T, Hashimoto S, Kay S A, Hogenesch J B and Iino M. Universality and flexibility in gene expression from bacteria to human. *Proc Natl Acad Sci U S A* 101:3765-9 (2004).



The basic dynamics that underlie and produce this power-law distribution have yet to be worked out, but Ueda and colleagues now propose that the proportional dynamic operating in gene expression changes might be described as a "rich-travel-more" mechanism, an offshoot concept to the "rich-get-richer" metaphor frequently invoked to explain other power law-based distributions, such as the growth of network connections in the World Wide Web.

The Ueda study looked at gene expression dynamics in a broad range of model organisms familiar to life sciences researchers, including the intestinal bacteria *E. coli*, the yeast *S. cerevisiae*, *Arabidopsis thaliana* (thale cress), the fruit fly *Drosophila melanogaster*, mouse and human, by studying changes in genome-wide RNA expression using GeneChip microarrays. The team's analysis of distribution of gene expression in diverse species revealed that there is an apparently universal principle in expression dynamics, and showed that this principle can generate the observed heterogeneous power-law distribution of gene expression. These findings dovetail nicely with other ongoing studies that indicate a similar mechanism is at work in the evolution of metabolic networks, and point the way toward an improved understanding of the systems-level features capable of generating complex and dynamic biological network structures.



Model showing similarities in gene expression dynamics in species ranging from *E.coli* (top left) to human (bottom right).

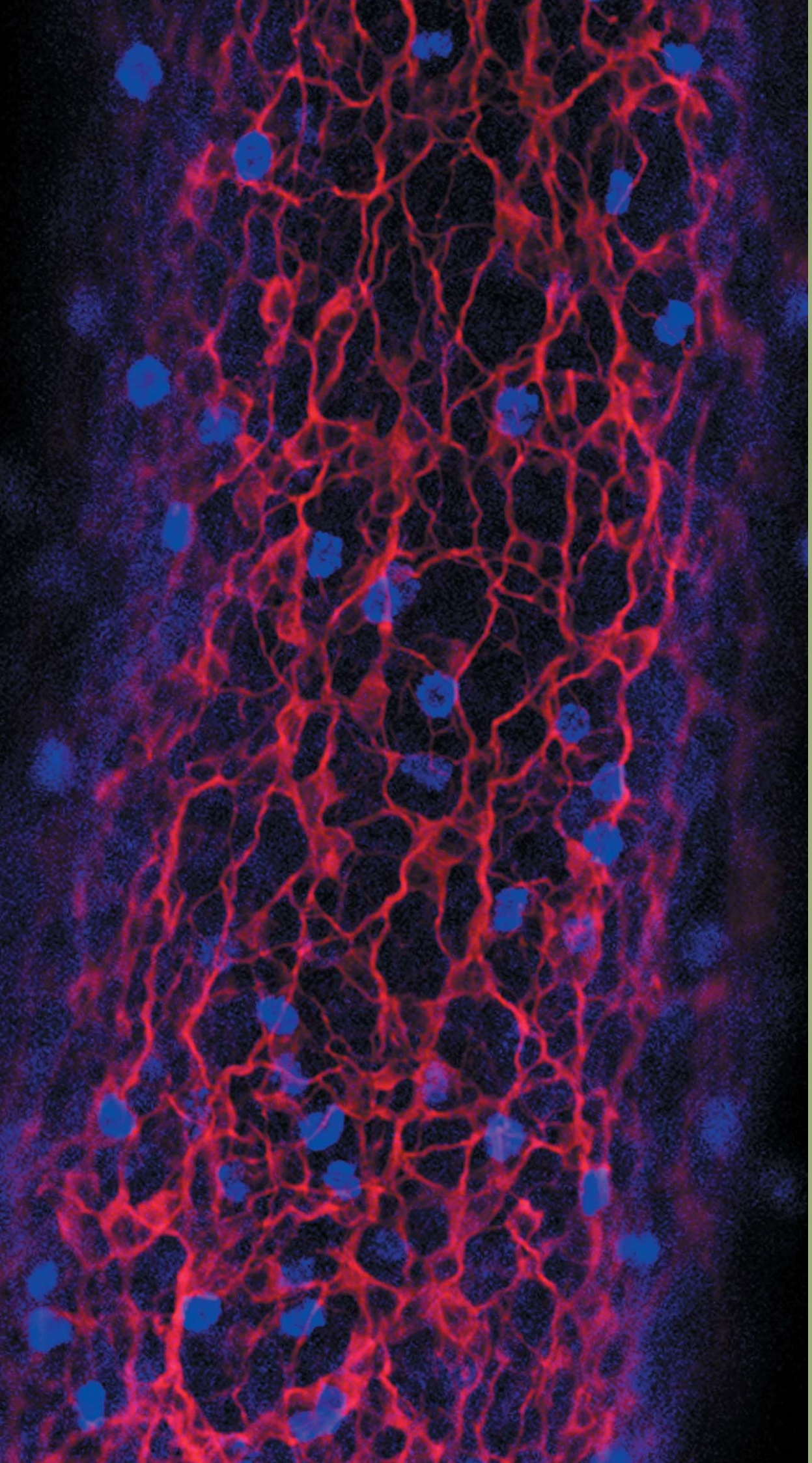
Scientist's story takes film prize

A television program on the life and scientific research of Kiyokazu Agata (Group Director, Laboratory for Evolutionary Regeneration Biology) won the MEXT Minister's Prize in the Educational Video category at the 45th Science and Technology Film/Video Festival held by the Japan Science and Technology Agency (JST). The award-winning program, titled "Learning the Secrets of Regeneration from Planaria" was first broadcast on Japan's Science Channel, a satellite and cable television station working to encourage public awareness of and interest in science and technology.

In the 29-minute program, Agata, who has authored a children's book on regeneration and stem cells in planaria and given many newspaper and television interviews to increase public understanding of these fields of research, describes how he first took an interest in biology as a junior high school student and his struggles to gain admission to Kyoto University after his graduation from high school. The program also features his research at the CDB, which focuses on the biological mechanisms underlying regeneration, using the planarian flatworm as his primary model system. Planarians are noted for their ability to regenerate entire new individuals from any body part when cut into sections. Agata has recently gained recognition for his identification of the gene, *nou-darake*, which restricts brain development to the head in these worms.

January-March Seminars

date	title	speaker
2004-01-05	The role of telomere shortening and telomerase activation in chromosomal instability and hepatocarcinogenesis	Karl Lenhard RUDOLPH
2004-01-06	Churchill, a zinc finger transcriptional activator, regulates the transition between gastrulation and neurulation	Guojun SHENG
2004-01-21	Regulation of multipotent cell lineages in the mammalian embryo	Patricia LABOSKY
2004-02-10	The AVE and the Cerberus-like gene family: searching for new players establishing a symmetries in the early mouse embryo.	Jose A. BELO
2004-02-20	PKA-RI spatially restricts Oskar expression for *Drosophila* embryonic patterning	Shoko YOSHIDA
2004-02-23	Neural crest migration in marsupials: Developmental mechanisms of evolutionary change	Kathleen K. SMITH
2004-02-24	Function of O-fucosylation in Notch signaling	Kenji MATSUNO
2004-02-24	Regulation of the Notch receptor by Suppressor of deltex	Martin BARON
2004-02-26	Cadherins as targets in skin autoimmune and infectious diseases	Masayuki AMAGAI
2004-03-03	Structure and function analysis of In vitro-induced organs in the vertebrate development	Makoto ASASHIMA
2004-03-09	EMAGE - The Edinburgh Mouse Atlas of Gene Expression: A Novel bioInformatics resource of virtual 2D and 3D gene expression patterns during mouse embryogenesis	Jeff CHRISTIANSEN
2004-03-10	Humoral regulations of Insulin-like peptides in *Drosophila* (DILPs)	Tomoatsu IKEYA
2004-03-10	Growth Factor Signaling during organogenesis: Looking through the eye into the heart	Yasuhide FURUTA
2004-03-18	Structure and function of channels analysed by cryo-electron microscopy	Yoshinori FUJIYOSHI



Kiyokazu AGATA

Shinichi AIZAWA

Takayuki ASAHARA

Hideki ENOMOTO

Chihiro HAMA

Shigeo HAYASHI

Masahiko HIBI

Shigeru KONDO

Shigeru KURATANI

Raj LADHER

Enteric neurons in E11.5 mouse midgut stained for TuJ1 (red) and phosphohistone H3 (blue)

Evolutionary Regeneration Biology

Kiyokazu AGATA Ph. D.

Kiyokazu Agata received his doctorate from Kyoto University in 1985 for his work on molecular cloning and gene expression of crystallin genes in chicken. From 1983 to 1991, he worked at the National Institute for Basic Biology, where he studied the molecular characteristics of dedifferentiated cells in transdifferentiation. He took an associate professorship at the Himeji Institute of Technology in 1991, and started his study of planarian regeneration with Kenji Watanabe. He remained at the Institute until 2000, when he left to assume a professorship at Okayama University. He joined the RIKEN Center for Developmental Biology as a group director in 2000.

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Publications

Hayashi S, Itoh M, Taira S, Agata K and Taira M. Expression patterns of Xenopus FGF receptor-like 1/nou-darake in early Xenopus development resemble those of planarian nou-darake and Xenopus FGF8. *Dev Dyn* 230:700-7 (2004).

Hwang J S, Kobayashi C, Agata K, Ikeo K and Gojobori T. Detection of apoptosis during planarian regeneration by the expression of apoptosis-related genes and TUNEL assay. *Gene* 333:15-25 (2004).

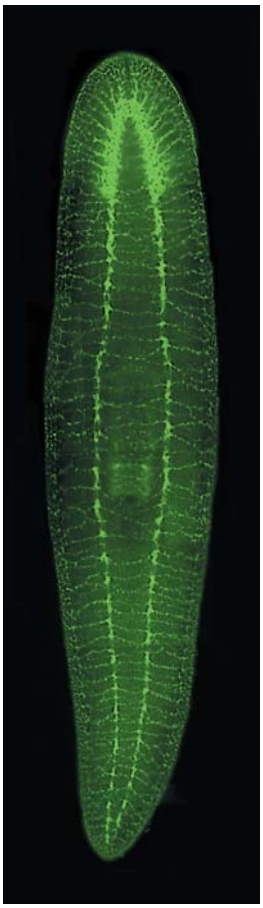
Inoue T, Kumamoto H, Okamoto K, Umesono Y, Sakai M, Sanchez Alvarado A and Agata K. Morphological and functional recovery of the planarian photosensing system during head regeneration. *Zoolog Sci* 21:275-83 (2004).

Although all animals possess some ability to regenerate, that capacity varies widely from species to species. The planarian flatworm, a relatively simple organism, exhibits remarkable regenerative ability attributable to a population of pluripotent stem cells capable of giving rise to all cell types in the body. Study of planarian regeneration may enable us to better understand how we rebuild tissues and organs damaged by injury, disease or as part of the natural aging process. The ultimate goals of our laboratory are to understand the mechanisms regulating the planarian stem cell system and to determine what signals are involved in both maintaining these cells' totipotency and in activating them during regeneration.

In recent years, planarian research has benefited from the application of molecular techniques such as whole-mount *in situ* hybridization and gene-knockdown by RNA interference. One of our laboratory's recent successes has been the identification of the *nou-darake* gene, a key element in modulating FGF signaling in stem cells to restrict brain tissue to the head region of planarians. These findings may provide valuable insights into the field of regenerative medicine, especially neural regeneration.

The study of planarians may also help to reveal how higher organisms develop complex cell systems, such as the nervous system, during evolution. A bilaterally symmetric animal, the planarian is believed to be the simplest extant taxon to form a distinct head. Although the planarian brain is very simple, it is capable of regulating behaviors in response to a variety of environmental signals. The establishment of EST databases for a variety of species, including the planarian and the fresh water sponge (one of the most primitive forms of multicellular life), promises to provide invaluable resources for the study of evolutionary biology and a basis for comparative analyses of the genomics underlying the shared and distinct features of these organisms.

By focusing on the development of the flatworm brain, we hope to use it as a model to shed light on the contribution of stem cells to the development of increasingly complex cellular systems through evolution, studies which we hope will also help to improve our understanding the role of the stem cell system in evolutionary regeneration biology.



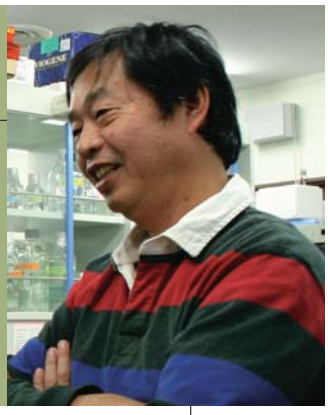
Central nervous system of planarian

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

Vertebrate Body Plan

Shinichi AIZAWA Ph. D.

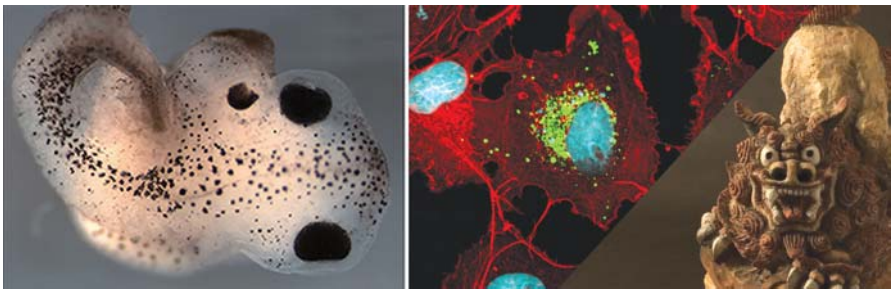
Shinichi Aizawa received his Ph. D. in biochemistry from the Tokyo Kyoiku University Department of Zoology in 1973. He spent the period from 1974 to 1979 as an investigator at the Tokyo Metropolitan Institute of Gerontology, then two years as a research fellow in the Laboratory of Genetic Pathology at 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 as a senior research associate. He was appointed professor in the Kumamoto University School of Medicine Department of Morphogenesis 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 team leader of the Laboratory for Animal Resources and Genetic Engineering. He also serves as managing editor for the journal, Mechanisms of Development.



All vertebrate species share certain features in the development of the brain, in that all vertebrate brains comprise four regions – telencephalon, diencephalon, mesencephalon and metencephalon (or cerebellum) – an organizational trait that is thought to have appeared with the advent of the vertebrate lineage. However, dramatic changes in this organization took place during the divergence of jawed (gnathostome) fishes from their jawless (agnathan) ancestors. Evolutionary modifications of the telencephalon first manifested in the reptiles, becoming even more pronounced with the development of a neocortex stratified into six layers regionalized by distinct physiological functions. However, the mechanisms that instruct the brain's laminar regions to conform to anterior-posterior and dorsal-ventral axis patterning programs, and the means by which each individual region is formed, remain unknown.

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

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



Overexpression of *shisa* and secondary axis inducer produces secondary head structure (left). Shisa retains the Wnt-receptor Frizzled within the endoplasmic reticulum, thereby suppressing Wnt signaling (middle). Shisa is a form of sculpture common to southern Japan, with a large head similar to the Egyptian sphinx (right).

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

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Publications

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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, Dr. 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.

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Publications

Murasawa S and Asahara T. Endothelial progenitor cells for vasculogenesis. *Physiology (Bethesda)* 20:36-42 (2005).

Asahara T and Kawamoto A. Endothelial progenitor cells for postnatal vasculogenesis. *Am J Physiol Cell Physiol* 287:C572-9 (2004).

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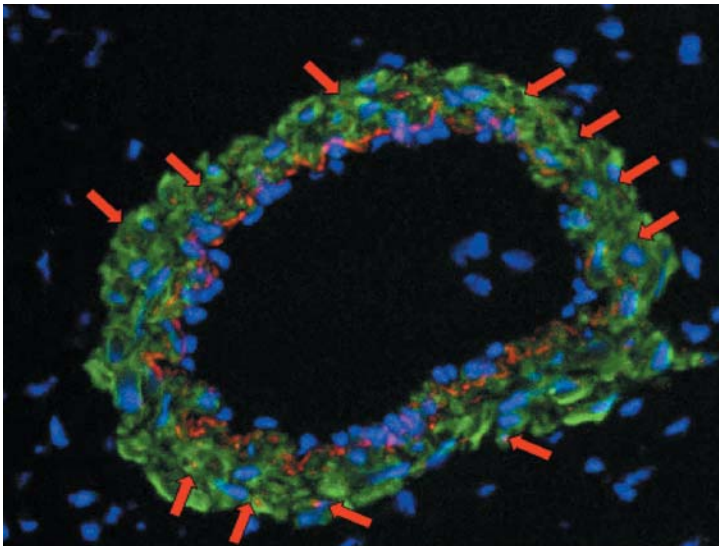
Young H E, Duplaa C, Romero-Ramos M, Chesselet M F, Vourc'h P, Yost M J, Ericson K, Terracio L, Asahara T, Masuda H, Tamura-Ninomiya S, Detmer K, Bray R A, Steele T A, Hixson D, el-Kalay M, Tobin B W, Russ R D, Horst M N, Floyd J A, Henson N L, Hawkins K C, Groom J, Parikh A, Blake L, Bland L J, Thompson A J, Kirincich A, Moreau C, Hudson J, Bowyer F P, 3rd, Lin T J and Black A C, Jr. Adult reserve stem cells and their potential for tissue engineering. *Cell Biochem Biophys* 40:1-80 (2004).

The emerging 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 contributes broadly to therapeutic application of human diseases. These studies are intended to translate basic research findings into potentially clinically significant knowledge. We hope that our preliminary studies will elucidate the specific circumstances and mechanisms responsible for vascular development in organogenesis.



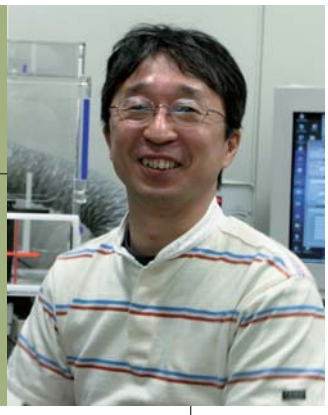
A blood vessel created by transplanted human vascular stem cells.

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

Neuronal Differentiation and Regeneration

Hideki ENOMOTO M. D. , Ph. D.

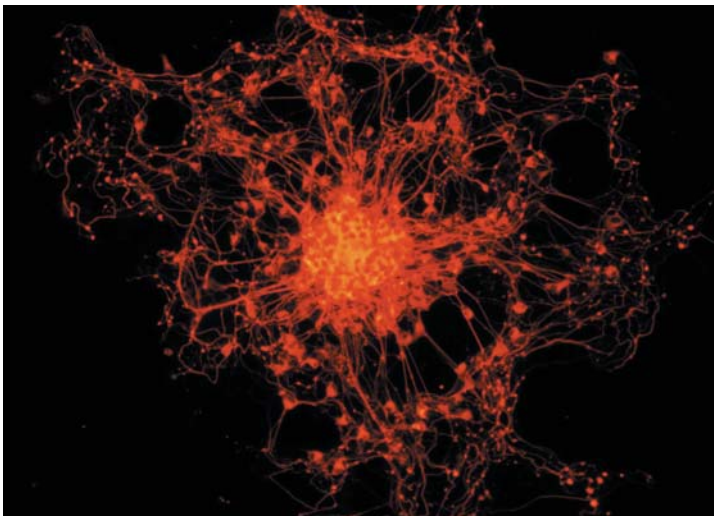
Hideki Enomoto received his M. D. from the Chiba University School of Medicine in 1988, and his Ph. D. from the same institution in 1996 for his work in the molecular cloning of the human *DAN* gene. After serving as the Chief of the Department of Pediatric Surgery, Matsudo Municipal Hospital in Chiba, 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, with GFRα1 showing the greatest ability to interact with the range of GFL family members. GFL signaling has been shown to affect neuronal growth 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.



Differentiating neurons from gut-derived neurospheres (red: class III β tubulin)

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

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Publications

Crowder R J, Enomoto H, Yang M, Johnson E M, Jr. and Milbrandt J. Dok-6, a Novel p62 Dok family member, promotes Ret-mediated neurite outgrowth. *J Biol Chem* 279:42072-81 (2004).

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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.

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Publications

Orihara-Ono M, Suzuki E, Saito M, Yoda Y, Aigaki T and Hama C. The *slender lobes* gene, identified by retarded mushroom body development, is required for proper nucleolar organization in *Drosophila*. *Dev. Biol.* (in press 2005).

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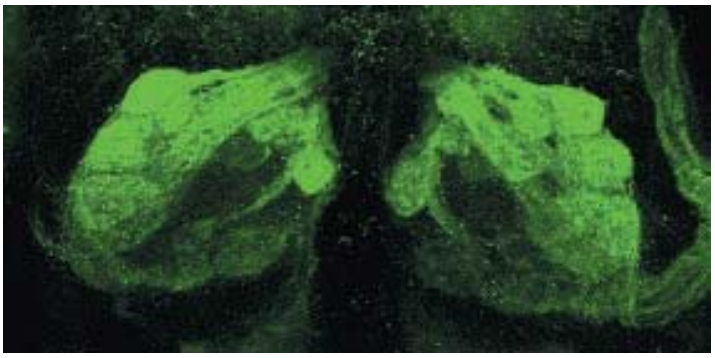
One of the most tantalizing questions in the field of neurobiology is how neural circuits of exquisite complexity are generated during nervous system development. Human brains consist of 10^{10} neurons, and each neuron projects an axon that extends along a predetermined pathway before finally finding 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, and addressed the issue of how vesicle transport is involved in neurite differentiation.

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 a single OR. Interestingly, axons from neurons that express a given OR precisely converge at one or two glomeruli, 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 a number of mutations that impair the projection of ORN axons into glomeruli. The genes responsible for these mutant phenotypes may be involved in axon guidance, synaptic targeting, glomerular formation or synapse formation. We have found one mutation of particular interest, which affects the asymmetric cell fate specification of ORNs that form pairs or clusters from single precursors during development and, as a consequence, change synaptic targets. It is hoped that analysis of this mutation will reveal a principle governing glomerular organization in the antennal lobe.

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.



A limited number of glomeruli express a cell-surface protein, which might be involved in the targeting specificity of olfactory receptor neurons.

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

Morphogenetic Signaling

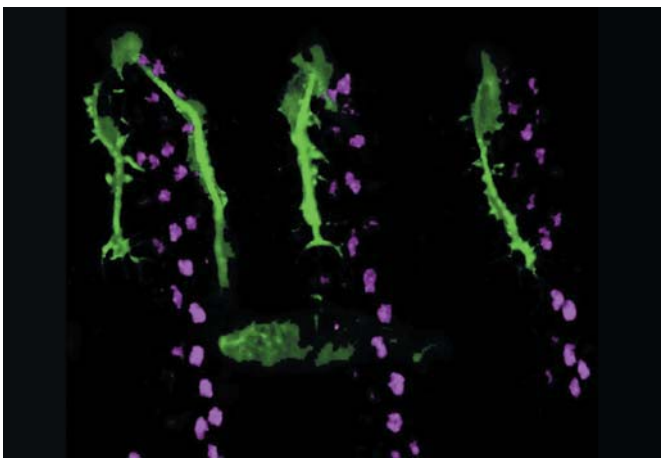
Shigeo HAYASHI Ph. D.

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 crystalin* 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.

A range of vital developmental processes, including the abilities 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 are a central question in the study of morphogenesis, the focus of research in our laboratory.

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 those 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, that 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*.



Dorso-lateral view of a *Drosophila* embryo showing trachea (green) and posterior (P) compartment of epidermis (magenta). Terminal branches of the trachea extend along the P compartment, guided by the signaling molecule hedgehog.

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

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Publications

Kato K, Chihara T and Hayashi S. Hedgehog and Decapentaplegic instruct polarized growth of cell extensions in the *Drosophila* trachea. *Development* 131:5253-61 (2004).

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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 post-doctoral fellow in Michael Karin's lab in the University of California, San Diego Department of Pharmacology, then returned to Japan to take an assistant professorship in the Division of Molecular Oncology at Osaka University Medical School. He was appointed associate professor in the same division in 1999, where he remained until he assumed his position as team leader at the RIKEN CDB.

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Publications

Bae Y K, Shimizu T and Hibi M. Patterning of proneuronal and inter-proneuronal domains by hairy- and enhancer of split-related genes in zebrafish neuroectoderm. *Development* (2005).

Shimizu T, Bae Y K, Muraoka O and Hibi M. Interaction of Wnt and caudal-related genes in zebrafish posterior body formation. *Dev Biol* 279:125-41 (2005).

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

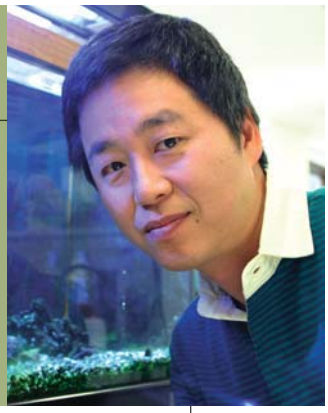
Although the molecular nature of the dorsal determinants has not been elucidated, they are known to activate the canonical Wnt pathway and thereby lead to the expression of genes involved in the induction of the dorsal organizer. The dorsal organizer generates secreted signaling molecules that participate in the generation of the DV axis in the mesoderm and endoderm, and the formation of the neuroectoderm. We are working to identify the molecules that are involved in the formation and function of the dorsal organizer. This search led us to identify the protein Secreted Frizzled (Sizzled)/Ogon 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 BMP inhibitors (such as Chordin), which are generated by the dorsal organizer. The induced neuroectoderm is characteristically anterior in character and is only subsequently subjected to posteriorization, which involves signaling by posteriorizing factors emanating from the non-axial mesendoderm. After neural induction and patterning, the proneuronal domains, in which neurogenesis takes place, are established. Within the proneuronal domains, a subset of cells is selected to become primary neurons by a Notch-mediated lateral inhibition mechanism. We have recently found that the homeobox gene *pnx* is regulated by the posteriorizing non-axial mesendoderm and Notch signaling, and that Pnx is involved in the development of posterior neurons. We are trying to determine the mechanism by which the formation of primary neurons is spatially regulated.

(Left) The maternal-effect mutant *tokkaebi* displays a completely ventralized phenotypes. (Right) Nuclear accumulation of β-catenin is affected in a *tokkaebi* embryo.

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

Positional Information

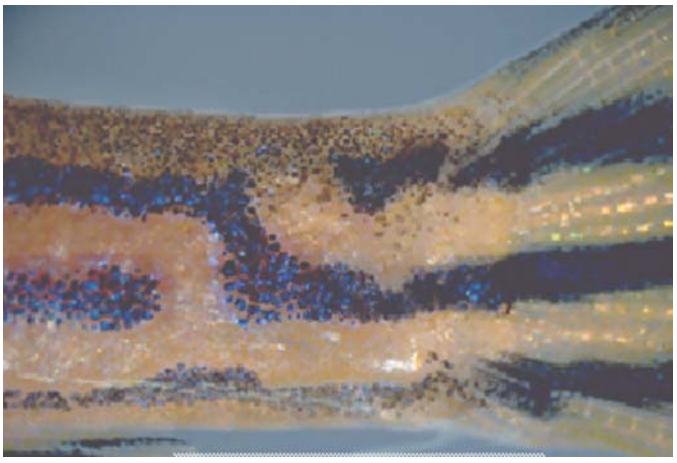


Shigeru KONDO Ph. D.

Shigeru Kondo received his doctorate from the Kyoto University Faculty of Medicine in 1988. He spent the period from 1989 to 1990 as a postdoctoral fellow in Masami Muramatsu's lab at the University of Tokyo, before taking an overseas fellowship at the Basel University Biocenter under Walter Gehring. He returned to Kyoto University in 1994 as assistant professor, where he remained until 1998, when he was appointed professor in the Tokushima University Faculty of Integrated Arts and Sciences. Kondo was appointed CDB team leader in 2001.

The question of how complex patterns arise from seemingly disorganized or formless initial structures represents an intriguing challenge to mathematicians, physicists, chemists and biologists alike. Theoretical work indicates that the mechanisms underlying pattern formation are similar in both biological and non-biological systems, and a number of mathematical models capable of describing pattern generation in chemical media have been proposed, but the greater complexity of living systems has made it much more difficult to demonstrate a mathematical basis for biological patterns. In 1952, the British mathematician Alan Turing proposed a simple mathematical equation capable of generating a wide range of patterns commonly found in the natural world, such as stripes, spots and reticulations. This model, known as the reaction-diffusion model, demonstrates that the interaction between a local activator and a long-range inhibitor can give rise to various periodic structures in response to differences in their individual diffusion rates.

Our team is interested in demonstrating the mathematical basis of pattern formation in development, and using mathematical models as predictive tools to aid in the identification of genes and molecules involved in the generation of spatial structures. The lab's research focuses on skin surface and morphogenetic patterning, both of which feature prominent examples of periodic structures that can be described in terms of standing and moving waves. By studying the molecular genetic mechanisms underlying pattern development in striped wild type and mutant organisms, including zebrafish and mouse, we hope to provide biological evidence of the mathematically explicable bases that underlie the formation of natural complex patterns.



Altered striping in zebrafish skin three weeks after chromatopohore ablation

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

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Publications

Suzuki N, Hirata M and Kondo S. Traveling stripes on the skin of a mutant mouse. *Proc Natl Acad Sci U S A* 100:9680-5 (2003).

Shoji H, Mochizuki A, Iwasa Y, Hirata M, Watanabe T, Hioki S and Kondo S. Origin of directionality in the fish stripe pattern. *Dev Dyn* 226:627-33 (2003).

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Evolutionary Morphology

Shigeru KURATANI Ph. D.

Shigeru Kuratani received his M. S. 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, 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.

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Kuraku S, Usuda R and Kuratani S. Comprehensive survey of carapacial ridge-specific genes in turtle implies co-option of some regulatory genes in carapace evolution. *Evol Dev* 7:3-17 (2005).

Nagashima H, Uchida K, Yamamoto K, Kuraku S, Usuda R and Kuratani S. Turtle-chicken chimera: An experimental approach to understanding evolutionary innovation in the turtle. *Dev Dyn* 232:149-61 (2005).

Takio Y, Pasqualetti M, Kuraku S, Hirano S, Rijli F M and Kuratani S. Evolutionary biology: lamprey Hox genes and the evolution of jaws. *Nature* 429:1 p following 262 (2004).

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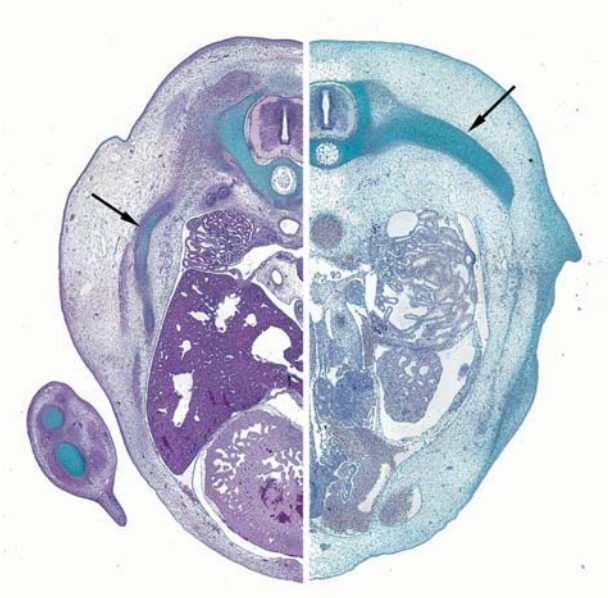
By studying the evolutionary designs of diverse species, I hope to gain a deeper insight into the secrets behind the fabrication of the vertebrate body. Integrating the fields of evolutionary morphology and molecular genetics, our lab seeks to expand the understanding of the relationship between genome and morphology (or body plan) through investigating the evolutionary changes in developmental processes. Our recent studies have focused on traits of the vertebrate head region, especially the jaw, as well as the turtle shell. By analyzing the history of developmental patterns, I hope to open new avenues toward answering as-yet unresolved questions about vertebrate development.

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 gnathostome (jawed fish) jaw and the turtle shell. Study of lamprey jaw development is intended to shed light on the true origins of the vertebrate head, as lampreys lack a number of important features, such as jaws, 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 acquisition of a shell.



Differences in rib growth (arrows) in chicken (left) and turtle (*Pelodiscus*; right)

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

Sensory Development

Raj LADHER Ph. D.

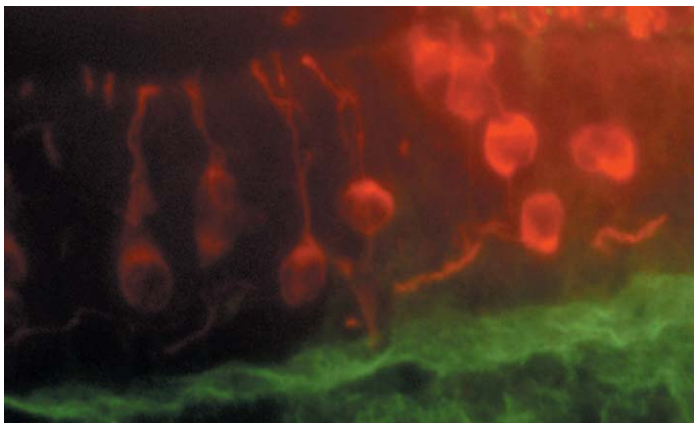
Raj Ladher received his B. Sc. in biochemistry from Imperial College, London in 1992 and his Ph. D. in developmental biology from the National Institute for Medical Research in 1996, for his thesis on the cloning of the *Xenopus* homeobox gene, *Xom*. He worked as a research associate at King's College, 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 RIKEN CDB in 2002.

Organogenesis is a monumental undertaking. From situating a given organ at 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 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.



Olfactory neurons (stained with Tuj1 -red) also form from a region of non-neural ectoderm - the olfactory placode. Shown in green is NCAM.

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

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Wright T J, Ladher R, McWhirter J, Murre C, Schoenwolf G C and Mansour S L. Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev Biol* 269:264-75 (2004).

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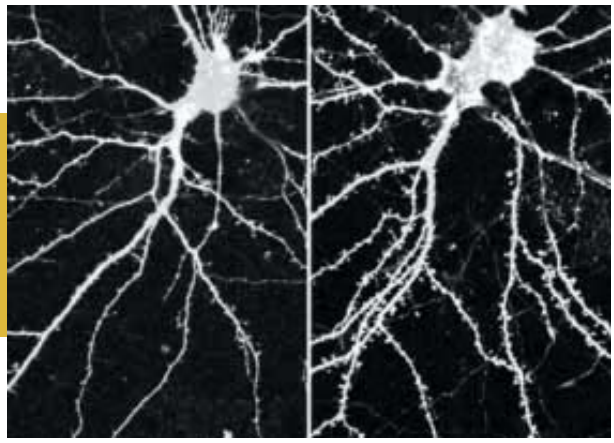
Dendritic spine dynamics

In the brainier branches of the phylogenetic tree, neural networks form from billions of interconnected axons and dendrites, neuronal projections that send and receive messages across junctional sites known as synapses. A synapse is formed when an axon (the projection that conveys outgoing signals) from one neuron establishes a stable junction with a dendrite (a signal-receiving neuronal branch) from another, allowing electrochemical messages to be exchanged. Dendrites are themselves covered with even smaller projections, called spines, which extend and make contact with other nerve cells. These dendritic spines are dynamic structures that are continuously projecting from and retreating back into the dendritic stem, stabilizing only on contact with another neuron under the right conditions.

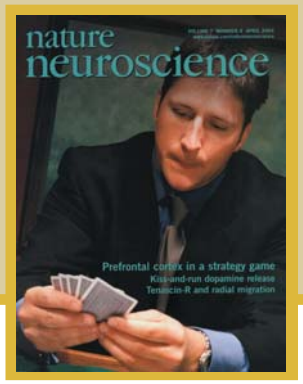
A report by Kentaro Abe, Masatoshi Takeichi and colleagues at the Laboratory for Cell Adhesion and Tissue Patterning showed that this synapse-forming dynamic is regulated by the activity of the αN-catenin molecule. Catenins are already known as key players in cell-cell adhesion, linking the cytoplasmic tails of cadherin transmembrane proteins to the actin cytoskeleton. This new study, published in the April issue of *Nature Neuroscience*, shows that αN-catenin, a form of catenin specific to the nervous system in mouse, also controls both the motility and the stability of dendritic spines.



Overexpression of αN-catenin in hippocampal neurons causes overproduction of dendritic spines (right) compared to control (left).

In many types of neurons, dendritic spines are formed when a dynamic fingerlike projection called a filopodium matures into a stable mushroom-headed structure, a process that was shown in a previous report by Takeichi et al. to rely on cadherin activity. In neurons from mice in which the gene for αN-catenin had been deleted, filopodia appeared and disappeared more frequently and protruded more dynamically than did those from normal mice, and as a consequence failed to form stable synapses. When αN-catenin was overexpressed, however, the opposite was found to occur: the overall density of spines increased and the mushroom caps in mature spines were slightly exaggerated, indicating that the rise in αN-catenin levels was linked to a concomitant increase in dendritic spine stability.

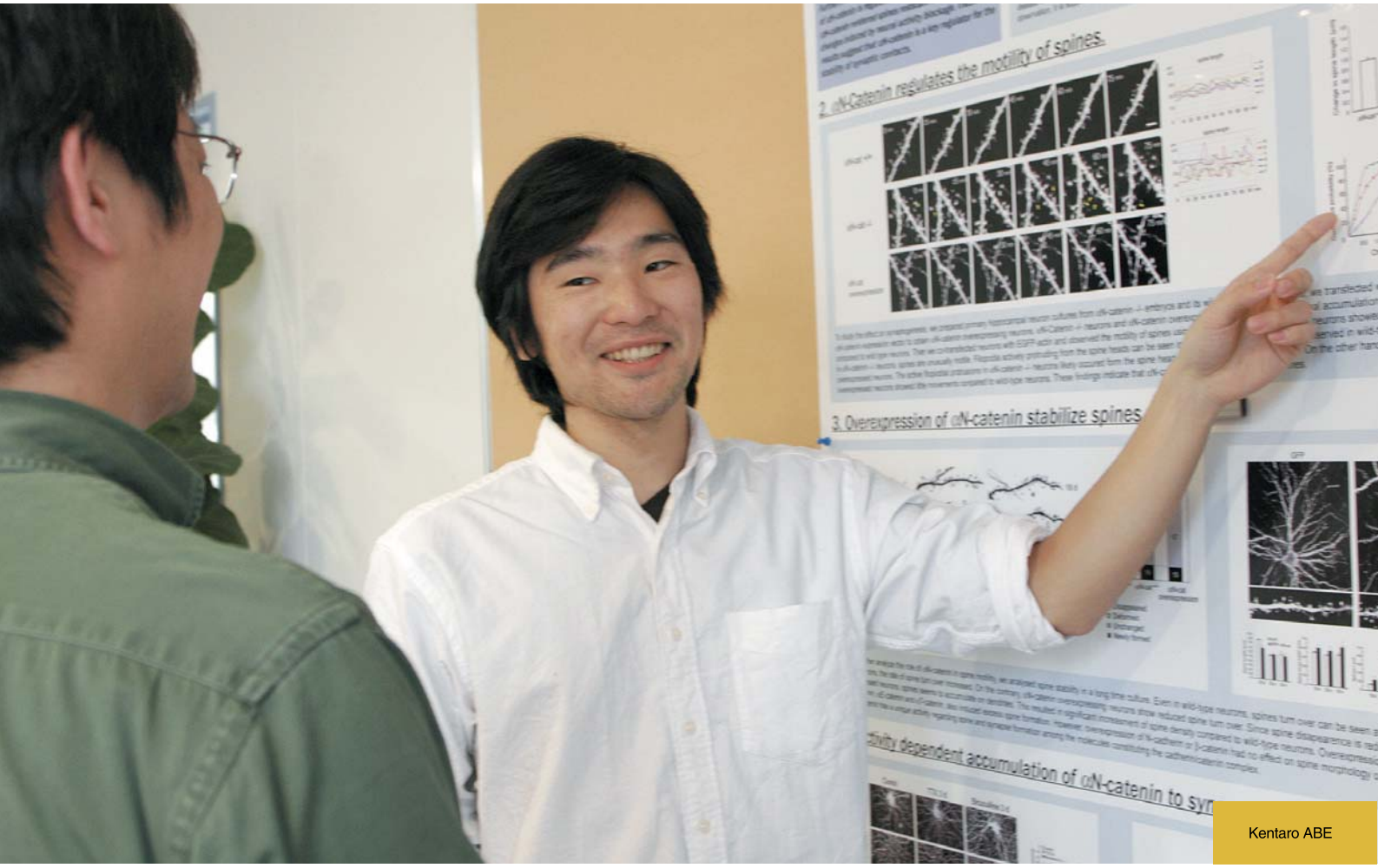
In addition to stabilization, spine morphology is also known to play an important role in synaptic plasticity, the ability of synapses to respond to changes in neural activity. The team used two neuroactive agents – tetrodotoxin (TTX), a neural blocker known to convert spines to filopodia in hippocampal neurons, and bicuculline, a GABA inhibitor that increases neural activity – to study the effects of altered activity levels on αN-catenin. TTX was found to reduce the concentration of αN-catenin at synapses, but



Abe K, Chisaka O, Van Roy F and Takeichi M. Stability of dendritic spines and synaptic contacts is controlled by αN-catenin.
Nat Neurosci 7:357-63 (2004).

not its overall expression, suggesting that lower activity affected αN-catenin distribution. Conversely, in the bicuculline-treated cells, synaptic αN-catenin levels were intensified and expression was slightly higher. Curious about whether the morphological changes seen in TTX-treated spines were the cause or the effect of the lowered αN-catenin concentration, the group next tested the effect of tetrodotoxin on αN-catenin-overexpressing hippocampal neurons and found them to be unresponsive to the drug. This increase in spine density was not seen on the overexpression of either N-cadherin or β-catenin (other important components of the neural cadherin-catenin complex), suggesting that αN-catenin acts as a primary mediator of neural activity-dependent changes in spine morphology, a process considered to be central to synaptic plasticity and the remodeling of neural circuits.

These studies have provided solid evidence of αN-catenin's role in neural network stability in vitro. Its physiological role, however, remains to be shown, as the lethality of the αN-catenin knockout mutation confounds in vivo studies. The lab next plans to use an alternative method that will allow them to bypass this lethality, and confirm their findings in live animals, work that promises to lead to a better understanding at a molecular level of how the brain rewires itself in response to changes in neural activity.

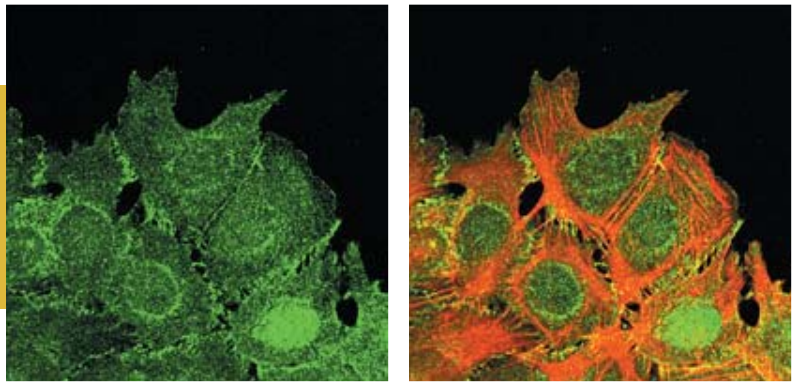


Kentaro ABE

Fat binds the bones of the cytoskeleton

The cytoskeleton gives structure, strength and mobility to the body of the cell through a network of microscopic struts, cables and a lattice-like cortex. One of the principal components of this skeletal frame is a protein called actin, which is most prevalent in cellular peripheries where it provides a stable but regulable meshwork enabling the cell to maintain its structural integrity while responding to external and internal stimuli through shape alterations and movements. Cells receive such environmental signals via an array of pores, channels and membrane proteins, including the cadherin family of cell adhesion proteins, which (among other functions) allow cells to recognize and bind to other cells of like type. In a study of these junctional regions published the *Journal of Cell Biology*, researchers in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Group Director) revealed new evidence of a direct link between cadherins and the actin cytoskeleton.

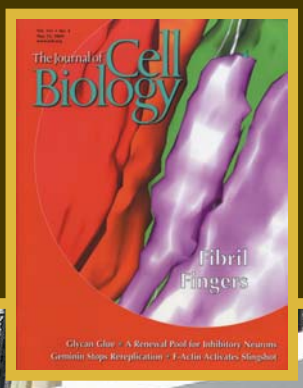
The cadherins form a superfamily of proteins that includes the classic cadherins, known for their function in cell-cell adhesion, and a number of other subfamilies with diverse activities. The largest cadherins are molecules known as Fat cadherins, which feature a domain projecting outward from the cell membrane that is nearly seven times the length of extracellular regions in classic cadherins. It has been suggested that this lengthening of the extracellular region may play a role in the regulation of the size of intercellular junctions in some tissues, such as the renal glomerulus, in which the size of the gaps between specialized cells known as podocytes has been linked to Fat cadherin activity.



Fat1 cadherin (green) localizes at cell-cell contact sites (left), colocalizing with F-actin (red/yellow, right) in epithelial cells.

On the inner side of the cell membrane as well, the Fat cadherins (there are three mammalian subtypes of the Fat molecule) are nearly entirely different from the classic cadherins in terms of protein structure, a uniqueness that has prompted some scientists to postulate a divergent function for the Fats independent of the cell binding and recognition activities of the classic cadherins.

Seeking to resolve the question of Fat function, Takuji Tanoue, working in the Takeichi group, focused on identifying the regions where mammalian Fat1 localized in cells, and found it to be present in filopodia, lamellipodia and cell-cell contact sites in certain types of cells. This distinct distribution at the dynamic edges of cells led the group to investigate the possibility of a connection between Fat and the actin cytoskeleton, given the tight overlap between their patterns of expression.



Tanoue T and Takeichi M. Mammalian Fat1 cadherin regulates actin dynamics and cell-cell contact. *J Cell Biol* 165:517-28 (2004).

Knocking down Fat1 activity by RNA interference (RNAi), the lab found that Fat1 was required for the proper organization of actin structures in the region as well as for the initiation and maintenance of tight cell-cell binding. A separate assay revealed that Fat1 is also needed to establish cell polarity at the margins of wounds during the healing process; correct polarization is essential for cells, such as those in damaged tissue undergoing repair, that need to know which side is facing "out" so that they can orient themselves accordingly.

Analyses at the molecular level showed Fat1 to have three consensus sequences for a binding site on a second protein, Ena/VASP, known to regulate the activity of the actin cytoskeleton. Further tests using fusion proteins determined that Fat1 does indeed bind directly with Ena/VASP, while studies of a mutant form of Fat1 with amino acid substitutions in the Ena/VASP binding site confirmed the association between these molecular partners at the cellular level.

Looking again at the peripheral zones where actin and Fat1 co-localize, Takeichi and Tanoue found that *fat1* regulates actin dynamics through its interaction with Ena/VASP, as shown by dramatic decreases in the formation of actin stress fiber formation in cells expressing mutant versions of Fat1 lacking a functional Ena/VASP binding site. RNAi knockdown of *fat1* further demonstrated its importance in the formation of tight cell-cell associations; associations between cells in which *fat1* was knocked down were significantly looser than those in normal cells, and actin organization appeared severely disrupted.

Taken together, Tanoue and Takeichi's work provides compelling evidence for the action of a Fat cadherin on one of the most extensively studied components of the cytoskeleton, shedding new light on the dynamics of actin regulation and suggesting an intriguing new role for cell adhesion molecules.

Takuji TANOUE

So, why *does* the lamprey have no jaw?

The *Hox* code characterizes a family of master control genes that function to establish regional identities in the body segments of animals ranging from worm to human. First identified in studies of fruit fly mutants, *Hox* genes are now known to specify body regions along the anterior-posterior (head-to-tail) axis in both invertebrate and vertebrate species. Findings from the Laboratory for Evolutionary Morphology (Shigeru Kuratani, Team Leader), published in the journal *Nature*, shed new light on the question of a specific role for *Hox* genes in the evolution of the jaw.

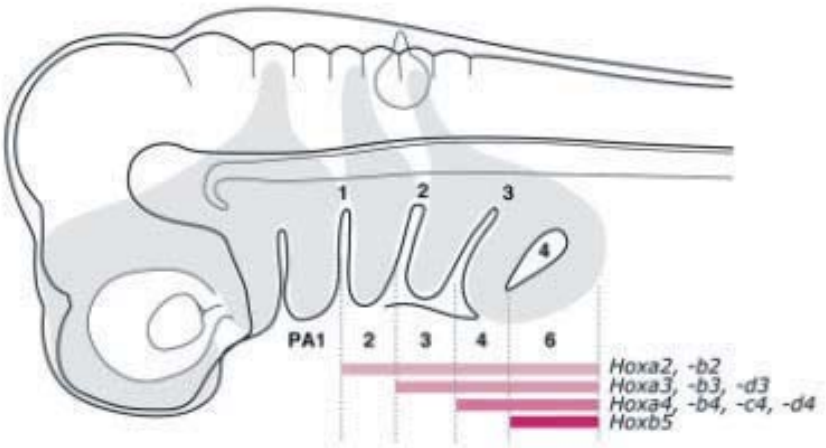
In jawed vertebrates, a group collectively known as "gnathostomes," the embryonic region from which the jaws arise does not express *Hox* genes. This region, called the first pharyngeal arch (PA1), is also found in the embryo of the lamprey, a jawless vertebrate (agnathan). A previous study by another group had reported the detection of the expression of a *Hox* gene in the lamprey PA1. The authors of that work suggested that the retreat of *Hox* gene expression from this region might have set the evolutionary stage for the emergence of gnathostomes, on the premise that *Hox* genes have an inhibitory effect on jaw development. Other experiments, in which either a *Hox* gene was artificially expressed in the normally *Hox*-free gnathostome PA1 and found to suppress jaw development, or, conversely, in which a *Hox* gene was inactivated in the normally *Hox*-expressing PA2, causing it to give rise to jaw-like structures, tended to support the contention of an evolutionary role for *Hox* expression in the innovation of the jaw.

(from left) Shigeru KURATANI, Yoko TAKIO, Shigehiro KURAKU

Takio Y, Pasqualetti M, Kuraku S, Hirano S, Rijli F M and Kuratani S. Evolutionary biology: lamprey Hox genes and the evolution of jaws.
Nature 429:1 p following 262 (2004).

The study by Kuratani and colleagues looked for *Hox* gene expression in the first pharyngeal arch of *Lethenteron japonica*, a different species of lamprey than the one used in the previous study (*Lampetra fluviatilis*) and found none; a discrepancy that might be attributable to inter-species differences. The real significance of this finding is that it strongly counters the hypothesis that a shift in *Hox* gene expression can explain the appearance of the gnathostome jaw.

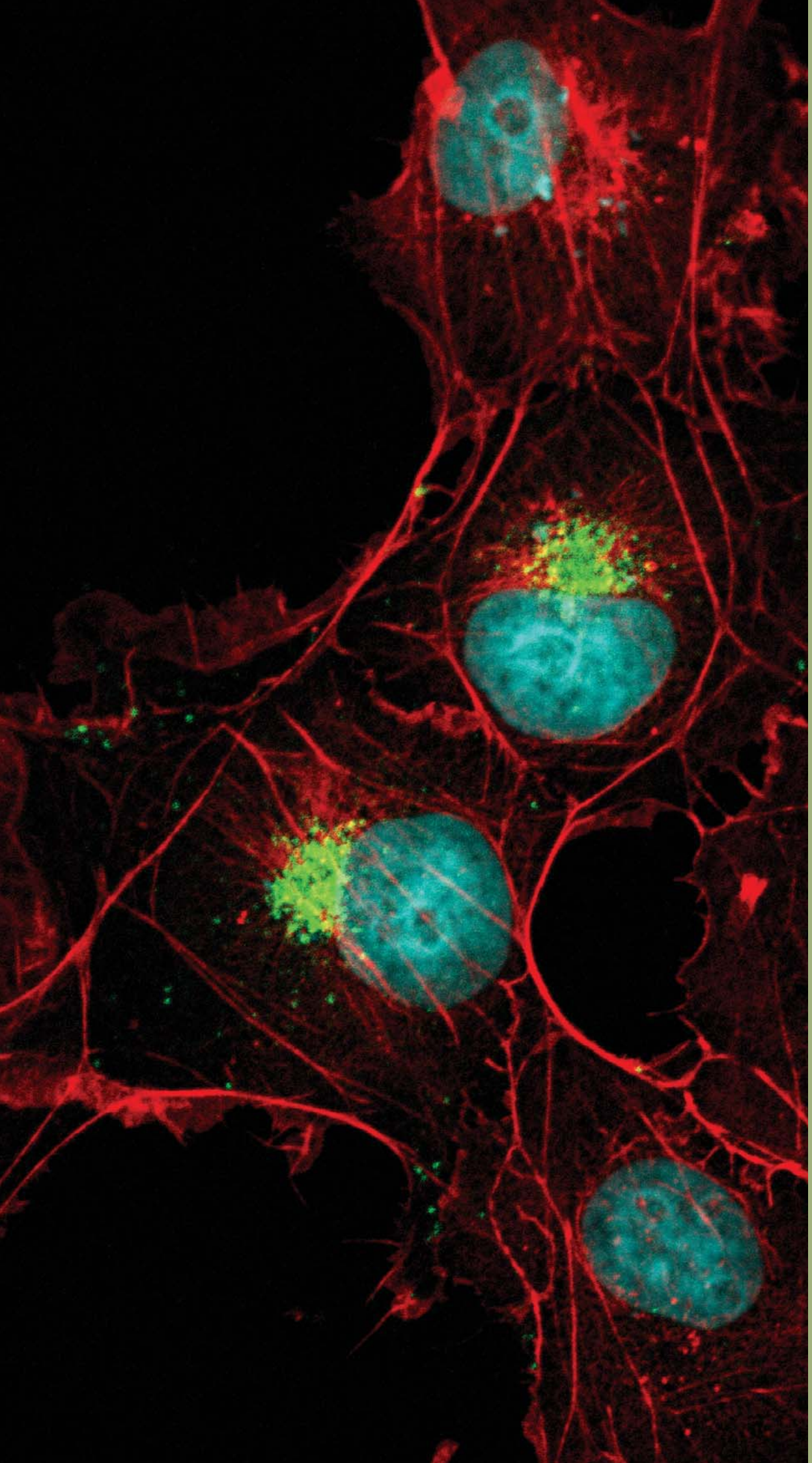
The expression of *Hox* genes is colinear; that is, the order of their expression down the body axis corresponds to their appearance on the chromosome. One of the most striking findings reported in the previous work suggesting that the loss of *Hox* expression in PA1 might have facilitated the branching off of the gnathostome lineage was that jawless chordates (such as lampreys and lancelets) broke the supposedly universal law of *Hox* colinearity. Testing this surprising assertion, Kuratani et al. isolated 11 Hox cDNAs in *L. japonicum* and checked their expression patterns in developing embryos. They failed to detect *Hox* activity in PA1, or any evidence of a disruption of colinearity, suggesting that this general principle is conserved in agnathans, as in all other known taxa. They propose that the lack of *Hox* expression in PA1 in both jawed and jawless vertebrates might instead simply reflect the fact that this structure gives rise to specialized structures distinct from those that originate in more posterior pharyngeal arches in both groups.



Cartoon showing *Hox* gene expression in pharyngeal arches of the gnathostome. Note that *Hox* expression is absent in the first pharyngeal arch (PA1), from which the jaw develops.

April-June Seminars

date	title	speaker
2004-04-01	Meiotic chromosome morphogenesis and function in *C. elegans*	Kentaro NABESHIMA
2004-04-08	A novel Polycomb MBT-1 as a dictator of differentiation of haematopoietic progenitor cells: implications for its relevance to leukemogenesis.	Toru MIYAZAKI
2004-04-16	Functional interdependency of fission yeast Alp14/TOG and Alp7/TACC in spindle function	Masamitsu SATO
2004-04-16	Analyses of gene functions with Sleeping Beauty transposon system and introduction of bi-allelic mutagenesis	Junji TAKEDA
2004-04-19	Constructing an organ through intracellular patterning	Yasushi HIROMI
2004-04-19	Clearing the language barrier: Advice for non-native speakers on preparing and submitting scientific manuscripts	Beatrice RENAULT
2004-05-12	Post-transcriptional regulation of oskar mRNA and RNP complexes during *Drosophila* oogenesis	Tamaki YANO
2004-05-13	How Shimadzu advanced technologies can work in your research project	H. JIKUYA, H. FUJIWAKE, Y. YAMAZAKI, A. HIRAKIMOTO
2004-05-14	The *Drosophila* microtubule associated protein Mini Spindles is required for RNA localization.	Woongjoon MOON
2004-05-19	Vasa and translational control in the *Drosophila* germ line	Paul LASKO
2004-05-24	Regulation of gonad formation and germ cell development	Mark Van DOREN
2004-05-24	Aspects of cross-talk between cadherin-mediated cell adhesion and the cytoskeleton	Alexander D. BERSHADSKY
2004-05-25	The *Snail* gene family in physiology and pathology	Angela NIETO
2004-05-28	microRNAs and growth control	Stephen M COHEN
2004-06-01	FGF signaling during mesoderm migration in the *Drosophila* gastrula	Arno MUELLER
2004-06-07	The putative RNA helicase Armitage is required for RISC assembly in *Drosophila* RNAi	Yukihide TOMARI
2004-06-09	The "odd-man out" of nodal-related proteins regulates convergent extension movements via the FGF receptor	Chika YOKOTA
2004-06-10	Ultrabithorax promotes membranous hindwing development by repressing elytra identity in the beetle *Tribolium castaneum*	Yoshinori TOMOYASU
2004-06-16	Axon growth inhibition signals from p75NTR	Toshihide YAMASHITA
2004-06-18	Physiological regulation of heterotrimeric G protein signaling in intact cells: An unexpected finding from electrophysiological measurement	Masaru ISHII
2004-06-28	New trends in ES cell technology	Woo-Suk HWANG, Sung Keun KANG, T WAKAYAMA, T ERA
2004-06-30	Novel tools for biomedical research and applications: Advanced biosensors and biodevices based on nanomaterials and microchip technology	Eiichi TAMIYA



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Shin-Ichi NISHIKAWA

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Hitoshi NIWA

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Tony PERRY

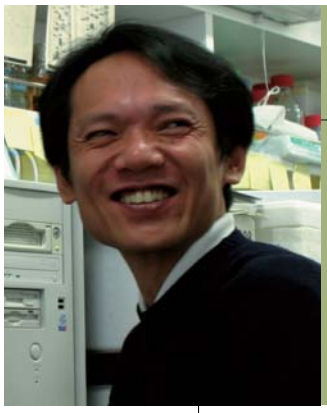
Mitinori SAITOU

Yoshiki SASAI

Hiroshi SASAKI

Hitoshi SAWA

Confocal fluorescence image of COS cells expressing Frizzled (Fz; Green) and Shisa, a molecule which regulates the establishment of receptors for the caudalizing factors, Wnt and FGF.



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 Gerard Edelman at the Rockefeller University. He returned to Japan in 1988 to take a position as a section chief in the Department of Molecular Genetics in the National Institute of Neuroscience. In 1998, he was appointed professor in the Department of Developmental Neurobiology at Tohoku University and remained there until taking his current position as group director at the RIKEN CDB.

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Izumi Y, Ohta N, Itoh-Furuya A, Fuse N and Matsuzaki F. Differential functions of G protein and Baz-aPKC signaling pathways in Drosophila neuroblast asymmetric division. *J Cell Biol* 164:729-38 (2004).

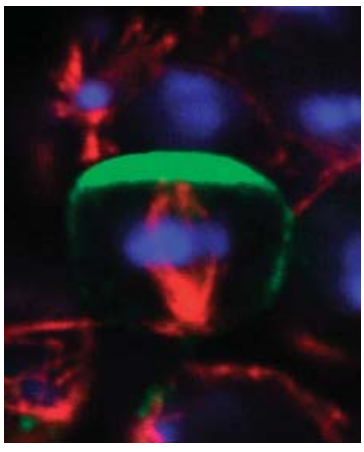
Fuse N, Hisata K, Katzen A L and Matsuzaki F. Heterotrimeric G proteins regulate daughter cell size asymmetry in Drosophila neuroblast divisions. *Curr Biol* 13:947-54 (2003).

Ohshiro T, Yagami T, Zhang C and Matsuzaki F. Role of cortical tumour-suppressor proteins in asymmetric division of Drosophila neuroblast. *Nature* 408:593-6 (2000).

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*, huge number of neural cells are 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 homologs 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 *Drosophila*, dividing neuroblasts localize the Miranda (green) / Prospero complex to be segregated into the daughter GMC.

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

Germline Development

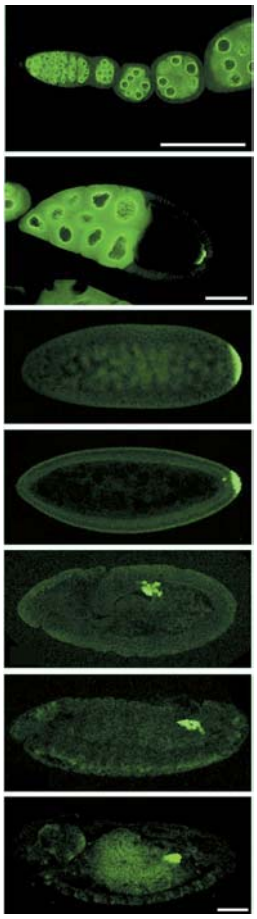
Akira NAKAMURA Ph. D.

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 post-doc 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 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, respectively.

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.



Drosophila germ plasm and germ cells visualized using GFP-Vasa fusion protein.

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

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Publications

Hanyu-Nakamura K, Kobayashi S and Nakamura A. Germ cell-autonomous Wunen2 is required for germline development in *Drosophila* embryos. *Development* 131:4545-53 (2004).

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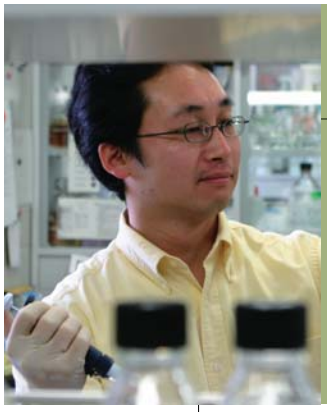

Chromatin Dynamics

Jun-ichi NAKAYAMA Ph. D.

Jun-ichi Nakayama received his bachelor's, master's and doctoral 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 2001as a PRESTO researcher in the Japan Science and Technology Corporation (JST). He was appointed team leader in the RIKEN CDB Laboratory for Chromatin Dynamics in 2002.

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Sadaie M, Iida T, Urano T and Nakayama J. A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast. *Embo J* 23:3825-35 (2004).

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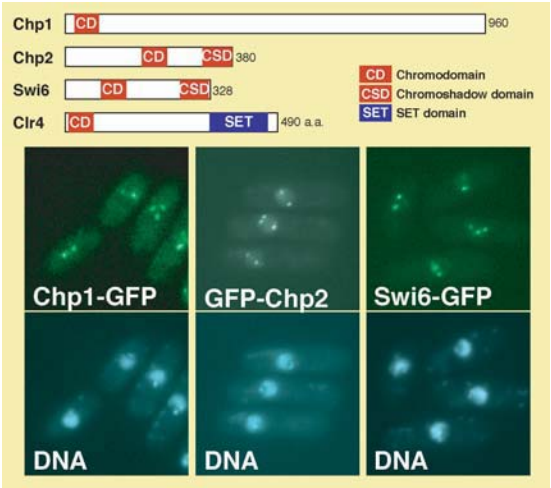
Multicellular organisms are made up of diverse populations of many different types of cells, each of which contains an identical set of genetic information coded in its DNA. Cell differentiation and the process of development itself depend on the ability of individual cells to maintain the expression of different genes, and for their progeny to do so through multiple cycles of cell division. In recent years, we have begun to understand that the maintenance of specific patterns of gene expression does not rely on direct modifications to the DNA sequence encoding the organism's genome, but rather takes place in a heritable, "epigenetic" manner. DNA methylation, chromatin modifications and 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 (*Saccharomyces 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.



Fission yeast (*Saccharomyces pombe*) chromodomain protein structure and localization

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

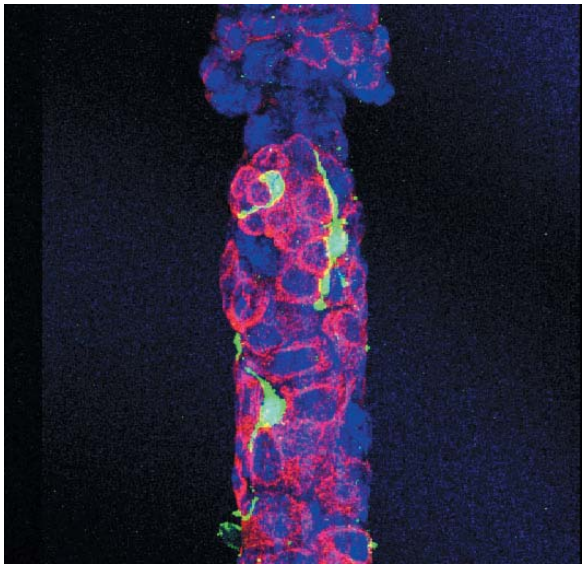
Stem Cell Biology

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. 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 which we hope will provide new insights into this fundamental process.



Visualization of a hair follicle

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Publications

Hirobe T, Osawa M and Nishikawa S I. Hepatocyte growth factor controls the proliferation of cultured epidermal melanoblasts and melanocytes from newborn mice. *Pigment Cell Res* 17:51-61 (2004).

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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.

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Kubota Y, Kuroki R and Nishiwaki K. A Fibulin-1 Homolog Interacts with an ADAM Protease that Controls Cell Migration in C. elegans. *Curr Biol* 14:2011-8 (2004).

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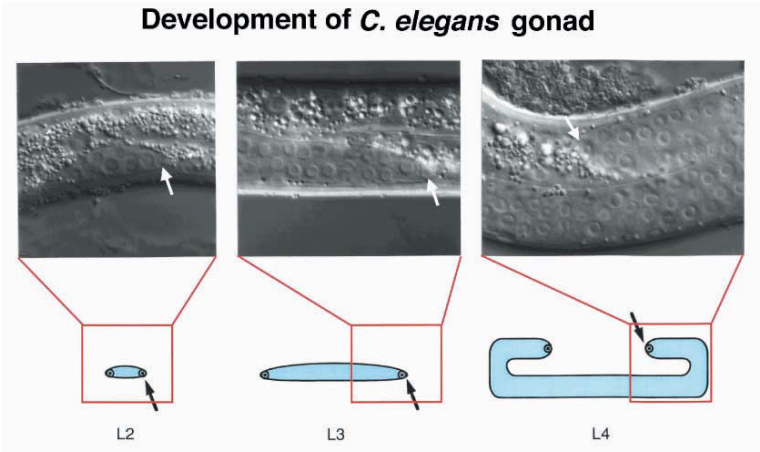
In many forms of organogenesis, we can observe the coordinated movement of epithelial cells in sheets. For example, in the development of the lung, tubular epithelial sheets repeatedly extend and subdivide in branching patterns and thereby give rise to the minutely ramified and intricate structure of 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 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 pattern 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 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.



Development of *C. elegans* gonad

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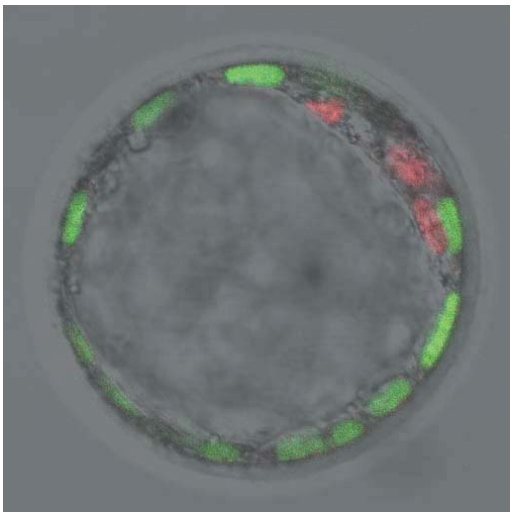
Pluripotent Cell Studies

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 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.

In previous work, we identified a peptide hormone that works to maintain the ability of ES cells to self-renew and developed an ES culture medium using fully characterized components. These developments were made in parallel with studies aimed at resolving the functions of genes involved in the maintenance of stem cells in an undifferentiated state and the induction of differentiation. We have also identified a transcriptional factor that directs differentiation into placenta and yolk sac. Given their ability to generate all of the body's cell types, ES cells have come to stand as a symbol for the emerging fields of cell replacement therapy and regenerative medicine, but they also represent an ideal system for the study of many of the processes of early mammalian embryonic development. The study of the basic biology of stem cells may one day bear fruit in the development of culture methods suitable for ES cells intended for clinical use or techniques for inducing differentiated cells to revert to a pluripotent state, while at the same time providing fundamental new insights into the differentiation of extra-embryonic tissues in the earliest phases of mammalian development.



Distribution of Cdx2 (green) and Oct3/4 (red) in blastocyst. Cdx2 localizes to trophectodermal regions, Oct3/4 to the inner cell mass.

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Miyagi S, Saito T, Mizutani K, Masuyama N, Gotoh Y, Iwama A, Nakauchi H, Masui S, Niwa H, Nishimoto M, Muramatsu M and Okuda A. The Sox-2 regulatory regions display their activities in two distinct types of multipotent stem cells. *Mol Cell Biol* 24:4207-20 (2004).

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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.

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Publications

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E and Sasaki H. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* 429:900-3 (2004).

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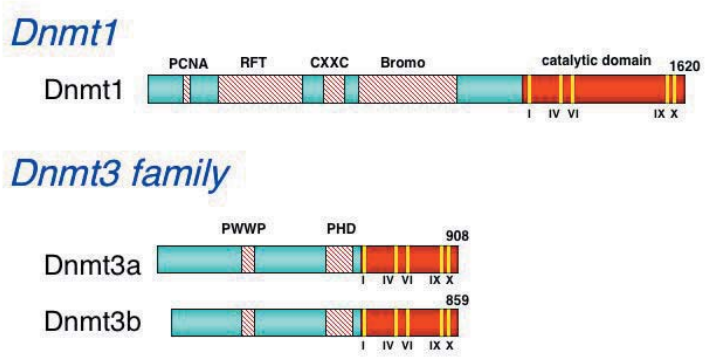
The development and physiological function of multicellular organisms relies on the ability of their component individual cells, which as a rule contain identical genetic information, to express subsets of genes in a selective manner. In order to maintain lineages of cells of different types, the gene 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 and structural remodeling of chromatin, nuclear structures that store the cell's DNA, allowing individual cells to regulate the switching on and shutting 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 fate determination 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.



Structures of mammalian DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b

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

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. In 1989 he became a postdoctoral fellow working on epididymal sperm maturation at Bristol University and in 1996 won a European Molecular Biology Travel Fellowship to work in the laboratory of Ryuzo Yanagimachi on the mechanism of oocyte activation, which remains one of his research interests. From there, Dr. Perry moved first to the Rockefeller University and then to the company Advanced Cell Technology, working primarily on novel methods of genome manipulation. In 2002 Dr. Perry took his present position as Team Leader of the Laboratory of Mammalian Molecular Embryology at the RIKEN CDB, where he works on mechanisms in mammalian preimplantation embryos.

Fertilization achieves the transformation of two specialized cells with little inherent developmental potential - a sperm and an unfertilized egg - by combining them to produce a state of totipotency: a single cell from which all cell types develop to produce an entire individual. The initial period of this remarkable process is known as oocyte activation, an intricate orchestration of sub-cellular events that include all the checks and balances that presage healthy growth of a new embryo. Oocyte activation can be observed under a light microscope as a series of dramatic changes in the morphology of the newly fertilized egg and provides a read-out of the underlying molecular mechanisms. Our laboratory combines molecular and cell biology with piezo-actuated micromanipulation of mouse gametes and embryos to study the events that occur during oocyte activation and their developmental consequences.

We are systematically elucidating interactions between sperm head components and the oocyte at fertilization, particularly during the moments soon after the sperm has penetrated the oocyte. The interests of our lab include attributing molecular identities to the proteins involved in these interactions and characterizing them functionally. This task is daunting, as beneath the membrane of a sperm head reside macromolecular assemblies that include a nucleus (containing the paternal genome and associated proteins) and a surrounding cytoplasmic matrix, the perinuclear matrix (PNM), which rapidly comes into contact with the oocyte cytoplasm at fertilization and is an immediate source of paternally-contributed molecules that may modulate development.

The study of these proteins holds forth the promise of enabling the identification of oocyte signaling pathways and processes that become operational at fertilization and establish what roles they play in subsequent development. Such roles may not be restricted to short-term development, as a growing body of evidence indicates that they have far-reaching consequences, even after the resulting adult reaches advanced age.

An early model of the sperm-borne oocyte activating factor

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Perry A C. Nuclear transfer cloning and the United Nations. *Nat Biotechnol* 22:1506-8 (2004).

Fujimoto S, Yoshida N, Fukui T, Amanai M, Isobe T, Itagaki C, Izumi T and Perry A C. Mammalian phospholipase Czeta induces oocyte activation from the sperm perinuclear matrix. *Dev Biol* 274:370-83 (2004).

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Mammalian Germ Cell Biology

Mitinori SAITOU M. D. , Ph. D.

Mitinori Saitou graduated from the Kyoto University Faculty of Medicine in 1995, and was awarded an M. D. in 1999 for his study of the structure and function of mammalian tight junctions under Prof. Shoichiro Tsukita in the Kyoto University Graduate School of Medicine. After a brief fellowship in the same department, he moved to the Wellcome Trust/Cancer Research UK Institute in 2000, where worked as a postdoctoral research associate in Azim Surani's laboratory, focusing on the origin and properties of the germ line in the mouse. He remained there until 2003, when he returned to Japan to take up his current position as head of the CDB Laboratory for Mammalian Germ Cell Biology. He received a three-year grant from the Japan Science and Technology Corporation (JST) PRESTO program that same year.

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Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M and Matsui Y. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. *Dev Biol* 278:440-58 (2005).

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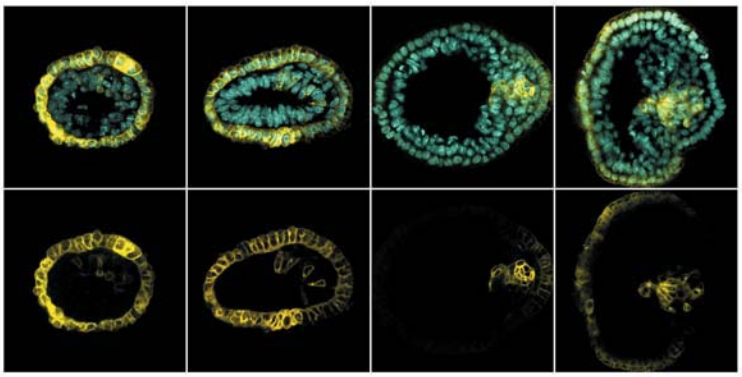
Saitou M, Barton S C and Surani M A. A molecular programme for the specification of germ cell fate in mice. *Nature* 418:293-300 (2002).

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 to a state of developmental totipotency, to undergo epigenetic reprogramming, and to divide meiotically. Ultimately, through fusion with another germline cell, they give rise to an entirely new organism.

Research in our laboratory is geared to elucidating the developmental bases of germline function at the molecular and systems levels from the very earliest stages of germline development. Specifically, our subjects of interest are the molecular mechanisms 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, and the development of technologies to enable the analysis of transcriptional regulatory networks at the single-cell level.

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 microarray analysis system currently being 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 one day in the future to be able to manipulate germ cell properties epigenetically.



Germ line segregation in the epiblast visualized by Blimp-1mEGFP transgene reporter. Fluorescent cells surrounding the epiblast are visceral endoderm.

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

Organogenesis and Neurogenesis

Yoshiki SASAI M. D. , Ph. D.

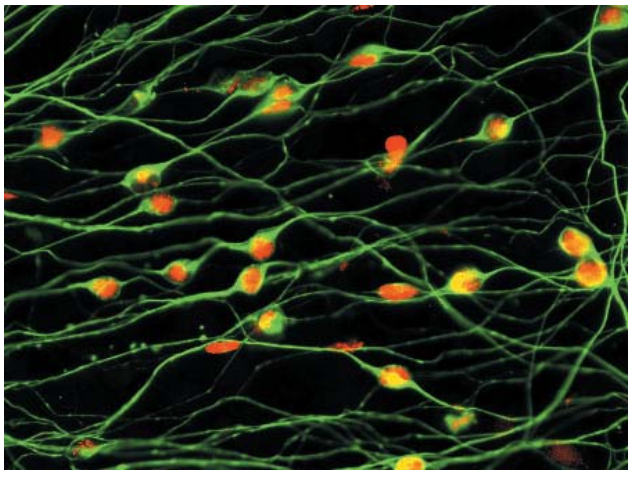
Yoshiki Sasai received his M. D. from the Kyoto University School of Medicine in 1986, subsequently performing internships in general practice and emergency medicine. He completed the Ph. D. course at the same institution in 1992, for work on neural specific transcriptional regulators. In 1993, he took 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. Dr. Sasai serves on the editorial boards of *Neuron, Development, 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 (back-belly) 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.

The 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 applications 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 it remains at quite 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.



Primate motoneurons

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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 Center for Developmental Biology.

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Sekimizu K, Nishioka N, Sasaki H, Takeda H, Karlstrom R O and Kawakami A. The zebrafish iguana locus encodes Dzip1, a novel zinc-finger protein required for proper regulation of Hedgehog signaling. *Development* 131:2521-32 (2004).

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

Research in our laboratory focuses on these signaling centers, especially those of the mouse, as a model for studying the regulation of embryogenetic processes. We focus particularly on the node and notochord, which are of central importance in the formation of the early embryo. Focusing on analyses of the roles played by the transcription factor Foxa2/HNF3β, and defects in head development that result in a loss of function mutant allele named *headshrinker*, we seek to determine the mechanisms that establish and maintain signaling centers during development.

The functional analysis of signaling molecules, such as the protein Sonic hedgehog, in embryonic morphogenesis and the identification of novel molecules and systems in the regulation of signaling centers are also subjects of interest for current and future study.

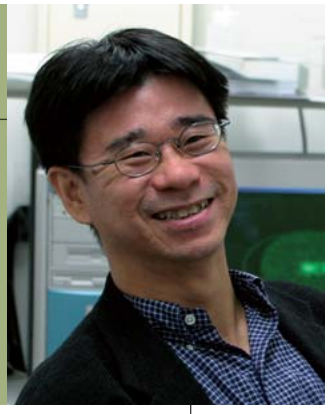
E7.5 transgenic mouse carrying Foxa2 enhancer region, showing strong gene expression (green) in notochord and node

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

Cell Fate Decision

Hitoshi SAWA Ph. D.

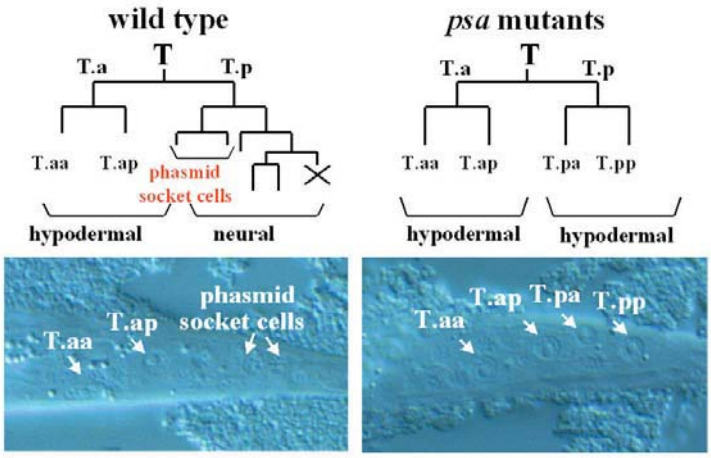
Hitoshi Sawa obtained his baccalaureate, master's and doctoral degrees from Kyoto University in the period from 1982 to 1991. He worked as a postdoctoral fellow at the California Institute of Technology on a Human Frontier Science Program grant during the period from 1991 to 1994, then at the Massachusetts 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 a 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.



Loss of Phasmid socket cells in *psa* mutant roundworm (right)

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

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XsalF comes to the fore in brain regionalization

Takayuki ONAI

In vertebrates, the nervous system is divided into distinct regions patterned in a head-to-tail direction. Embryologists have long been interested in working out the means by which this regionalization is achieved, and for years the dominant theory has involved a two-step mechanism in which neural tissues are first induced lengthwise down the entire body axis, followed by a transformation step where a second signal specifies the posterior identities of the neural tissue, which can be induced by a number of factors including members of the Wnt and FGF gene families, and retinoic acid.

While there is much evidence to support this model, a number of recent studies have indicated that the specification of the forebrain (an anterior structure, presumed to be induced by the initial signal in the activation-transformation model) requires additional regulatory inputs as well. New work by scientists in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director) showing anterior neural specifying activity in the African clawed frog, *Xenopus laevis*, lends weight to the revisionist argument.

In an article published in *Developmental Cell*, Takayuki Onai et al. in the Sasai lab reported that *XsalF*, the *Xenopus* homolog of *spalt*, a homeotic gene known to function in anterior-posterior segment identity in *Drosophila*, regulates the expression of forebrain and midbrain-specific genes. A series of experiments in which *XsalF* was misexpressed, deleted and its function blocked, showed direct linkage between *XsalF* expression and forebrain/midbrain identity.

XsalF was originally identified in a screen of the frog anterior neural plate, a structure that appears early in neural development.

Onai T, Sasai N, Matsui M and Sasai Y. Xenopus XsalF: anterior neuroectodermal specification by attenuating cellular responsiveness to Wnt signaling. *Dev Cell* 7:95-106 (2004).

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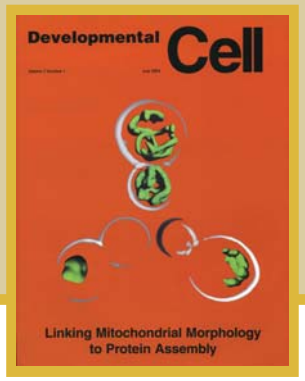


Sequencing of the gene, and analysis of the timing and spatial pattern of its expression showed that it codes a transcription factor related to Spalt and is expressed in the incipient forebrain and midbrain at precisely those developmental stages when brain regions are specified. When members of the Sasai lab overexpressed the gene by injecting its mRNA into early embryos, they found it caused the expanded expression of genes specific to anterior brain regions while suppressing the expression of more posterior markers.

Onai et al. next went on to test the effects of the loss of *XsalF* function. Disabling the gene by the deletion of functional domains resulted in embryos with significant reductions in anterior neural structures. Conditional loss-of-function experiments showed that the gene's expression was required in the early and mid neurula stages, when the developing brain undergoes regionalization. They then confirmed the specificity of this requirement for *XsalF* by injecting morpholinos (short nucleotide chains that block the function of a targeted gene), which gave similar results to the earlier loss-of-function studies.

These preliminary findings prompted the lab to look into the molecular mechanisms behind XsalF's anterior specifying activity, which they suspected was linked to the inhibition of the Wnt cascade (a signaling pathway that posteriorizes neural tissues). They focused on two factors, GSK3β and Tcf3, known to antagonize Wnt signaling in certain contexts, and found that the expression of both factors was dependent on

Interference with *XsalF* gene function results in incomplete head development in the *Xenopus* embryo (bottom); normal embryo shown at top .

XsalF. Gain- and loss-of-function studies reconfirmed the connection between XsalF and these Wnt antagonists, showing that XsalF alters the receptivity of anterior neural cells to Wnt signaling by regulating the expression of GSK3β and Tcf3, making these cells resistant to the posteriorizing effects of Wnt.

The signaling networks at work in this competitive regional determination appear to be intricate and involved in two linked, but distinct, aspects of regions-specific transcriptional regulation – the switching on of fore – and midbrain-specific genes, and the suppression of posterior genes.

NRH1 and Wnt signaling: A happy convergence

The African clawed frog, *Xenopus laevis*, begins development as a compact ball of cells that undergoes a dramatic transformation through cell migrations and positional rearrangements that result in the separation of the embryo into three distinct germ layers, which go on to give rise to all of the tissues and structures in the adult animal's body. During this transformation, known as gastrulation, the embryo changes from a roughly spherical shape to an elongated, streamlined form through a process called convergent extension (CE), in which polarized cells migrate to and merge at the embryo's midline, driving it to lengthen along its anterior-posterior axis

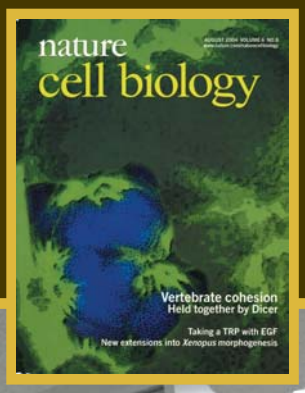
A number of genes involved in the regulation of convergent extension have been identified in amphibians and other vertebrates, such as zebrafish, but the picture of the underlying molecular mechanisms remains incomplete. In a report published in *Nature Cell Biology*, Noriaki Sasai and colleagues in the Laboratory for Organogenesis and Neurogenesis show that the product of the gene *NRH1* is essential to the regulation of CE movements in the frog.

Embryo injected with NRH1 morpholino (top) shows shortened body axis due to failure of convergent extension. Wild type embryo is shown below.

While performing a screen of genes expressed in the posterior neuroectoderm, Sasai et al. identified a gene encoding a protein that showed similarities to the gene for p75 NTR, a neurotrophin receptor. (Neurotrophins are molecules that function in the survival, growth and migration of neurons.) However, on testing its affinity for neurotrophin ligands, the group found that, unlike p75 NTR, their protein (named NRH1) did not bind with neurotrophins, which led them to begin a search for other biological roles.

Experiments in which NRH1 was overexpressed by injecting its messenger RNA directly into very early (4-cell stage) embryos resulted in shortening of the body axis and the failure of mesodermal and neural plate marker gene expression to converge on the midline or extend axially. Interestingly, interfering with *NRH1* function by introducing morpholinos to block production of its protein had similar effects – the inhibition of convergent extension. That both gain and loss of NRH1 function resulted in the failure of CE activity, suggesting that the gene's function in this process is tightly regulated.

These first findings led Sasai to investigate possible interactions between *NRH1* and genes involved in the Wnt/PCP (planar cell polarity) signaling pathway, which is also known to play an important role in the regulation of CE movements in both fish and frog through the activity of downstream small GTPases.



Sasai N, Nakazawa Y, Haraguchi T and Sasai Y. The neurotrophin-receptor-related protein NRH1 is essential for convergent extension movements.
Nat Cell Biol 6:741-8 (2004).

(from left) Tomoko HARAGUCHI, Yoko NAKAZAWA, Noriaki SASAI, Michiru MATSUMURA

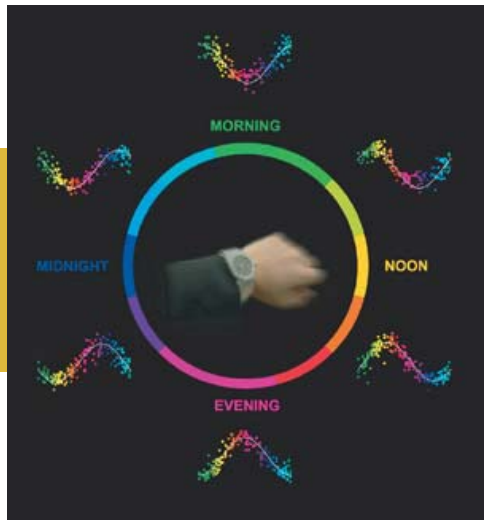
These proteins, which include Rho, Rac and Cdc42, interact with the cytoskeleton and play important roles in the dynamics of cell morphology and motility. Overexpression and loss-of-function of *NRH1* in the marginal zone (where convergent extension originates) respectively resulted in increased and decreased *Rho*, *Rac* and *Cdc42* activity, confirming the link between NRH1 and Rho-family small GTPases. Loss of *NRH1* function could be rescued by the co-injection of Frz7, a Wnt receptor functioning upstream of Rho, Rac and Cdc42 in the PCP pathway and, similarly, NRH1 complemented a dominant-negative Frz7 phenotype, indicating the two proteins play compensatory and mutually independent roles in the activation of small GTPases.

Further experiments showed that NRH1's effects on CE movements are also mediated by a second branch of the Wnt/PCP pathway, in which MKK7 and JNK work to phosphorylate c-Jun in the animal cap (a region of prospective ectoderm located on the roof of the blastocoel, a hollow in the spherical early embryo). As with the small GTPases, the activation of the MKK7-JNK cascade could also be effected by either Frz7 or NRH1, but it was found that NRH1 functioned independently of Xdsh, another upstream regulator of Rho-family small GTPase activity in the Wnt/PCP pathway. While the transduction mechanisms by which NRH1 interacts with Wnt/PCP signaling factors and the specifics of the inter-related but apparently independent roles of NRH1 and PCP signaling in the control of cell movements within the developing embryo remain to be worked out, the Sasai study represents a significant first step toward untangling the genetic knitwork behind this complex series of events.

Got the time

A look inside a wristwatch reveals that timekeeping is a complex affair, involving the coordination of mechanical parts providing the impulses and feedback needed to achieve precisely recurring movement. Biological clocks are equally complex, regulated by a network of genes and transcriptional factors that interact to stabilize the rhythms of numerous physiological systems. Unlike the wristwatch, however, there is no visible readout or display showing an individual's body time, a lack that has stood as one of the major obstacles to realizing the promise of chronotherapy, which seeks to deliver drug treatments at optimal body times.

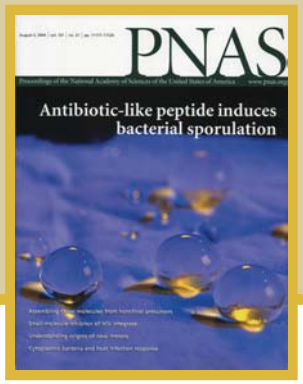
A new study by Hiroki R. Ueda (Laboratory for Systems Biology) and colleagues has provided proof of principle that just such a display of individual body time may one day be possible. The report, published in the *Proceedings of the National Academy of Sciences* describes the analysis of the expression of more than 100 time-indicating genes in the mouse. The results of this genome-wide study enabled the authors to develop a "molecular timetable" that provides an accurate representation of the animals' body time based on the sampling of gene expression levels at a single point in time.



Body time represented in a gene expression profile of time-indicating genes. In the morning (center top) 'dawn-indicating genes' (green) are highly expressed, and 'dusk-indicating genes' (pink) are expressed at low levels. In the evening (center bottom), their expression levels are reversed.

Many genes exhibit variable expression cycles roughly over the course of a 24-hour day, a phenomenon known as circadian rhythmicity. In their study, Ueda et al. first identified genes that are expressed in high-amplitude circadian patterns. Such genes demonstrate cyclical expression irrespective of variations in exposure to light, maintaining their amplitude and periodicity even when the animals are kept in constant darkness. The team identified 168 genes fulfilling these criteria in samples from mouse liver, and calculated their peak expression times in terms of both external time and the animal's subjective inner clock. They found that the genes could be organized into a kind of daily schedule by their patterns of peak expression – some were expressed most highly at daybreak, some at dusk, some at other timepoints throughout the cycle of a single day.

By plotting the expression levels of the entire set of genes against a 24-hour curve, Ueda and colleagues found that it is possible to determine body time with a high degree of accuracy. To test the robustness of this molecular chronometry they measured the expression levels of the 168 genes at the lights-out phase in animals kept in conditions of alternating 12-hour periods of light and dark. This phase



Ueda H R, Chen W, Minami Y, Honma S, Honma K, Iino M and Hashimoto S. Molecular-timetable methods for detection of body time and rhythm disorders from single-time-point genome-wide expression profiles. *Proc Natl Acad Sci U S A* 101:11227-32 (2004).

is presumed to be a hotbed of circadian regulatory activity, as the mouse's internal rhythm runs in cycles that are naturally shorter than 24 hours, and darkness serves as a stimulus for resetting the circadian clock. Such stimuli are referred to as *Zeitgeber* mechanisms, from the German word meaning “time-giver”. Their analysis of expression levels at this critical *Zeitgeber* Hour 12 produced estimated body times to an accuracy of about 1 hour in all animals studied, indicating that the molecular timetable provided is strongly resistant to environmental noise (in this case, variations in exposure to light).

They next tested the system in mice with a homozygous mutation in the gene *Clock*, whose loss of function is known to perturb the natural circadian rhythm. They found rhythm disruptions in the expression profiles of all of the *Clock / Clock* mice, suggesting that the molecular timetable approach may also have applications in the diagnosis of circadian rhythm disorders.

(from left) Hiroki UEDA, Yuichi MINAMI

This new approach to the horology of the body has been validated in mice of varying genetic backgrounds and in principle can be applied to any organism exhibiting biological clock activity, a range that spans the living world from bacteria to plants to humans. And indeed a test of the method in wild type and *Clock*-mutant fruit flies gave similar results to those in the mouse studies.

The development by Ueda and colleagues of a universally-applicable, specific, sensitive and accurate method capable both of detecting individual body time and of diagnosing circadian disorders represents a major step toward the fulfillment of the longstanding dream of scientists and physicians: to put a readable face on the body's clock. Its application in medical treatment may one day allow doctors to tailor drug administration to a patient's body time, which promises to optimize efficacy and reduce adverse effects.

Breaking new ground in germ cell guidance

Many types of cells are able to make their way through the body, propelled by molecular motors and guided by pathfinding mechanisms. One of the great migratory phenomena in development is the journey undertaken by primordial germ cells in the *Drosophila* embryo. These cells form externally to the embryo at its posterior pole, are carried within by a process of invagination and navigate unerringly through the body to contribute to the formation of the gonad, acquiring the characteristics of more mature germ cells en route. The puzzle of how these cells find their way through the developing body has challenged biologists for years, but is now beginning to yield to their painstaking efforts toward identifying the underlying mechanisms that guide these travelers on their way.

Kazuko Hanyu-Nakamura and colleagues in the Laboratory for Germline Development (Akira Nakamura, Team Leader) fit a new piece into this jigsaw with findings published in the journal *Development*, which described an intriguing model for germ cell migration involving a pair of guidance molecules, Wunen and Wunen2, and their discrete activities in germ and somatic cells.

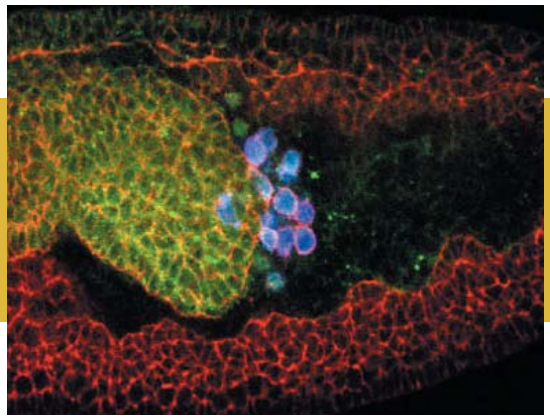
The study began with a screen for mutant flies, which uncovered a phenotype in which the flies' primordial germ cells, called “pole cells,” showed defects during their migration: the pole cells died in large numbers at a stage when they would normally begin to associate with the gonadal mesoderm. The genetic deficiency responsible for the defect was identified as a maternal effect mutation, meaning that its function (or loss of function) relies entirely on transmission from the mother fly. A closer analysis of the fail-

(from left) Kazuko HANYU-NAKAMURA, Akira NAKAMURA

Hanyu-Nakamura K, Kobayashi S and Nakamura A. Germ cell-autonomous Wunen2 is required for germline development in Drosophila embryos. *Development* 131:4545-53 (2004).

ing pole cells indicated that they began their development and migration normally, and that their deaths did not display a significant increase in the molecular activity most commonly associated with programmed cell death – activation of the apoptosis mediator, caspase-3.

Hanyu-Nakamura et al. pinpointed the site of the mutation to the locus for the guidance gene, *wunen2*. The products of this gene and its relative, *wunen*, have been known for some time to play important roles in the guidance of *Drosophila* pole cell migration by somatic cells. The genes encode lipid phosphate phosphatases (LPPs), which it had been proposed degrade an extracellular substrate, thereby creating an environment unfavorable to germ cells and steering them toward their gonadal destination. The overexpression of either gene in somatic cells had also been shown to cause the death of pole cells, indicating their importance to germ cell viability. A maternal-effect function for *wun2*, however, had never been reported, spurring the team to examine its role more closely.



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

What they found surprised them. While *wun* and *wun2* cause pole cell die-offs when overexpressed in somatic cells, the team discovered that maternal *wun2* activity is actually required to sustain pole cells in a cell-autonomous manner: cells lacking maternal *wun2* died in significantly greater numbers during migration. This suggested that *wun2* had nearly opposite effects on germ cell survival depending upon whether it was expressed in somatic or primordial germ cells. Hanyu-Nakamura proposes that this may be caused by a situation in which zygotically expressed Wun and Wun2 in somatic cells and maternally supplied Wun2 in germ cells exist in a kind of competitive homeostasis, which might be the result of the shared uptake and dephosphorylation of a common substrate necessary for the survival of germ cells. In this model, too much Wun or Wun2 in somatic cells would deprive germ cells of the survival signal, causing them to die; too little Wun2 in the germ cells themselves would have the same effect. The putative substrate has yet to be identified, but if the Hanyu-Nakamura model can be validated, it may well represent a new paradigm for explaining the function of LPPs in developmental processes from axon growth and patterning to extraembryonic vasculogenesis.

There and back again

(from left) Yoshiko TAKAHASHI, Yukiko NAKAYA

Epithelium and mesenchyme represent two extremes in the organization of groups of cells. Epithelial cells array themselves into flat sheets or rolled tubes, while mesenchymal cells appear less coordinated in their structure-forming activities and form fewer and looser connections with other cells. These tissue-level differences are reflected at the level of the individual cell as well. Under the microscope, mesenchymal cells are amorphous and lack the distinct apical-basal polarity that characterizes their epithelial counterparts. Both types of cells contribute to the body's function in distinct ways, with epithelium being the essential structural and physiological component of organs and tissues such as the kidney and the lining of the gut, and mesenchyme forming all migratory cells, including metastatic cancer cells, as well as providing support for the epithelium in various contexts. Despite (or perhaps because of) these differences, our anatomy contains countless examples of interaction between epithelial and mesenchymal cells. Indeed, nearly every one of the body's structures, from internal organs to limbs to teeth, is made up of a mesenchymal and an epithelial component

Developmental biologists are particularly interested in the ability of each of these cell types to be converted into its counterpart, epithelial cells become mesenchymal, while in other situations the reverse transformation occurs. In September 2004, Yoshiko Takahashi and colleagues in the Laboratory for Body Patterning reported a heretofore unknown mechanism by which mesenchymal-epithelial transitions (METs) are regulated in the embryogenesis of the chicken. Their study, published in *Developmental Cell*, describes how a pair of intracellular molecules affects the ability of mesenchymal cells to convert into epithelial cells during the formation of somites early in embryogenesis. Somites are transitory structures that appear in a head-down direction in strictly timed and ordered pairs as the chicken develops, dividing the embryo into body patterning segments, and later giving rise to the vertebrae, ribs and the muscles of the trunk.

Nakaya Y, Kuroda S, Katagiri Y T, Kaibuchi K and Takahashi Y. Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1.
Dev Cell 7:425-38 (2004).

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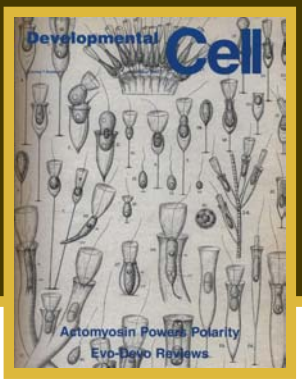


The presomitic mesoderm, from which somites form, consists of a pair of strips of mesenchymal cells running down the embryo's back. After the boundaries of an individual somite have been established, the cells in the border region undergo a transformation, acquiring the polarized structure and properties of epithelial cells. This results in a somite body in which an outer layer of epithelial cells surrounds a mesenchymal core. Takahashi et al. focused on this mesenchymal-epithelial transition to study how mesenchymal cells known for their formlessness are able to make the transition to become highly organized epithelial cells. They used a method that they had previously developed for introducing DNA into somitogenic cells by electroporation to study the role of a group of molecules known as Rho-family small GTPases. This family, which includes Rho, Rac and Cdc42, is known to play an important role in the dynamic organization of the cytoskeleton, the framework of elements that determines many aspects of a cell's morphology and structure.

The team found that the expression of constitutively active Cdc42 prevented mesenchymal cells from undergoing their normal epithelialization, while interference with Cdc42 function by a specific inhibitor caused an unusually high number of the mesenchymal cells to take on epithelial characteristics, demonstrating the central role of this GTPase in somitogenic MET. These findings were made possible by a novel experimental system developed in the Takahashi lab that takes advantage of the ability to introduce cDNA along with a fluorescent protein directly into a living embryo's cells by momentarily and reversibly disrupting their outer membranes by the application of a tiny electrical current, a process called electroporation. This technology allowed the team to track cells that had been electroporated with the mutant forms of Cdc42 and observe as they contributed to somite formation.

Somites forming in 2-day chicken embryo

Electroporation of dominant negative and constitutively active forms of Rac1 also indicated a role for that molecule in the mesenchymal-epithelial transition. Rac1 appears to act in a tightly constrained dose-dependent fashion to regulate MET, as both constitutively active and dominant negative forms of the protein disturb somitic epithelialization. The team further demonstrated that developing somites in the chicken are able to activate Rac1, suggesting a function for the protein in normal somitogenesis. When they extended their study to investigate possible crosstalk between Rac1 and Paraxis, the only transcription factor known to be essential for somitic MET, they found that Paraxis does indeed require Rac1 in its function as an epithelialization factor, strengthening the argument for its importance to the somitogenic process.

The development of a system for introducing DNA directly into somite-forming cells and the demonstration of the central role played by cytoskeleton-regulatory molecules in the cell shape changes characteristic of the mesenchymal-epithelial transition represent significant steps toward a clearer comprehension of how cellular reorganizations are achieved during development. And with epithelial-mesenchymal transitions featuring in fundamental biological processes from organ development to cancer metastasis, such knowledge may one day contribute to our ability to understand these processes, and perhaps even to right them, when they go awry.

Building a better tangle

Chromosomes, with their distinct morphologies and well-known function as the storehouses of genomic DNA, are one of the most familiar structures in the biology of the cell. In eukaryotes, these organelles are manufactured from complexes of DNA, histones and other proteins, called chromatin. This complex organization spools and folds lengthy strands of genetic material into compact aggregates capable of fitting in the tiny space within the nucleus while fulfilling their function as depots and providers of information used by the cell's transcription machinery. The ability to condense itself so that genes needed for protein production remain accessible, while others remain knotted into the deeper recesses is central to chromatin organization, and is evidenced by the existence of two structurally different forms – a less condensed form called euchromatin and its more densely-packed counterpart, heterochromatin. In most contexts, heterochromatic regions do not permit the expression of any genes they might contain, owing to their extreme compactness and epigenetic modifications. Heterochromatin is, nonetheless, important to the organization of non gene-encoding chromosomal regions necessary to the cell's ability to survive and replicate.

The chromosomes of the fission yeast, *Schizosaccharomyces pombe*, contain a number of heterochromatic regions, including centromeres, telomeres and the mating-type region. The configuration and function of chromatin in these domains is studied as a model of how chromatin organization achieves its repressive effects in other species, including our own. Generally, the formation of higher-order chromatin structure can be divided, at least, into two processes; establishment and maintenance. Researchers in the Laboratory for Chromatin Dynamics (Jun-ichi Nakayama, Team Leader) now report the identification of a role for the protein, Chp1, in the establishment of heterochromatin. This protein had previously been implicated as important to chromatin organization by studies that showed that yeast lacking the *chp1* gene suffered defects in chromosome segregation and centromeric transcriptional silencing, and that the Chp1 localizes to centromeric heterochromatic regions. It is also related to other known chromatin assembly molecules by virtue of its possession of a conserved motif, known as the

(from left) Jun-ichi NAKAYAMA, Mahito SADAIE

Sadaie M, Iida T, Urano T and Nakayama J.
A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast.
Embo J 23:3825-35 (2004).

chromodomain, shared by many of the protein players involved in epigenetic control of gene expression. Different chromodomain-containing proteins have been thought to play discrete roles in the chromosome's various heterochromatic regions.

Nakayama and colleagues started by analyzing the localization of three chromodomain proteins, Chp1, Chp2, and Swi6. Swi6 is a homolog of heterochromatin protein 1 (HP1) in mammals, and has been shown to play a crucial role in the formation of heterochromatin. The researchers found that Chp1 does indeed associate with all three heterochromatic regions in *S. pombe*, the first demonstration of its presence outside of the centromere. A detailed analysis of the differences in the localization patterns of Swi6 and Chp2 in mutant strains of fission yeast lacking Chp1 suggested that this protein plays a vital role in the localization of Swi6 and Chp2 specifically to the centromeric heterochromatin.

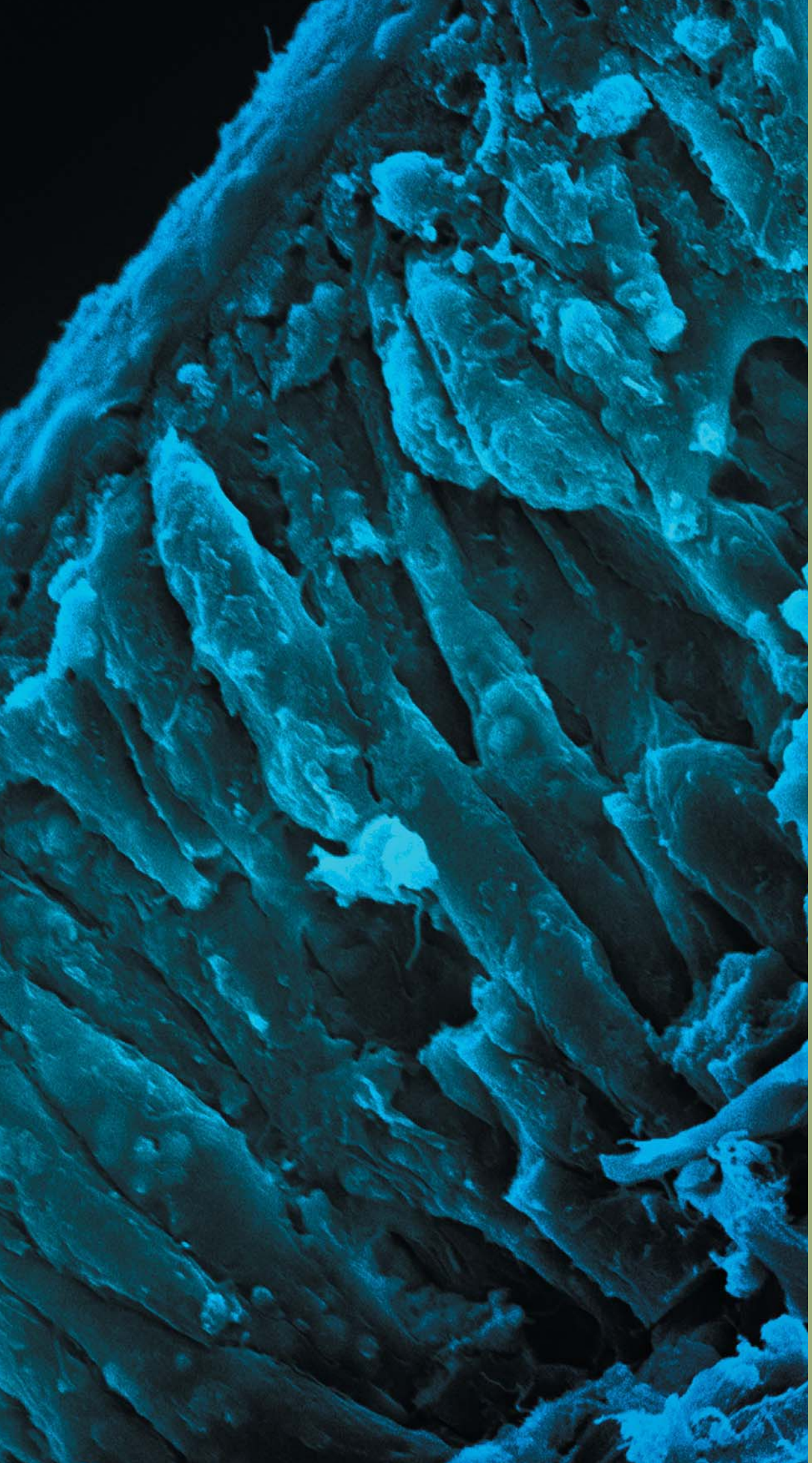
This sparked the team's interest in whether its function might be linked to RNA interference (RNAi), a process responsible for post-transcriptional gene silencing and known to be linked to both the establishment of heterochromatin and to centromere-specific gene silencing activity in fission yeast. Further experiments showed that loss of *chp1* function had similar effects on the accumulation of RNA transcripts to that of the loss of RNAi machinery components, indicating a role in either the production or processing of centromeric RNA, either of which might involve heterochromatin establishment. These similarities suggest that RNAi machinery and Chp1 work together; however, it remained unclear why mutations in *chp1* or RNAi cause centromere-specific defects. Given Chp1's universality of association at centromeres, telomeres and the mating-type region, they reasoned that the protein must have common function in all heterochromatic domains.

Histone proteins in chromatin, heterochromatin in particular, are subject to a form of epigenetic modification called methylation, which affects the expression of genes within the methylated region, generally by inactivating them. The introduction of methyl modification on histones is thought to be an initial and critical step in the establishment of heterochromatin. Nakayama and colleagues designed experiments to analyze the establishment steps using the histone methyltransferase, Clr4. When the function of the gene, *clr4*, is disturbed, methylation is drastically reduced; reintroduction of *clr4* restores this defect. However, in a *chp1* mutant, the restoration of *clr4* failed to re-establish methylation not only at centromeres, but also at the mating-type region and telomeres. These experiments elegantly demonstrated the common function of Chp1 in the establishment of all heterochromatic domains. Interestingly, tests of the chromodomain proteins Swi6 and Chp2 revealed that both were essential, possibly overlapping, factors in the maintenance of H3-K9 methylation. They concluded that these three chromodomain proteins play distinct and cooperative roles in the establishment and maintenance of heterochromatin structure.

This study reveals a surprising, indeed vexing, intricacy and specialization of epigenetic function that belies the seeming simplicity of *S. pombe*. The region-specificity and diversity of the activities of different proteins on the establishment and maintenance of methylation in the yeast chromosome underscores the importance of gene silencing – for what non-critical function would evolve such elaborate machinery? – and highlights the dazzling complexity that characterizes even seemingly simple biological systems. New questions, of course, await further study. It remains to be seen whether higher eukaryotes use similar mechanisms to establish and maintain heterochromatin structure, and if so, whether counterparts for Chp1 exist in these species. Whatever the answers may be, studies of fission yeast will continue to be needed to help develop a better understanding of the molecular mechanisms underlying chromatin-based epigenetic phenomena.

July-September Seminars

date	title	speaker
2004-07-01	Characterization of the stem cell system of basal flatworms	Peter LADURNER
2004-07-02	Non-canonical Wnt signaling pathways control cell polarity in *C. elegans*	Michael A. HERMAN
2004-07-02	Genetic analysis of epidermal morphogenesis in *C. elegans*	Andrew D. CHISHOLM
2004-07-02	Telomere binding proteins and their functions in the nematode *C. elegans*	Junho LEE
2004-07-05	Isolation of non-hematopoietic stem cells from mouse bone marrow osteoblastic zone	Kazuhiro SAKURADA
2004-07-06	Molecular mechanism of vascular development	Thomas N. SATO
2004-07-06	Development of wiring specificity of the olfactory system in *Drosophila*	Liqun LUO
2004-07-12	Functional expression of odorant receptors	Hiroaki MATSUNAMI
2004-07-12	Transcription regulation: a genomic network	Nicholas LUSCOMBE
2004-07-14	Gli activator and repressor functions in Hedgehog signaling	Chi-chung HUI
2004-07-14	ziro1b plays an evolutionarily conserved role controlling Hh expression during retinal development in zebrafish	Shuk Han CHENG
2004-08-11	BMP signaling via ALK2 and ALK3 in the surface ectoderm cells is required for lens formation	Shunichi YOSHIKAWA
2004-08-12	Casting a smile and wagging the tail. . . how to make skeletal muscle in different areas of the vertebrate embryo	Susanne DIETRICH
2004-08-12	Transcriptional control of early nuclei formation in the embryonic midbrain	Frank SCHUBERT
2004-08-13	Frizzled 5 function during nervous system development in the mouse	Carole J. BURNS
2004-08-23	The role of neuromuscular activity and neurotrophic factors on motoneuron development	Ronald W. OPPENHEIM
2004-08-23	Annexin 5- more than just a marker for apoptosis?	Bent BRACHVOGEL
2004-08-24	Bcl-2 and caspase regulation of neuronal death and degeneration: Lessons from gene-disrupted mice	Kevin A. ROTH
2004-08-25	The cell biology of neurogenesis	Wieland B. HUTTNER
2004-08-30	Control of hematopoietic stem/progenitor cell expansion by Lnk adaptor protein	Satoshi TAKAKI
2004-09-09	Changes in neurogenesis during arthropod evolution	Angelika STOLLEWERK
2004-09-10	Inversion of Volvox as a model for studying morphogenesis	Ichiro NISHII
2004-09-21	Tsukushi functions as a novel organizer inducer	Kunimasa OHTA
2004-09-27	Pattern formation in development without positional information	Robert KAY
2004-09-28	The role of lymphangiogenic growth factors in embryogenesis and cancer	Marc G. ACHEN
2004-09-29	Mechanism of first polarity establishment in the mouse preimplantation embryo	Takashi HIIRAGI



Guojun SHENG

Asako SUGIMOTO

Yoshiko TAKAHASHI

Masatoshi TAKEICHI

Hiroki R. UEDA

Teruhiko WAKAYAMA

Shigenobu YONEMURA

Animal Resources and Genetic Engineering

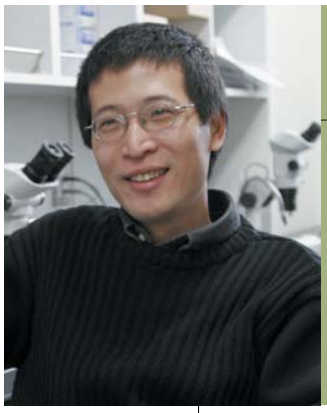
Genomics Support Unit

Leading Project Research Units

Animal Facilities

Administrative Support

Scanning electron micrograph of ectodermal cells from the rostral end of a stage 8 chicken embryo



Early Embryogenesis

Guojun SHENG Ph. D.

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

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Publications

Sheng G, dos Reis M and Stern C D. Churchill, a zinc finger transcriptional activator, regulates the transition between gastrulation and neurulation. *Cell* 115:603-13 (2003).

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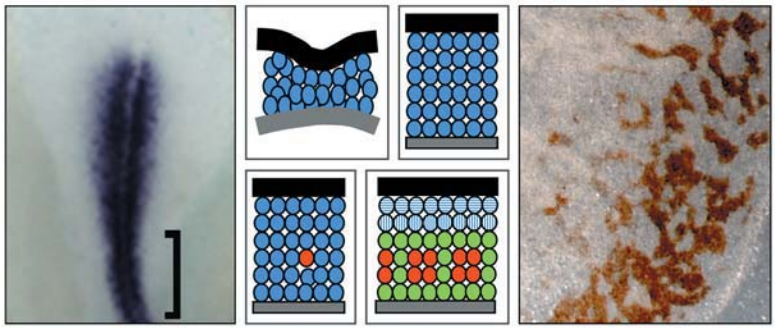
We study the molecular and cellular mechanisms of gastrulation, an early developmental process conserved throughout the animal kingdom. During gastrulation, three germ layers (ectoderm, mesoderm and endoderm) are formed from a uniform layer of epiblast cells. Each of these germ layers undergoes further 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 focuses on two aspects of gastrulation: the initial patterning of the neural ectoderm and mesoderm by signals from the organizer and the primitive streak, and the differentiation of posterior mesodermal precursors into hematopoietic and angiogenic cell lineages.

In the chicken embryo, signals from the organizer (Hensen's node) and the primitive streak induce molecular markers specific to either neural ectoderm or mesoderm cells, and control their distinct cell migratory behaviors. This induction is a multi-step process involving gradual commitment, ultimately leading to the assignment of a terminal fate. We are studying the signaling processes involved in the induction of cell fate-specific gene expression and behavioral changes in these systems.

The extraembryonic mesoderm gives rise to the earliest functioning cell types in the developing embryo, and serves as a simple model for the study of how subdivisions occur immediately after initial germ layer induction. Our team is investigating how two types of extraembryonic mesoderm cells (blood cells and endothelial cells) are specified by focusing on signaling inputs from adjacent tissues as well as these cells' autonomous differentiative properties.

In a third area of interest, we are beginning to study FGF molecules, which are involved in the initial induction and later cellular differentiation of both mesoderm and neural ectoderm. We are currently investigating the biochemical nature of this pathway's diverse roles by focusing on the properties of different ligand/receptor combinations as well as intracellular mediators of FGFR signaling.



Left panel: mesoderm cells in the streak (bracket indicating region contributing to extraembryonic mesoderm cell lineages); Middle panel: diagram of extraembryonic mesoderm cells' migration and differentiation; Right panel: cells differentiating into blood cells.

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

Developmental Genomics

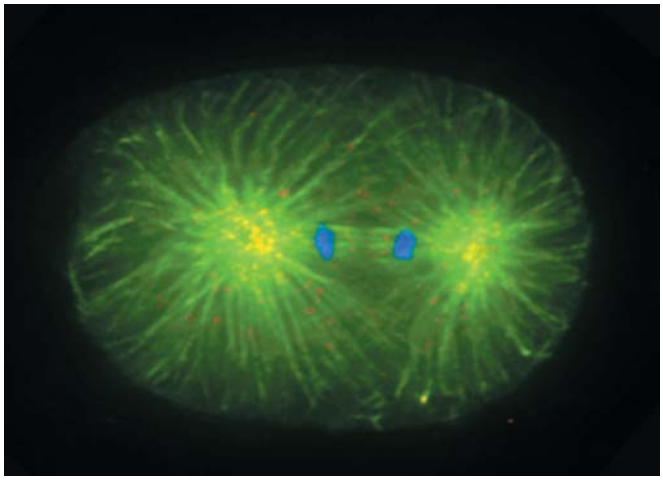
Asako SUGIMOTO Ph. D.

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.

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. The lab also looks to take findings from these studies as a base for advancing the understanding of developmentally important mechanisms.

Our laboratory has developed a high-throughput system for interfering with gene function using RNAi, which allows us to perform comprehensive screens for individual genes and gene groups with important functions in developmental processes and to profile phenotypes resulting from their loss of function. Through the systematic analysis of phenotypes (a field of study known as phenomics), we seek to gain a more detailed understanding of how an organism's genome frames and determines the set of developmental programs that operate in the building of the body.

We are now beginning to perform live imaging and other cell biological studies of discrete sets of genes identified in our phenomics analyses as being involved in microtubule dynamics in cell division and the regulation of hypodermal cell morphology. By studies such as these, we hope to provide a more detailed picture of the regulation of dynamic processes by networks of genes.



The first cell division of a *C. elegans* embryo
Green: microtubule. Red: Protein phosphatase 4 (PPH-4.1). Blue: DNA.

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

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Maruyama R, Endo S, Sugimoto A and Yamamoto M. Caenorhabditis elegans DAZ-1 is expressed in proliferating germ cells and directs proper nuclear organization and cytoplasmic core formation during oogenesis. *Dev Biol* 277:142-54 (2005).

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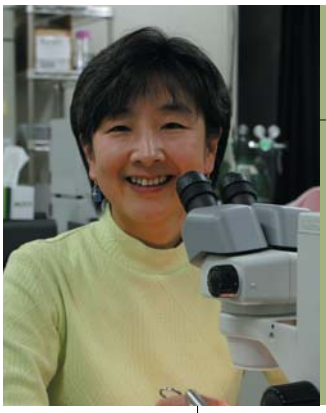

Body Patterning

Yoshiko TAKAHASHI Ph. D.

Yoshiko Takahashi received her B. Sc. from the University of Hiroshima, before moving to the Kyoto University Department of Biophysics where she received her master's and doctoral degrees in developmental biology. She pursued consecutive postdoctoral fellowships in developmental biology at the Institut d'Embryologie du CNRS (1988 to 1991), the University of Oregon Institute of Neuroscience (1991 to 1993) and Columbia University (1994). She returned to Japan as an associate professor at Kitasato University in 1994, where she worked until 1998, when she took an associate professorship at the Nara Institute of Science and Technology Graduate School of Biological Sciences. She was appointed team leader at the RIKEN CDB in 2001.

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Nakaya Y, Kuroda S, Katagiri Y T, Kaibuchi K and Takahashi Y. Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1. *Dev Cell* 7:425-38 (2004).

Tonegawa A, Kasai T and Takahashi Y. Systematic screening for signaling molecules expressed during somitogenesis by the signal sequence trap method. *Dev Biol* 262:32-50 (2003).

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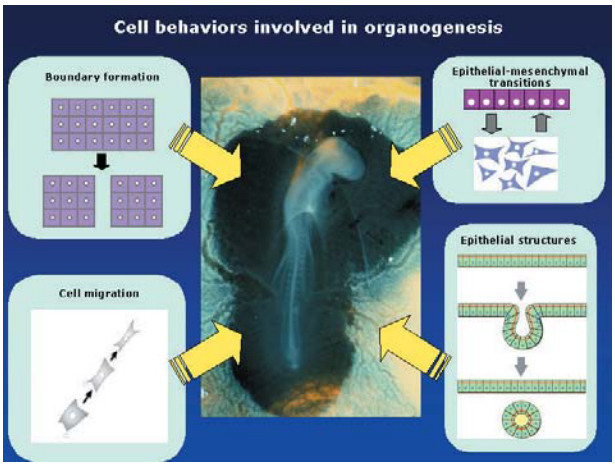
The vertebrate body arises from a seemingly unremarkable clump of cells that gradually organize into tissues displaying a wide variety of shapes and functions. On closer observation, the individual cells that take part in these morphogenetic processes also exhibit highly dynamic and varied activity. The research in our lab concentrates on investigations into the early stages of the body's development, when its structural plans are formed and executed, as evidenced by the behavior of cells in our primary model systems, the chicken and the mouse.

Visible patterns are defined by boundaries, but the means by which boundaries form in biological patterning processes is poorly understood. We study the formation of somites, embryonic regions that serve as precursors to much of the adult musculoskeletal system in vertebrates, seeking insights into the molecular underpinnings of boundary formation during that process. Previous work by our lab identified a boundary-inducing activity in inter-somite regions named the "segmenter", which we have linked to Notch signaling and the activity of the Ephrin/Eph ligand-receptor complex.

Cells in the early embryo are mobile, migrating throughout the body as it takes shape, following sets of rules that direct their movements to specific destinations, allowing them to build functional complex structures, tissues and organs. We are studying how one such rule, which dictates that vascular development follows pathways laid down by neurons, operates in the establishment of a three-dimensional network formed by cells of the neural crest (precursors of the peripheral nervous system) and embryonic blood vessels through the study of cellular behaviors and the regulatory effects of inductive factors.

The physiological functions of many organs, such as lung and the lining of the gut, rely on the activity and organization of epithelial cells. In such organs, these cells form highly-ordered sheets, which in many cases roll up into tubular structures. Conversions of epithelial cells into mesenchymal cells, and vice-versa, collectively referred to epithelial-mesenchymal transitions (EMT), make the formation of even more complex structural arrangements possible. We are now investigating epithelial and tubular structures and the molecular mechanisms participating in EMT to clarify how these activities contribute to the building of the body.



Cell behaviors involved in organogenesis

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Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph. D.

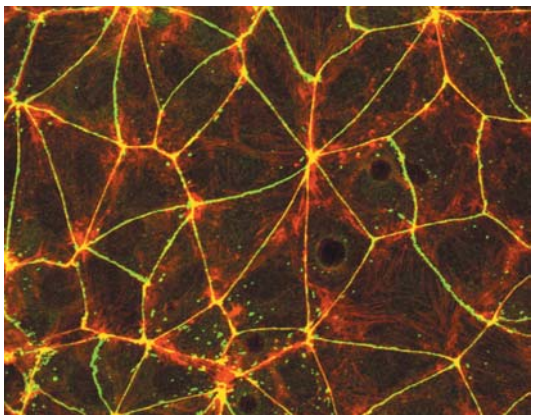
Dr. Masatoshi Takeichi is director of the RIKEN Center for Developmental Biology (CDB; Kobe, Japan) as well as director of the Cell Adhesion and Tissue Patterning research group. He completed the B. Sc. and M. S. programs in biology at Nagoya University before receiving a doctorate in biophysics from Kyoto University in 1973. After attaining his Ph. D., Dr. Takeichi took a research fellowship at the Carnegie Institution Department of Embryology under Dr. Richard Pagano. He then returned to Kyoto University, attaining a full 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 the study of 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, which is important for regulating many types of morphogenetic cell behavior. Cadherin activity is modulated by interactions with cytoskeletal or signaling factors mediated by members of the catenin family of cadherin-associated proteins. We are studying the mechanisms underlying the crosstalk between cadherins and cytoskeletal or signaling systems, with the goal of uncovering novel regulatory mechanisms specific to cell-cell adhesion.

We are also 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, how this process is involved in the physiological regulation of synaptic activities, and the role of neuron type-specific expression of cadherin subtypes in neural network development. We are also interested in the roles of other cell surface molecules, such as OL-protocadherin and nectins, in interneuronal recognition processes.

A third 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. Using the neural retina and brain cortices as model systems, our team is attempting to determine how cell migration and positioning are controlled by cell adhesion and signalling molecules during the formation of laminar structures in these tissues.



Epithelial cell-cell junctions are delineated by actin filaments, an important regulator of cell adhesion. Caco cells were double-stained for actin (red), and ZO-1 (green), a tight junction protein. Photographed by T. Otani.

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Publications

Abe K, Chisaka O, Van Roy F and Takeichi M. Stability of dendritic spines and synaptic contacts is controlled by α N-catenin. *Nat Neurosci* 7:357-63 (2004).

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Systems Biology

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

Hiroki R. Ueda received his M. D. from Faculty of Medicine, 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 Pharmaceutical, on a project studying biological clock mechanisms in fly and mouse. He was appointed team leader at the CDB in 2003.

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Ikuko TADA

Publications

Ueda H R, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y, Iino M and Hashimoto S. System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat Genet* (2005).

Ueda H R, Chen W, Minami Y, Honma S, Honma K, Iino M and Hashimoto S. Molecular-timetable methods for detection of body time and rhythm disorders from single-time-point genome-wide expression profiles. *Proc Natl Acad Sci U S A* 101:11227-32 (2004).

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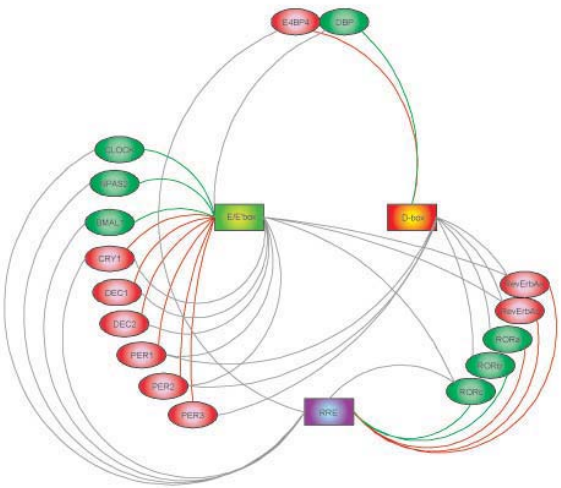
Recent large-scale efforts in genome-sequencing and expression analysis have produced an embarrassment of riches for life science researchers – biological data can now be accessed in quantities that are orders of magnitude greater than were available even a few years ago. Now, the growing need for integration of data sets has set the stage for the advent of systems biology, in which discrete biological processes and phenomena are approached as complex, interactive systems. We see systems biology research as a multi-stage process, beginning with the identification and analysis of individual system components and their networked interactions, and leading to the ability to control existing systems and design new ones based on an understanding of structure and underlying principles.

Our lab takes the mammalian circadian clock as a relatively simple and self-contained initial model for the study of a biological system. In addition to its advantages as a basic research model, the function of the circadian clock is intimately involved in the control of metabolic and hormonal cycles, and its dysregulation is linked to the onset and symptomatology of numerous human diseases, including sleep disorders. An improved understanding at the system level promises to provide biomedical and clinical investigators with a powerful new arsenal to attack these conditions.

To address complex and dynamic biological systems such as the circadian clock, it is necessary to make comprehensive and precise measurements of the system's dynamics and to work out the organization of its underlying gene network. Our team previously conducted a genome-wide screen and statistical analysis of gene expression to identify the clock-controlled genes that are rhythmically expressed in the central (suprachiasmatic nucleus; SCN) and peripheral (liver) circadian clocks. Analysis of the transcriptional regulation of gene expression in the morning, daytime, evening and night periods revealed a gene network of inter-regulating activators and inhibitors of time-linked gene expression. Our recent work has further demonstrated a number of general design principles underlying the transcriptional dynamics of clock-related genes. We next hope to apply these findings to the study of more involved and elaborate developmental processes.



Topology of transcriptional circuits composed of 16 transcription factors underlying mammalian circadian clocks. Transcriptional activators (green circles) and repressors (red circles), and transcriptional activation and repression (gray lines) are shown.

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

Genomic Reprogramming

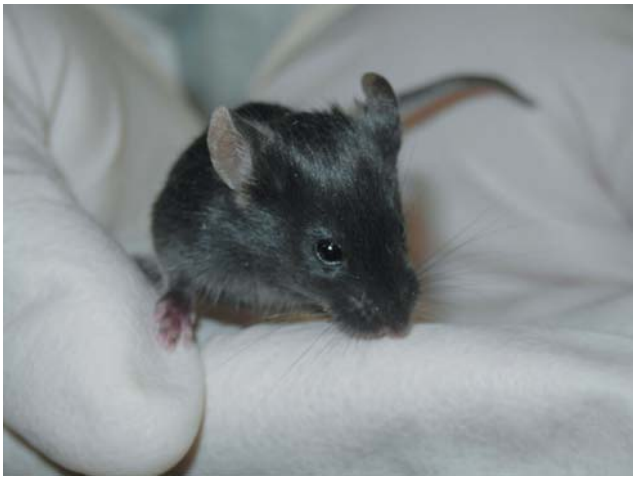
Teruhiko WAKAYAMA Ph. D.

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 producing 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 an clones of 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 doctors 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.



Wakame, a mouse cloned from somatic cells at Riken CDB

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

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Publications

Wakayama S, Kishigami S, Van Thuan N, Ohta H, Hikichi T, Mizutani E, Yanagimachi R and Wakayama T. Propagation of an infertile hermaphrodite mouse lacking germ cells by using nuclear transfer and embryonic stem cell technology. *Proc Natl Acad Sci U S A* 102:29-33 (2005).

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Cellular Morphogenesis

Shigenobu YONEMURA Ph. D.

Shigenobu Yonemura received his B. Sc., M. Sc. and Ph. D. from the University of Tokyo, earning his doctorate in 1988 for thesis work under Prof. I. Mabuchi. He spent a year as a postdoctoral fellow at the same institution before moving to pursue a fellowship at Johns Hopkins University from 1989 to 1990. He returned to Japan as an assistant professor in the Department of Cell Biology at the National Institute for Physiological Sciences, where he remained until 1995. He joined the Kyoto University Faculty of Medicine the same year, first as an assistant professor, then as a lecturer in the Department of Cell Biology, a position he retained until his appointment as CDB team leader in 2001.

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Publications

Yasuda S, Oceguera-Yanez F, Kato T, Okamoto M, Yonemura S, Terada Y, Ishizaki T and Narumiya S. Cdc42 and mDia3 regulate microtubule attachment to kinetochores. *Nature* 428:767-71 (2004).

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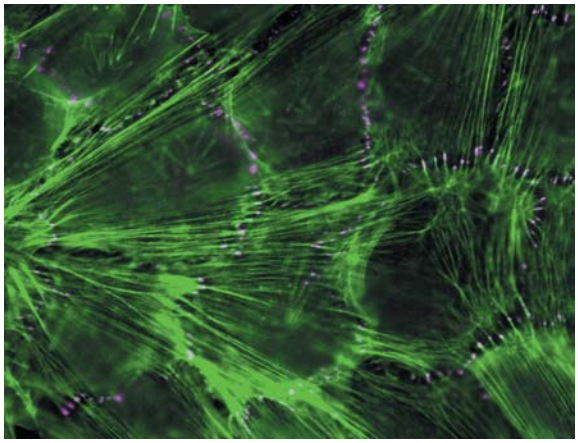
The body's cells exhibit a variety of shapes, a diversity that reflects the truism that form is at least a partial determinant of function. Cells' interiors contain networks of protein polymers called cytoskeletons. Although these structures are thought to play a major role in cellular morphogenesis responsive to changes in the cell's environment, there are still a number of unresolved questions regarding the mechanisms underlying cytoskeletal regulation. We address this issue taking an advantage of imaging techniques, such as fluorescence and electron microscopy, including methods developed by our lab.

Rho family proteins are known to be essential for actin cytoskeleton organization and cell division, but only very limited information about their behavior within cells had been available. We developed an antibody and fixation protocol for capable of localizing Rho and have now begun to use it to elucidate the distribution of Rho in cells and tissues. Animal cells cleave by forming a furrow between the two daughter nuclei following nuclear division. We have found that Rho accumulates at the putative furrow region in a microtubule-dependent manner prior to the initiation of furrowing. This is the first evidence that Rho localization is regulated by microtubules. Further, we have determined the signaling pathway connecting microtubules and Rho by knocking down a number of microtubule-associated proteins. Regulation by Rho appears also to be deeply involved in the determination of the cell division plane.

Cell-cell junction structures associated with actin filaments called adherens junctions are commonly found between adjacent cells in an epithelial sheet. Actin filaments are able to generate tension by interaction with a motor protein, myosin, and the general importance of actin association in the formation of the adherens junction has gained increasing acceptance, but we still do not know the specific reason why actin filaments are so indispensable. We have recently found that in fibroblastic NRK cells the adherens junction forms only at certain tension levels. Without the right amount of tension, adhesion molecules and associated proteins disengage and disperse. Rho activity was found to be involved in this junction formation, and another junction associated protein, vinculin, accumulated in a tension-dependent fashion in epithelial cells, suggesting not only that tension can be transmitted from cell to cell across the adherens junction but that it is also required for proper junction formation.



Cell-cell junctions in fibroblastic NRK cells (green: actin filaments, magenta: ZO-1)

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

Animal Resources and Genetic Engineering

Shinichi AIZAWA Ph. D.

The study of model organisms and systems is integral to biological research. Such systems provide scientists with the means to search for broadly shared mechanisms underlying developmental and regenerative processes across species, and conversely to identify those traits that earn each species its unique branch on the phylogenetic tree. The mouse is one of the most important and widely used model organisms in science today, prized for its amenability to genetic manipulation, its high level of homology with humans, and the trove of data regarding its physiology, genetics and development that has accumulated over nearly one century of intensive scientific research.

The Laboratory for Animal Resources and Genetic Engineering (LARGE) provides an important suite of services related to the generation of experimental mice to labs within the CDB and around Japan. In its role as a CDB support laboratory, the LARGE team produces transgenic and knockout mouse models to the specifications of scientists working in a wide range of genetic, embryological and biomedical research projects, maintaining the highest quality standards and rapid turnaround to ensure fast and easy access to researchers working within the Center and throughout the country. In addition to these core functions, the LARGE staff provides a number of other services, such as cloning by nuclear transfer and cryopreservation of mouse zygotes and sperm. The lab also performs a number of maintenance and logistical functions, such as the specific pathogen free (SPF) housing, cleaning, processing and distribution of animals.

The LARGE team continues to expand its services and initiate new programs, notably the generation of target vectors from sequence information alone, and the independent production of novel genetically-modified constructs, a drive that generates on the order of 40-50 new mutant strains per year. Such strains serve as research platforms with the potential to provide new insights into a range of important research problems, from the developmental mechanisms of organ development to the genetic bases of human disease. The lab will also function as part of Japan's system of Mouse Embryo Banks, with a special emphasis on producing, storing and cataloging embryos for use in developmental biology and regenerative medical research.

Chimera mice

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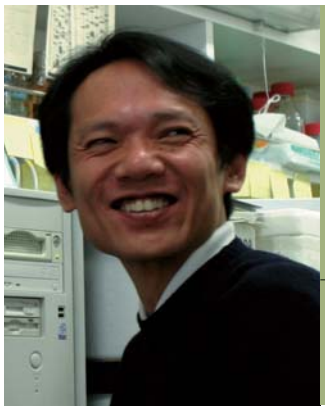
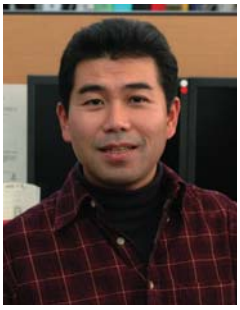


Genomics Support Unit

Fumio MATSUZAKI Ph. D.

In October of 2004, the CDB established a technical support unit merging the functions of the existing Sequencing Lab with an additional new subunit created to provide a range of bioinformatics and data processing services to the research staff at the Center. The Genomics Unit is organized into two complementary subunits, devoted to genome resources and analysis and functional genomics, each headed by a separate leader. The Genomics Unit is under the supervision of Fumio Matsuzaki, who also serves as Group Director of the Laboratory for Cell Asymmetry.



Genome Resource and Analysis

Hiroshi TARUI Ph. D.

The Genome Resource and Analysis Subunit aims to support a wide range 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 by using a DNA sequencing system capable of analyzing more than 100,000 genes per year. We are also equipped to custom make DNA microarray systems. Gene resources derived from a variety of species can also be stored and distributed to researchers on request. We constantly strive to build upon existing technologies to provide solid research support and explore new ideas and possibilities, which we hope will maximize the flexibility and efficiency of our responses to researcher requests.

Staff

Subunit Leader
Hiroshi TARUI

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Junko UEDA
Mikako DOHI

Functional Genomics

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

The Functional Genomics Subunit is available to perform genome-wide expression analyses for all laboratories in the CDB, and is working to develop advanced DNA chip technologies such as single-cell genome-wide expression analysis to enable expanded services in the future. In addition to our DNA chip service, we seek to improve on existing assay technologies, by developing systems such as transfection assays using tens of thousands of RNAi vectors and full-length cDNA clones.

Staff

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Kenichiro UNO

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.

Cell Plasticity

Mitsuko KOSAKA Ph. D.

Our research studies the possibility of achieving the regeneration of ocular tissue, such as neural retina, by using pigmented epithelial (PE) cells. We have recently found that postnatal avian or mammalian iris PE cells can transdifferentiate into lens or neuron under appropriate conditions. Multipotent stem cells in the iris PE may provide a source for autologous retinal transplantation. Some of our recent findings indicating that iris stem cells express genes characteristic of highly undifferentiated pluripotent stem cells also offer new avenues for exploration for researchers studying the genetic regulation of differentiative potency. We hope this project will open new avenues in the field of stem cell biology and ocular regenerative medicine.

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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.

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Sayaka SEKIYA

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Masato SUGANO

Part-Time Staff
Yumiko TENNOJIDANI

Animal Facility

The mouse provides a valuable platform for investigations into many of the fundamental processes and mechanisms of mammalian physiology and metabolism, and is widely used as a model organism in developmental biology research. With the sequencing of the mouse genome, mice can now be modified for use in research into the developmental consequences of genetic alterations, and as models of human disease.

The CDB animal facility provides the technology, equipment and staff to enable the humane, hygienic, efficient and economical handling of large numbers of experimental mice. This 24,000 cage SPF facility has the capacity to handle and care for up to 100,000 mice. Cutting edge technology, such as semi-automated cage cleaning, makes it possible to maintain high standards of hygiene and sterility, minimizing the risk of outbreaks of contagion among the mouse populations. The facility also provides services for the production of transgenic and knockout mice to specifications and on demand.

Research Aquarium

The CDB research aquarium serves as home to a number of water-dwelling species used in research into development and regeneration, including the zebrafish (*Danio rerio*) and the African clawed frog (*Xenopus laevis*). These, and other aquatic species, provide useful models for studies ranging from developmental genomics to classical embryology.

The aquarium includes seven climate-controlled rooms geared to providing optimal environments for the handling and breeding of freshwater and marine species, and utilizes reverse osmotic technology to maintain consistent tank-water purity. In addition to the commonly used zebrafish and *Xenopus* frog, the facility also houses specimens from more novel models used in evolutionary development research, such as the lamprey, *Lethenteron japonicum.*

Research Promotion Division

Director: Toshio Nakajima, Ph. D.

The Kobe Research Promotion Division serves as the main administrative department of the Kobe Institute, in which the Center for Developmental Biology is located. Within the KRPD, the Planning Section assists with intellectual property and licensing, organization of scientific meetings, domestic press releases, budget implementation, website updating and the coordination of external funding. The Finance Section is responsible for purchasing,contracts and asset management, and ensuring compliance with RIKEN and governmental fiscal regulations. The General Affairs section handles personnel issues, general administration and facilities management.

Safety Control Division

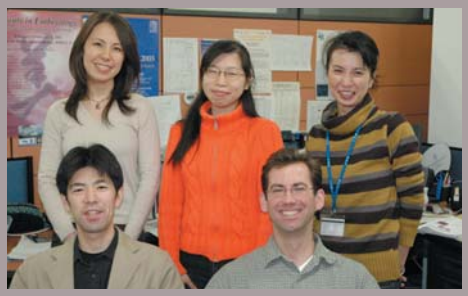
The CDB Safety Control Division provides training and supervision for lab and workplace safety, implements and monitors disaster prevention measures, and educates staff on and ensures compliance with national and institutional regulations governing laboratory practice and technology. The office also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

CDB Library

The CDB Library serves as the local branch of the RIKEN library network, and maintains a collection of books and periodicals for use by the Center's staff. The Library also serves as an access gateway to online publications and information sources, and provides a range of services including interlibrary loans and copies, and assistance with information search and retrieval.

Advanced Center for Computing and Communication

The ACCC maintains the information and network environment of the CDB, managing the Center's mail and file servers and intranet, monitoring network security, and integrating with the RIKEN wide area network. The ACCC office additionally provides advice and help with software and hardware issues to both research and administrative staff.



Office for Science Communications and International Affairs

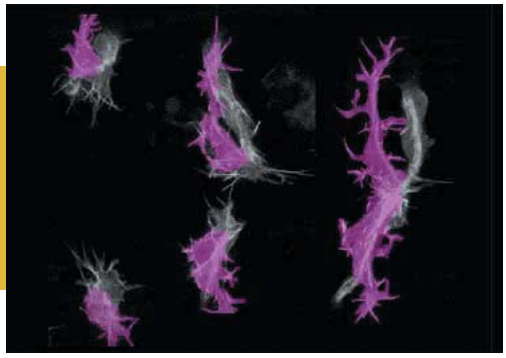
The SCIA conducts science communication and public outreach activities in Japanese and English, including the production of website content, print and software materials, and the development of content and facilities for use in site visits and other events. The office also assists non-Japanese staff with cultural and communications related issues within and outside of the CDB, and helps to maintain a bilingual work environment.

How tracheal cells know where to grow

The embryo is a hive of activity, with cells stretching, wandering and assembling to form the higher-order structures and networks that ultimately build the body. Some cells crawl along a matrix, making their way to distant locations, while others, such as neurons, extend projections while the cell body remains in place. These types of shape change and migration are acknowledged as fundamentally important developmental phenomena, but scientists have long puzzled over the guidance mechanisms that make sure that cells and their processes end up in the right places. A number of migratory systems have been shown to rely on molecules known as morphogens, which can act as either attractors or repellants for migrating cells and steer them to their destinations.

During its embryonic development, the fruit fly, *Drosophila*, assembles a trachea – a tubular respiratory network that delivers oxygen to the rest of the body in the larva and adult. This organ arises from ten pairs of tracheal placodes in thoracic and abdominal segments, which send forth six primary branches that migrate in stereotypical patterns. The dorsal branches move to points on the inner surface of the epidermis along the medial axis of the embryo's dorsum (back) to fuse with a partner from the opposite side. Each dorsal branch is tipped with a specialized cell that leads the cells behind it, but the exact means by which these terminal cells find their way across the interior face of the epidermis to the dorsal midline has remained unknown.

In a study aimed at resolving this question published in the journal *Development*, Shigeo Hayashi (Group Director; Laboratory for Morphogenetic Signaling) and colleagues at the CDB and the National Institute of Genetics looked at molecules known to be involved in tracheal branching for their potential roles as cell migration path determinants. In order to study these molecular signals, Kagayaki Kato, a RIKEN special postdoctoral fellow in Hayashi's lab, first tracked cell movements during the process of tracheal development.



Time-lapse images of the extending tracheal terminal branch (pseudocolored in purple) of the *Drosophila* embryo. Images of a dorsal branch fusion point at three successive time points (left to right) taken from GFP-labeled tracheal cells are shown.

Watching pairs of GFP-tagged cells found at the tip of the dorsal branch, Kato saw that they migrated over the underside of the dorsal epidermis (DE), where they made contact with partner cells from the opposite side of the body. Throughout the process, these tip cells remained closely associated with the epidermis, indicating that guidance signals might be of epidermal origin. The team opted to focus on one of the tip cells, called the terminal cell, which stretches out, seemingly in response to directional signals.

At first, terminal cell filopodia project equally in all directions, but only those which extend ventrally (toward the belly of the embryo) stabilize; other filopodia tend to withdraw back into the cell body after a short time. It is this stabilization that allows the terminal cell to sprout its branch exclusively in a ventral direction. Hayashi et al. looked at the epidermal region immediately adjacent to the spot where the dorsal branch tip cells congregate for specific patterns of gene expression and noted that their migration and subsequent activity seemed to home to a space underlying a dorsal epidermal region marked by the expression of a pair of morphogens, Decapentaplegic (Dpp) and Hedgehog (Hh), which are expressed in stripes in the DE. An experiment in which extra terminal cells were generated supported

Kato K, Chihara T and Hayashi S. Hedgehog and Decapentaplegic instruct polarized growth of cell extensions in the Drosophila trachea. *Development* 131:5253-61 (2004).

the idea that these cells display a preference for Hedgehog-positive zones, as terminal branch cells that were displaced from one such region would make their way through non-Hh-expressing territory to the closest Hh-positive segment.

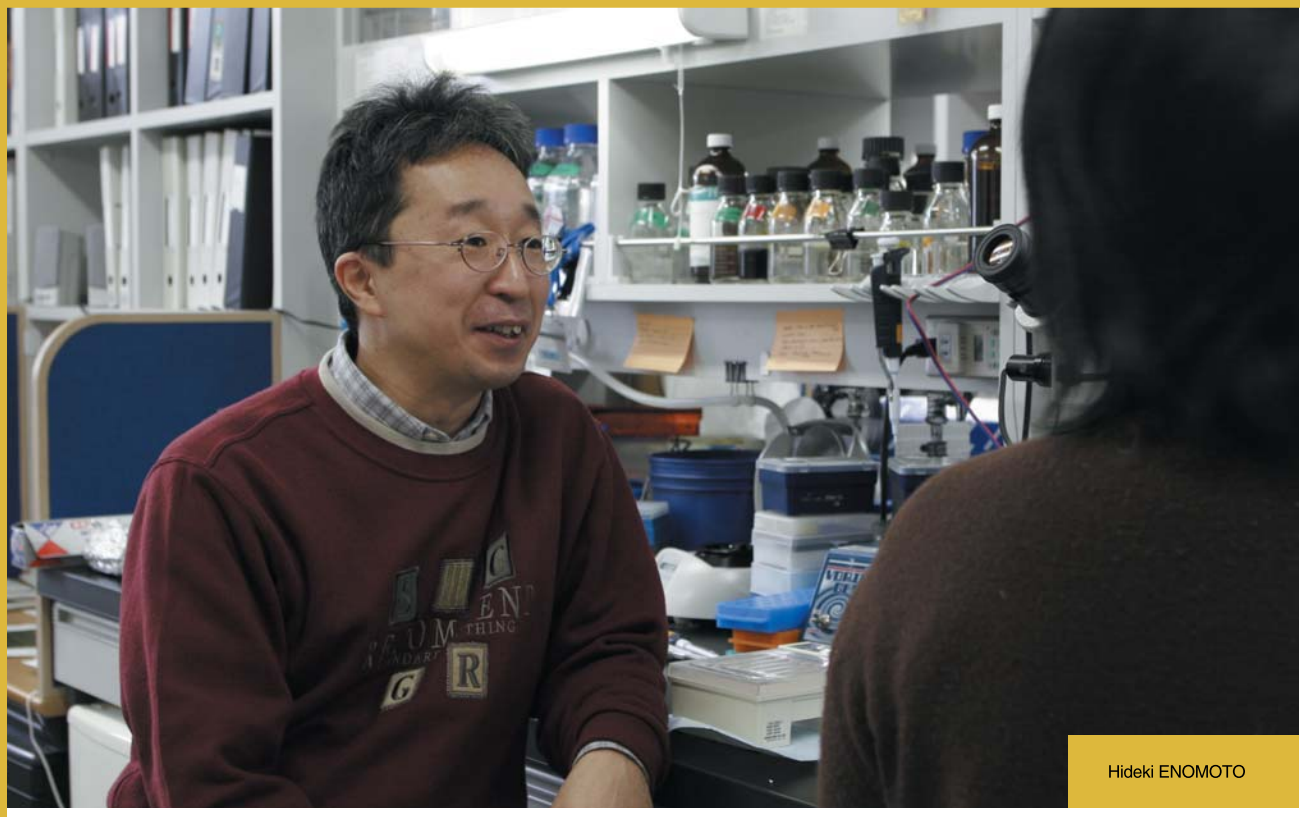
Suspecting a role for *hedgehog* in directing the outgrowth of terminal branches, the team next made tests in which the gene was broadly misexpressed or its signal transduction interfered with, and obtained results that tended to confirm their hypothesis that external Hh influences the direction of terminal branch outgrowth. Cells rendered unresponsive to Hh signaling displayed aberrant dorsal branch migration, with filopodial extensions radiating in all directions, unrestricted to their usual ventral orientation, indicating that tightly restricted expression of Hh is required for normal terminal branch migration.

Returning to the earliest stage of multidirectional outgrowth, the researchers examined whether Dpp, which is expressed in the overlying dorsal epidermis, might act as an inhibitor of terminal branch outgrowth, further ensuring that foraying branches ultimately only travel downward to the Hedgehog expressing regions. Dpp is already known to be important for dorsal branch specification, so Hayashi and colleagues designed tests to investigate whether it plays a specific role in branch migration. Experiments in which Dpp was overexpressed showed that branches failed to extend as normal, while Dpp downregulation resulted in misdirection of the terminal branch along the anterior-posterior axis.

The team has proposed a model to explain the localization to and subsequent behavior of dorsal branch cells at the intersection of expression of two developmentally crucial morphogens, in which Hedgehog sets up a permissive environment allowing the cells to travel into the posterior compartment while Decapentaplegic exerts the opposite effect, posting molecular No Entry signs during the early exploratory phase of branch outgrowth. The team suspects that such coordinated permissive/repulsive mechanisms may be found in the patterning of organs and complex structures in other species as well and may represent a relatively simple strategy by which the developing body lets its cells know where to grow.

(from left) Kagayaki KATO, Shigeo HAYASHI

No getting around RET



Hideki ENOMOTO

Neurons depend on external molecular signals for their very survival. These molecules, collectively referred to as neurotrophic factors, include a family of four GDNF Family Ligands (GFLs) that bind to specific receptor sites on the surfaces of neural cells. These sites allow GFLs to signal through a receptor complex composed of the RET tyrosine kinase and a GFRα -family receptor. Tyrosine kinases, such as RET, are well known for their function in phosphorylation cascades that span the cell membrane. The role of the GFRα co-receptors in these complexes was long thought to be limited to acting as a co-receptor for RET, but GFRs have recently been suggested to play other roles as well.

The individual functions of the RET and GFRα subunits in these receptor complexes, which are important in developmental milieux from peripheral neurogenesis to the developing kidney, remains a thorny question complicated by the fact that GFRα is expressed in many cells lacking RET in vivo (RET-independent GFRα) and that, in vitro, cells expressing GFRα1 without RET have been shown to respond to GDNF signals. A report by Hideki Enomoto (Team Leader; Laboratory for Neuronal Differentiation and Regeneration) and colleagues at the CDB and the Washington University School of Medicine published in the journal *Neuron* challenged the view that RET-independent GFRα1 plays a significant physiological role in either development or regeneration.

Enomoto first devised an elegant experimental system to make it possible to generate mice specifically lacking RET-independent GFRα1. The study of GFRα deficiencies in vivo is dogged by the lethality of the phenotype, in which the absence of enteric neurons and functioning kidneys results in death soon after birth. In vitro studies and the proximity of RET-independent GFRα and RET-expressing cells in some developmental regions, however, have prompted strong speculation that GFRα might be able to operate even in the absence of RET indigenous to the cell. It has been suggested that this might take the form of either trans signaling, in which the GFRα receptor captures diffusible GFLs and presents them to a neighboring RET-expressing cell, or through a separate signaling mechanism mediated by GFL-activated neural cell adhesion molecules (NCAMs).

Enomoto H, Hughes I, Golden J, Baloh R H, Yonemura S, Heuckeroth R O, Johnson E M, Jr. and Milbrandt J. GFRα1 expression in cells lacking RET is dispensable for organogenesis and nerve regeneration. *Neuron* 44:623-36 (2004).

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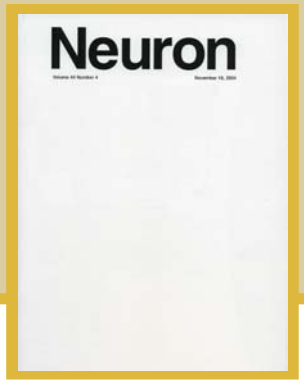
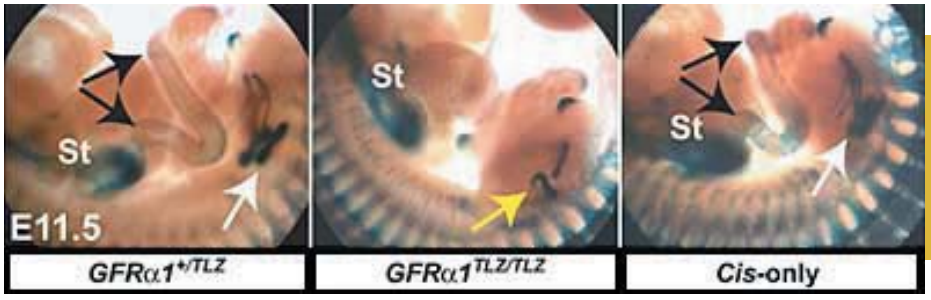


Given this body of work showing the likelihood of a physiological role for RET-independent GFRα1 activity, Enomoto et al. decided to test whether the in vitro evidence would be borne out in living mice. The team showed that mice homozygous for a transgene deleting an important segment of the GFRα1 gene died, while heterozygotes (which carried only a single copy of the transgene) were healthy and fertile. On comparing specific embryonic regions in hetero- and homozygous mice, they found associations between RET-expressing and RET-independent GFRα1 cells in kidney, enteric and motor neurons, as well as the expected disturbances in development. However, when they next generated mice that were only capable of expressing GFRα1 in the RET-expressing cells (by cloning GFRα1 cDNA into a region under the control of the Ret promoter and crossbreeding the resulting animals with GFRα1 heterozygotes), they were surprised to discover the mice were born healthy and free of any evident developmental defects in the kidney or nervous system. They found no trace of GFRα1 mRNA in non-Ret-expressing cells in these mice (which they named Cis-only mice, for their lack of trans signaling), while GFRα1 transcripts were detected as expected in RET-positive cells, proving that the conditional expression scheme had worked.

Analysis of individual regions known to be susceptible developmental failure on loss of GFRα1 function, such as the kidneys, motor and enteric neurons and certain parts of the central nervous system during development and following injury, showed that Cis-only mice develop and regenerate structures that are both morphologically normal and fully functional.

Investigating the second question of a possible alternate RET-independent GDNF receptor complex thought to involve neural cell adhesion molecules (NCAMs), they next examined Cis-only mouse olfactory bulbs. These bulbs are reduced in size in NCAM-deficient mice as the result of impaired migration of neural precursors through a zone called the rostral migratory stream and swell with cells that have failed to reach



GFRα1-expressing cells (blue) in heterozygote (left), homozygote (middle) and Cis-only (right) mutants. Enteric neurons and kidneys fail to develop in the null-mutant, but are normal in heterozygote and Cis-only mice.

their normal destination; this phenotype is seen only weakly in mice lacking GFRα1 (which is thought by some to regulate NCAM-mediated cell adhesion), but not in mice lacking RET. Again, the Cis-only mice showed no discernible differences from wild type.

This comprehensive series of experiments makes a convincing case against any essential physiological role for RET-independent GFRα1, but leaves open the question of why GFRα1 would be more widely expressed if it indeed plays no role without RET. It may be the case that GFRα receptors associate with other partners that have yet to be identified. Whatever the answer, by laying to rest a theory that had been strongly supported by in vitro evidence, the Enomoto report serves to underscore the importance of differences between the behavior of cells in the body and cells in a dish.

Two wrongs make a right

The developing embryo is a highly trafficked zone, with primordial tissues, individual cells and cellular processes traveling purposefully as they form the body's structures. Fortunately, these itinerants don't need to rely entirely on their own sense of direction; the embryonic landscape is clearly marked with molecular signposts that instruct migrating cells where and where not to go.

In the developing larva of the nematode *C. elegans*, the secreted ADAM-family protein MIG-17 is known to play precisely this sort of role in directing the migration of cells at the leading edge of the early gonad. These cells, called distal tip cells (DTCs), normally traverse the larva's interior along a stereotypical U-shaped route, a path the remaining gonadal cells subsequently follow. However, in *mig-17* mutants the DTCs tend to meander after rounding the first corner of the U, resulting in abnormal gonad morphology. Kiyoji Nishiwaki (Team Leader; Laboratory for Cell Migration) and colleagues have reported the discovery of a form of protein in the nematode extracellular matrix that seems to work together with MIG-17 to guide migrating DTCs. This study, published in the journal *Current Biology* provides new insight into one potential mechanism by which cells can be steered along highly specific and intricate paths.

The Nishiwaki had its first clue to the guidance function of the protein from a genetic screen they conducted to identify mutations that suppressed migration defects in *mig-17* null mutants. The screen

(from left) Yukihiko KUBOTA, Kiyoji NISHIWAKI, Rie KUROKI

Kubota Y, Kuroki R and Nishiwaki K. A Fibulin-1 Homolog Interacts with an ADAM Protease that Controls Cell Migration in C. elegans.
Curr Biol 14:2011-8 (2004).

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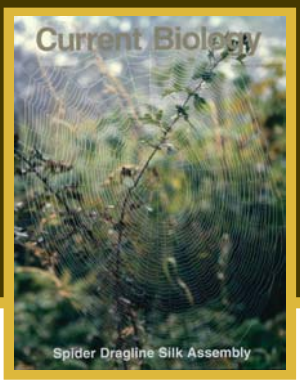
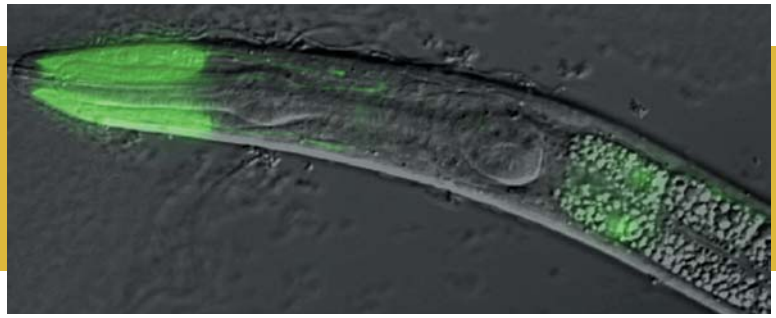


uncovered a pair of mutations that allowed normal DTC migration in the absence of MIG-17; these were subsequently identified as mutants of the gene *fbl-1*, a *C. elegans* homolog related to the mammalian fibulins, a family of genes that encode proteins associated with the basement membrane, extracellular matrix and blood plasma. Closer investigation of the *fbl-1* mutants revealed that only one of two isoforms of the protein, FBL-1C, rescued the MIG-17 deficiency phenotype. Protein isoforms are naturally occurring variant configurations of gene products; in the case of FBL-1, the isoforms FBL-1C and FBL-1D differ only in their C-terminal domains.

Nishiwaki et al found that when they induced a gain of FBL-1 function by inserting multiple copies of mutant genes into *mig-17* mutants, they partially restored DTC migration, a finding that suggested the mutant protein functions to enable proper migration, in contrast to the wild type, which interferes with the process in *mig-17* deficient worms. Analysis of distribution patterns of both FBL-1 isoforms confirmed that FBL-1C localizes in the gonadal basement membrane, while FBL-1D does not, shoring up the case for a specificity of action to gonadal guidance. Interestingly, the basement membrane localization of wild type FBL-1C was dramatically reduced in *mig-17* mutants, while the isoform of a mutant version of the protein was unresponsive to MIG-17 activity.



Expression of fbl-1::GFP in muscle and gut cells in the head region

These findings permit a number of interpretations of the role of *fbl-1* in *mig-17* control of cell migration. The simplest model is one in which FBL-1 is a direct substrate of MIG-17; the results of Western blots for FBL-1 in wild-type or mutant *mig-17* animals, however, tend to argue against this. It seems more likely that MIG-17 acts on an as-yet unidentified intermediary substrate, which affects the affinity of the gonadal basement membrane for FBL-1C. Isoforms from the *fbl-1* mutant are immune to the effects of MIG-17, and appear to mimic the effects of MIG-17 activity on the gonadal basement membrane in its absence. The exact mechanisms by which FBL-1 interacts with MIG-17 and other ADAM-family proteases remain to be worked out, but the Nishiwaki study indicate a new role for fibulins in cell guidance, as well as illustrating that, even at the genetic level, sometimes multiple errors can lead to a correct result.

Not the end of the line?

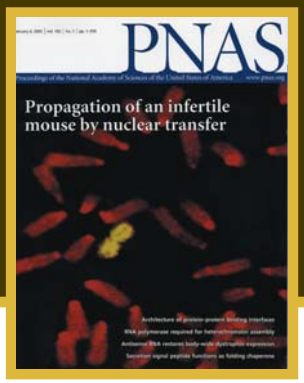
(back row, from left) Hiroshi OHTA, Takafusa HIKICHI, (front) Teruhiko WAKAYAMA

Infertile mice, whether the result of systematic mutagenesis or the fortuitous product of routine mating, represent a valuable research resource, but one that by its very nature defies attempts at long-term preservation by breeding. The cloning of such unique animals by somatic cell nuclear transfer has been proposed as the sole available means by which such mice can be maintained as a genetic resource over multiple generations. However, the rates at which viable offspring are generated by cloning, generally at less than 2% of all cloning attempts, are consistently too low to be used to maintain reliable supplies of offspring, particularly in inbred strains. Given the extent of their use as laboratory models of both physiology and disease, the problem of how to preserve lines of genetically infertile mice is one of substantial scientific and economic importance.

In a report published in the *Proceedings of the National Academy of Sciences*, Teru Wakayama (Team Leader, Laboratory for Genomic Reprogramming) and colleagues at the CDB and the University of Hawaii presented an alternative to simple cloning as a possible solution to the steep challenge of preserving lines of animals that genetically lack germline cells. The team showed that by applying a combination of nuclear transfer and embryonic stem cell technology, it is possible to produce viable offspring from a sterile hermaphroditic mouse.

The story began with a serendipitous discovery – a genetically male mouse with abnormalities in its Y chromosome that caused it to develop male and female sex characteristics and gonads, but lack both sperm and

Wakayama S, Kishigami S, Van Thuan N, Ohta H, Hikichi T, Mizutani E,Yanagimachi R and Wakayama T.
Propagation of an infertile hermaphrodite mouse lacking germ cells by using nuclear transfer and embryonic stem cell technology.
Proc Natl Acad Sci U S A 102:29-33 (2005).



eggs. Novel mutants with infertile phenotypes are of significant scientific interest, but their inability to reproduce limits the ability to study them extensively, as each individual is, in a sense, the first and last of its line. Wakayama, one the pioneers of mouse cloning, and colleagues determined to try to preserve a genetic copy of the animal by traditional cloning techniques as well as by cloning by nuclear transfer from embryonic stem cells (ntES), but despite multiple attempts, the team was unable to produce live pups.

They next tried a new twist on cloning by ntES technology, which involves the generation of an ES-like cell line from somatic cells of donor a donor mouse by nuclear transfer; such ntES cells demonstrate the same developmental potential as ES cells created using traditional methods. By injecting embryonic stem cells derived from the albino hermaphrodite donor into early-stage host embryos (called blastocysts) possessing either two (diploid) or four (tetraploid) sets of chromosomes, the team sought to take advantage of a phenomenon called “tetraploid complementation,” in which the contribution of a tetraploid embryo that

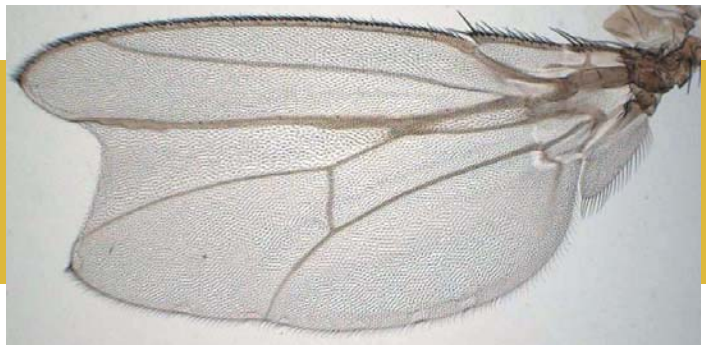
Male chimeric mouse generated through tetraploid complementation. Cells taken from the animal’s tail-tip revealed a normal, full complement of Y chromosomes.

receives a transfer of embryonic stem cells is restricted to extraembryonic tissues, such as placenta, making the transferred ES cells the sole contributors to somatic development. These attempts produced a total of 27 live first-generation chimeric pups, twelve of which were selected to study germline transmission of the albino hermaphrodite nuclear sire’s genome. While several of the diploid chimeras were able to produce offspring through normal mating, none of the resulting pups were perfect copies of the original hermaphrodite phenotype. Several albino pups, however, were born in the second generation, indicating that an infertile genome could contribute to a viable germline via ntES. (Albinism is a recessive trait and was used as an indicator of germline transmission, as the host blastocysts came from mice with colored coats.)

While providing proof of principle that nuclear transfer embryonic stem cells represent a promising technique for the preservation of mouse lines, the study also raises important questions about the fidelity of genomic reproduction by ntES cloning and the extent to which tetraploid complementation can be relied upon to produce “pure” strains of mice for laboratory use.

Bound for destruction

The Notch pathway is an important molecular signaling mechanism whose existence has been known, or at least hinted at, for nearly a century since the identification of a mutant strain of *Drosophila* fruit flies with "notched" wings in Thomas Hunt Morgan's lab in 1910. Later studies revealed that the Notch gene encodes a receptor protein that extends through both sides of the cell membrane and which is capable of interacting with a ligand partner, such as the protein Delta, presented on the surface of a neighboring cell. This "juxtacrine" interaction causes the cleavage of an intracellular region of the Notch protein, loosing it into the cytoplasm and triggering the activation of transcription factors within the cell's nucleus. In addition to its effects on wing structure in flies, Notch signaling is known to be important in a number of neural cell fate determination and developmental processes, and is conserved in species from human to roundworm. In all processes in which it participates, Notch signaling shows the ability to sense a small change in cell fate and amplify it, acting as a sort of contrast enhancement mechanism in cell fate determination.



Adult Drosophila wing in which Nedd4 was overexpressed. Nedd4 inhibited wing margin formation and promoted expansion of the wing vein. The defects resemble the typical Notch mutant phenotypes.

Notch is activated by a protease that is present ubiquitously in the cell membrane. What has long remained a mystery, however, is the question of how Notch receptors that have not been activated by a ligand are protected from digestion by that protease. In a report published in *Current Biology*, Shigeo Hayashi (Group Director, Laboratory for Morphogenetic Signaling) and colleagues at the RIKEN CDB have identified the means by which unstimulated cells protect the Notch receptor from activation.

Recent studies by other labs had shown that a number of stages in the Notch cascade were subject to ubiquitination, in which proteins are tagged by a complex of ubiquitin proteins. This system is best known for its function in marking proteins for degradation by a waste disposal unit known as a proteasome. Hayashi et al. sought to study the possibility that ubiquitination might play a part in rendering the unbound Notch receptor inert. Their attention was drawn to Nedd4 (a member of the ubiquitin ligase family of molecules that directly bind to proteins marked for degradation), as it had previously been shown that Nedd4 plays a role in the processing of other types of transmembrane proteins. Proteins in the cell membrane must first be internalized through a process known as endocytosis before they can be digested by the proteasome, and indeed other types of ubiquitin ligase have been shown to operate in the endocytosis of ligand-activated Notch.

Sakata T, Sakaguchi H, Tsuda L, Higashitani A, Aigaki T, Matsuno K and Hayashi S. Drosophila nedd4 regulates endocytosis of notch and suppresses its ligand-independent activation.
Curr Biol 14:2228-36 (2004).

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The group first showed that an increase in *nedd4* activity resulted in the nicked wing phenotype characteristic of Notch loss of function and in reductions of the total amount of the Notch intracellular domain in the cytoplasm of treated cells. Taken together, these results suggested that Nedd4 works as an antagonist of Notch signaling at an early stage, prior to the proteolytic cleavage of the receptor's intracellular domain. Further investigation revealed the specific domain by which Nedd4 interacts with Notch and pinpointed the site of origin for this interaction at the cell membrane, a finding congruent with the idea of Nedd4 as an agent of endocytosis. Nedd4's role as a suppressor of Notch was illustrated even more plainly when the lab showed that its inhibition results in the upregulation of ligand-independent activation of the Notch pathway.

Nedd4's place in the greater scheme of Notch signaling became clearer when the group next turned to examine the interaction between Nedd4 and Deltex (Dx), a putative ubiquitin ligase known to bind and activate the Notch receptor. Hayashi's group found evidence that Nedd4 and Dx vie with each other to regulate Notch activity during endocytosis, and that Nedd4 actually destabilizes Dx in the presence of Notch. This competition between two ubiquitin ligases to permit or suppress activation of a signaling pathway represents a neat solution to the problem confronting cells of how to prevent molecular loose cannons from fouling their precisely ordered workplans.

(from left) Tadashi SAKATA, Hiromi SAKAGUCHI, Leo TSUDA, Shigeo HAYASHI

October-December Seminars

date	title	speaker
2004-10-05	Probing cell morphogenesis dynamics at the molecular level: Actin turnover regulation at the leading edge and processive actin capping by a Formin homology protein, mDial	Naoki WATANABE
2004-10-05	Molecular mechanisms of the presynaptic active zone structure and function	Toshihisa OHTSUKA
2004-11-01	Oligodendrocyte precursor cells, neural stem cells, and cancer stem cells	Toru KONDO
2004-11-02	Function and regulation of centralspindlin, a kinesin/RhoGAP complex, incytokinesis	Masanori MISHIMA
2004-11-02	Neuro-angiogenesis: cellular and molecular studies of cross-talk between the nervous system and vascular system	Yosuke MUKOYAMA
2004-11-09	Genetic analysis of circadian behavior in mouse	Kazuhiro SHIMOMURA
2004-11-10	Life or death decisions: the genetic network regulating programmed cell death in *C. elegans*	Joel H. ROTHMAN
2004-11-18	Structural basis for inositol 1,4,5-trisphosphate receptor mediated calcium release from the endoplasmic reticulum	Mitsuhiko IKURA
2004-11-22	*C. elegans*: a wonderful system for studying the assembly and maintenance of myofibrils	Guy BENIAN
2004-11-25	Hepatic progenitors in liver development and regeneration	Atsushi MIYAJIMA
2004-12-03	Molecules, maps and training: Seeing again after optic nerve regeneration	Sarah DUNLOP
2004-12-06	FAK, a multitasking protein: crosstalk with p53 and a role in skin barrier formation	Dusko ILIC
2004-12-07	Embryonic analysis of WISE, a novel Wnt modulating factor in trigeminal ganglion formation	Yasuyo SHIGETANI
2004-12-07	Control of dendrite development	Yuh-Nung JAN
2004-12-07	Proteomic analysis of embryonic stem (ES) cells by multi-dimensional LC-MS/MS technology	Tomonori IZUMI
2004-12-09	Performing reliable signaling operations with a few molecules	Upinder S. BHALLA
2004-12-13	Role of chromatin remodeler Mi-2b in T cell development and CD4 gene regulation	Taku NAITO
2004-12-15	Evolution of the vertebrate immune system	Chris AMEMIYA
2004-12-17	Downstream functions of the floral homeotic protein AGAMOUS in *Arabidopsis*	Toshiro ITO

The CDB is located in the international city of Kobe. This port city has been the major entrance to western Japan for over a century, giving the city a cosmopolitan atmosphere and unique charm. With 1.5 million residents, the city is the sixth-largest in Japan, offering a perfect mix of urban sophistication and access to some of Japan's more beautiful natural and historical attractions. Situated in the heart of the Kansai region of western Japan, Kobe is just a short drive or train ride away from Osaka and Kyoto. An exceptional public transportation system provides convenient access to destinations within Kobe and around Japan, and the Kansai International Airport serves as the region's hub for overseas travel.

Photos courtesy of the city of Kobe.

Community Outreach

Rapid advances in the study of development and regeneration and the contributions these fields seem certain to make to clinical medicine have drawn heightened public attention, accompanied by increased ethical scrutiny of issues such as animal cloning and the research use of tissue derived from early human embryos. Every institute conducting developmental biology research has a strong interest in providing accurate and easy-to-understand information to the public to ensure continued support for its work; publicly funded organizations have the added duty of accountability to taxpayers. In Japan, the trend of *rikabanare* (lack of interest in science) presents additional challenges to educators and science communicators. The CDB has developed a number of communications tools, programs and exhibits, including software, publications, mock lab demonstrations and software simulators to achieve greater public awareness and understanding of its research mission and achievements.

Open House

The CDB held its second annual Open House event on April 17, 2004, welcoming more 1,600 visitors to the Center for a day of scientific exhibits, talks and demonstrations.

The event, which took place as a part of Japan's national Science and Technology Week activities, offered simple and fun explanations of topics in development, regeneration and regenerative medicine, from embryogenesis and stem cells to model organisms used in research. All exhibits and demonstrations were staffed by scientists who explained their work and laboratory techniques to visitors of all ages and walks of life.

A number of scientific demonstrations allowed non-scientists to get firsthand experience with basic scientific principles and research equipment, including a simple experiment letting people visualize their cells under a microscope, as well as demonstrations of the freezing power of liquid nitrogen and the natural patterns formed by chemical and physical reactions.

Large sections of all three of the CDB's main laboratory buildings were open, with many labs granting a peek into life as a researcher to the general public or to guided group tours.

Intensive Lecture Program

As part of its commitment to fostering the next generation of developmental biology researchers, the CDB has entered into agreements with a number of universities and graduate school programs in the Kansai area, allowing CDB research staff to give lectures at partner institutions and host graduate students in their labs. The CDB extended the range of its interactions with academia in September 2004 by holding a two-day intensive lecture program intended to provide students with a first-hand look at the work being done at the Center. In addition to a series of lectures by CDB staff, the program, held on September 6 and 7, included laboratory visits, scientific exhibits and demonstrations, and was attended by 130 students from local graduate programs and across Japan.

Mock Lab Demonstrations

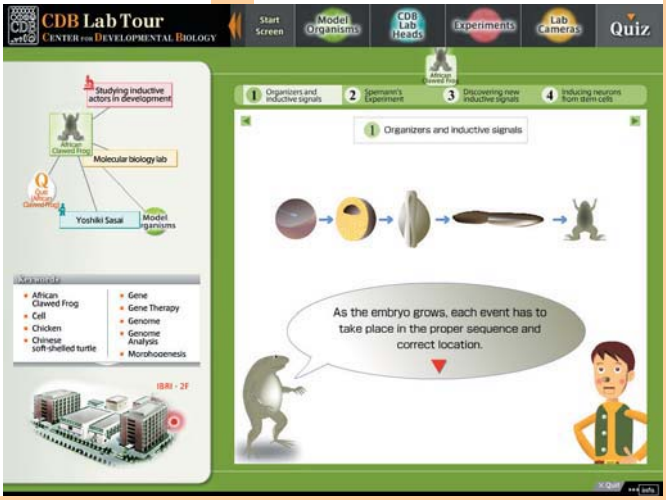
In April 2004, the Center opened a mock laboratory for use in demonstrations and educational tours. The laboratory is designed and equipped to offer as realistic as possible a simulation of an actual working laboratory. All laboratory equipment, from incubators to fluorescence microscopes, is fully functional, allowing visitors to join in firsthand demonstrations of experimental methods. Live cells and organisms from GFP-expressing roundworms to beating cardiomyocytes derived from mouse ES cells are maintained by the mock lab staff under the supervision of Naoki Namba in the Office for Science Communications and International Affairs, giving students and guests of the Center an opportunity to see actual model organisms and systems as they are used in CDB labs.

Museum Exhibits

As part of its mission to increase public awareness, understanding and interest in science, the CDB has worked with science museums and exhibition centers around the country to develop fun and easy-to-understand exhibits focusing on the Center's research. In 2004, short-term exhibits were produced for display at science museums in Osaka and Tokyo, and for a special event hosted by the Ministry of Education, Sports, Culture, Science and Technology.

Virtual Laboratory Tour

The CDB has developed a software system allowing people located anywhere with access to an Internet connection to tour the Center's lab's, read interviews with the PIs, and learn about development and regeneration though interactive walkthroughs, simulations and quizzes. The software is intended for users of junior high school age and older, and was first released in Japanese on a CD-ROM prepared for the 2004 Open House. A bilingual Japanese-English version capable of being viewed over the Internet is scheduled for release in early 2005, which will further broaden the range of access to this engaging tour of the CDB.



International Activities

Intraregional and International Meetings

The fields of developmental biology and regenerative medicine and the growth of the biotechnology and life science research sectors in countries across the Asia-Pacific region have been subjects attracting great attention in recent years. The CDB, as one of Asia's largest research institutes committed to the study of the biology of development and regeneration welcomes the increase in activity within Asia and other parts of the world, and in 2004 a number of labs within the Center sought to promote the exchange of information and provide networking opportunities to fellow scientists by hosting regional or international meetings at the CDB or in the Kansai region of Japan.

Beauty in Embryology

Beauty in Embryology: Patterning and Shaping of the Body (February 3)

Organizer: Yoshiko Takahashi (Laboratory for Body Patterning)

The first event to be held in the Center's new 150-seat lecture theater, this meeting featured talks by some of the world's leading authorities on subjects from neural crest development to the evolution of body plans.

Asian Reproductive Biology Society Workshop (April 12 to 14)

Organizers: Tuyen Bui Cach (Nong Lam University) and Teruhiko Wakayama (Laboratory for Genomic Reprogramming)

The Asian Reproductive Biology Society (ARB) held its first workshop in Ho Chi Minh City in a meeting co-hosted by the CDB and Nong Lam University (Vietnam). The meeting was attended by scientists from across Asia who presented and discussed their work in the development and application of reproductive biology technologies.

Asian Reproductive Biology Society Workshop

New Trends in Hematopoietic Stem Cell Research (April 23)

Organizers: Shin-ichi Nishikawa and Timm Schroeder (Laboratory for Stem cell Biology)

A one-day meeting that brought researchers studying the biology of blood formation together from around the world to discuss their recent work and the state of the science, which impacts on important medical questions from immunology to cancer.

Kobe Meeting on Vertebrate Brain Development (July 23)

Organizer: Shinichi Aizawa (Laboratory for Vertebrate Body Plan)

Scientists from leading laboratories gathered for a day of open lectures treating the evolution and ontogeny of the vertebrate head.

East Asia *C. elegans* Meeting (July 30)

Organizers: Hitoshi Sawa (Laboratory for Cell Fate Decision), Asako Sugimoto (Laboratory for Developmental Genomics), Junho Lee (Yonsei University, Korea), Joohong Ahnn (KJIST, Korea)

Heads of CDB labs studying roundworm development worked with colleagues from South Korea to organize the first Asia regional scientific meeting on *C. elegans* biology with support from the CDB and Hyogo Prefecture, providing the chance for scientists from 7 countries, including Japan, South Korea, China and Taiwan to present and discuss their work.

Asia-Pacific Developmental Biology Research Symposium

Asia-Pacific Developmental Biology Research Symposium (November 8)

Organizers: Masatoshi Takeichi (Laboratory for Cell Adhesion and Tissue Patterning), Kiyokazu Agata (Laboratory for Evolutionary Regeneration Biology)

CDB scientists organized a regional meeting co-sponsored by the Japanese and International Societies of Developmental Biologists to encourage interaction between scientists from around the Asia Pacific region.

Asia-Oceania Fish Meeting (November 15-16)

Organizers: Masahiko Hibi (Laboratory for Vertebrate Axis Formation), Hitoshi Okamoto (RIKEN Brain Science Institute)

This meeting, organized by heads of laboratories from the RIKEN CDB and Brain Science Institute (Wako, Japan), featured a two-day program of meeting program of talks by speakers from South Korea, China, Singapore, Taiwan, Australia, New Zealand and Japan, covering the development, neurobiology and genomics of the zebrafish and medaka model organisms.

Asia-Oceania Fish Meeting

Institutional Affiliations

The CDB is engaged in programs of cooperation with research organizations in other parts of the Asia-Pacific region and around the world. This activity is based primarily on cooperation between individual researchers and laboratories. In 2004, the Center entered into scientific exchange agreements with research and academic institutions in India, Germany and Canada, bringing its total number of such international affiliations to five. To date, the CDB has established formal agreements for collaboration with the following organizations overseas:

The University of Texas Graduate School of Biomedical Sciences at Houston, Texas, USA

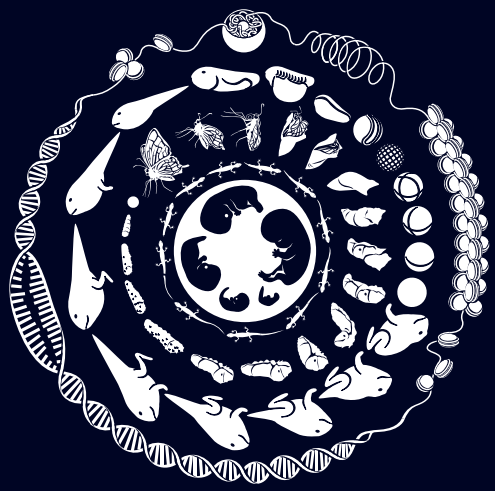
Temasek Life Sciences Laboratory Limited, Singapore

National Centre for Biological Sciences, TIFR, Bangalore, India

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

McGill University Faculty of Science, Montreal, Canada

A number of labs within the CDB also participate in regular joint workshops and meeting programs focusing on stem cell biology and tissue engineering with research centers and labs in Scotland, France and the United States.



DEVELOPMENTAL
REMODELING

The 2nd CDB Symposium 2004

March 29 - 31, 2004 RIKEN CDB, Kobe Japan

Session 1 Nuclear Reprogramming
En Li (Novartis Institute for Biomedical Research, USA)
Teruhiko Wakayama (RIKEN CDB, Japan)
Nobuaki Kikyo (University of Minnesota, USA)

Session 2 Chromatin Dynamics
Steven Henikoff (Fred Hutchinson Cancer Research Center, USA)
Jun-ichi Nakayama (RIKEN CDB, Japan)
Tetsuji Kakutani (National Institute of Genetics, Japan)
Renato Paro (University of Heidelberg, Germany)

Session 3 Tissue Plasticity
Barry M. Gumbiner (University of Virginia, USA)
Richard Fehon (Duke University, USA)
Naoto Ueno (National Institute for Basic Biology, Japan)
Paul Martin (University College London, UK)

Session 4 Regeneration
Jeremy P. Brockes (University College London, UK)
Elly Tanaka (Max Planck Institute, Germany)
Susan Bryant (University of California, Irvine, USA)

Session 5 Morphological Remodeling
Donald D. Brown (Carnegie Institute of Washington, USA)
James W. Truman (University of Washington, USA)
Koji Tamura (Tohoku University, Japan)
Cheng-Ming Chuong (University of Southern California, USA)

Application Deadline
December 22, 2003

More information is available at
http://www.cdb.riken.go.jp/sympo2004

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The Second Annual CDB Symposium

DEVELOPMENTAL REMODELING

The RIKEN Center for Developmental Biology (CDB) was established to promote research into the fundamental mechanisms of animal development and regeneration. To keep up with advances and identify future directions in these rapidly evolving fields, the CDB inaugurated a program of annual symposia covering diverse aspects of developmental biology and regeneration and aimed at promoting the free and timely exchange of research achievements. The second symposium in the series focused on the theme of "Developmental Remodeling," and was held from March 28 to 31, 2004 in the CDB Auditorium.

2005 CDB SYMPOSIUM

Origin and Development of the Vertebrate Traits

April 11 - 13 , 2005

Center for Developmental Biology
RIKEN Kobe Institute (Kobe, Japan)

The 2005 CDB Symposium will address subjects relating to vertebrate evolutionary developmental biology, with special focuses on the evolution of morphogenesis (Day 1), the neural crest, sensory systems and the brain (Day 2), and the genetic and cellular bases for the chordate body plan (Day 3). It is hoped that the broad scope of the presentations and disciplines covered will stimulate fruitful discussion and yield fresh new insights into the evolution of vertebrates, including ourselves.

Session 1
Nuclear Reprogramming
En LI
(Novartis Institute for Biomedical Research, USA)
Teruhiko WAKAYAMA
(RIKEN CDB, Japan)
Nobuaki KIKYO
(University of Minnesota, USA)

Session 2
Chromatin Dynamics
Steven HENIKOFF
(Fred Hutchinson Cancer Research Center, USA)
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(RIKEN CDB, Japan)
Tetsuji KAKUTANI
(National Institute of Genetics, Japan)
Renato PARO
(University of Heidelberg, Germany)

Session 3
Tissue Plasticity
Barry M. GUMBINER
(University of Virginia, USA)
Richard FEHON
(Duke University, USA)
Naoto UENO
(National Institute for Basic Biology, Japan)
Paul MARTIN
(University College London, UK)

Session 4
Regeneration
Jeremy P. BROCKES
(University College London, UK)
Elly TANAKA
(Max Planck Institute, Germany)
Susan BRYANT
(University of California, Irvine, USA)

Session 5
Morphological Remodeling
Donald D. BROWN
(Carnegie Institute of Washington, USA)
James W. TRUMAN
(University of Washington, USA)
Koji TAMURA
(Tohoku University, Japan)
Cheng-Ming CHUONG
(University of Southern California, USA)

Per E. Ahlberg
(Uppsala Univ., Sweden)
Shin Aizawa
(RIKEN CDB, Japan)
Clare V. H. Baker
(Univ. of Cambridge, UK)
Marianne Bronner-Fraser
(Caltech, USA)
Ann Burke
(Wesleyan Univ., USA)
Michael J. Depew
(King's College London, UK)
Scott E. Fraser
(Caltech, USA)
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(Harvard Univ., USA)
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(Univ. of Oxford, UK)
Philip W. Ingham
(Univ. of Sheffield, UK)
Shigeru Kuratani
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Thurston Lacalli
(Univ. of Victoria, Canada)
Patrick Lemaire
(IBDM, France)
Yasunori Murakami
(IGBMC, France)
Filippo Rijli
(IGBMC, France)
Nori Satoh
(Kyoto Univ., Japan)
Rich Schneider
(UCSF, USA)
Yoshiko Takahashi
(RIKEN CDB, Japan)
Christine Thisse
(IGBMC, France)
Cheryll Tickle
(Univ. of Dundee, UK)
Hiroshi Wada
(Univ. of Tsukuba, Japan)
Sayuri Yonei / Koji Tamura
(Tohoku Univ., Japan)
H. Joseph Yost
(Univ. of Utah, USA)

For more information, contact:
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Kyoto University Graduate School of Medicine (Japan)

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Center for Genome Research
University of Edinburgh (UK)

Yoshimi Takai
Department of Molecular Biology and Biochemistry Osaka University
Graduate School/Faculty of Medicine

The CDB Advisory Council (DBAC), chaired by Dr Igor Dawid (National Institute of Child Health and Human Development, NIH) submits regular external reports regarding the scientific administration and the state of research progress at the Center. The ten-member Council includes top scientists working in related fields from Japan and around the world and serves as a review board for the evaluation of CDB research activities and governance.

The Advisory Council held its second plenary meeting from June 1 to 4, 2004 to conduct an extensive review of the research programs and administrative organization of the Center. The report gave high marks to the CDB's organization, facilities and research programs, stating, "The research at CDB is broad but focused at the intersection of developmental biology with stem cell research and translational biology. Both the intellectual and physical resources available to the individual scientist at the CDB are outstanding. The Center constitutes a large community of researchers in related fields where interactions and cross-fertilization can occur to enhance the originality and productivity of research. Such interactions should continue to be fostered and encouraged. Laboratory resources at the Center are likewise exceptional. Facilities, equipment, animal resources and all types of support are as strong, or stronger, than in any comparable institute anywhere in the world. Thus, scientists have near-optimal conditions under which they can carry out their research."

In his concluding remarks Dr Dawid stated that "The RIKEN CDB has rapidly established itself as a center of excellence in the area of developmental biology. The major issue the Center faces in the future is to receive assurance of its continued full operations. Given such assurance, and with several comparatively minor adjustments suggested herein, the CDB will strengthen its standing in the scientific community and contribute greatly to advances in its field."

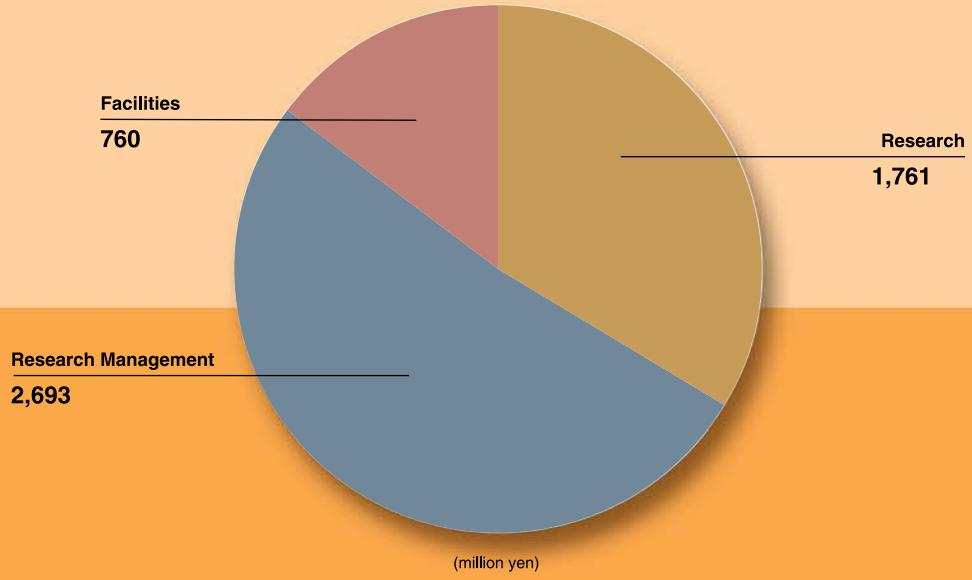
Institutional Review Board

The Institutional Review Board includes representatives from local academic, research and lay organizations as well as CDB research staff, and meets regularly to review and discuss the ethical and social implications of programs and investigations being conducted at the CDB. The results of the Board's discussions are submitted to the Center Director and taken into consideration when planning research activities. The IRB finalized its review of plans for three research projects involving the use of human embryonic stem cells by CDB labs in autumn of 2003; these plans received final authorization in March of 2004.

2004 Budget and Staff

The Center for Developmental Biology was founded in April 2000 under the auspices of the Millennium Project research initiative launched by former Prime Minister Keizo Obuchi. The Millennium Projects were established to drive research in the fields of information technology, environmental science and the study of aging, areas of vital importance to both Japan and the world in the 21st century. The organization and operations of the CDB were consigned to RIKEN, which coordinates the construction management and administration of the Center. The Millennium Project will conclude its five-year term in March 2005, and a full review of research achievements will be conducted.

2004 CDB Budget



(million yen)

In addition to these intramural funds, individual labs and investigators are encouraged to apply for external funding as well, from sources such as the Japan Society for the Promotion of Science (JSPS), other government funding agencies, private foundations and industry. In 2004, 29 of the 30 CDB labs received some form of extramural funding.

2004 CDB Staff

Laboratory heads	32
Research scientists	110
Research associates	8
Technical staff	116
Assistants	24
Visiting researchers	5
Collaborative scientists	36
Student trainees	67
Part time staff	34
Research Promotion Division	43
Other	85
Total	**560**

RIKEN Intramural Activities

RIKEN is one of Japan's largest publicly-funded scientific research organizations, comprising a number of institutes and centers throughout the country. Seeking to capitalize on the strengths of the diversity of its research programs – which span physics, chemistry and the life sciences – RIKEN strongly encourages scientists working at different locations to meet, communicate and forge collaborations. The CDB has taken an active approach to making the most of this multidisciplinary environment through its participation in programs intended to build links within RIKEN at the individual, laboratory and institutional levels.

Joint Forums

CDB researchers joined staff from other RIKEN centers for three joint forums in 2004. In these meetings, scientists working in fields from development to neuroscience to immunology convene to share and discuss their latest work.

Collaborative Research

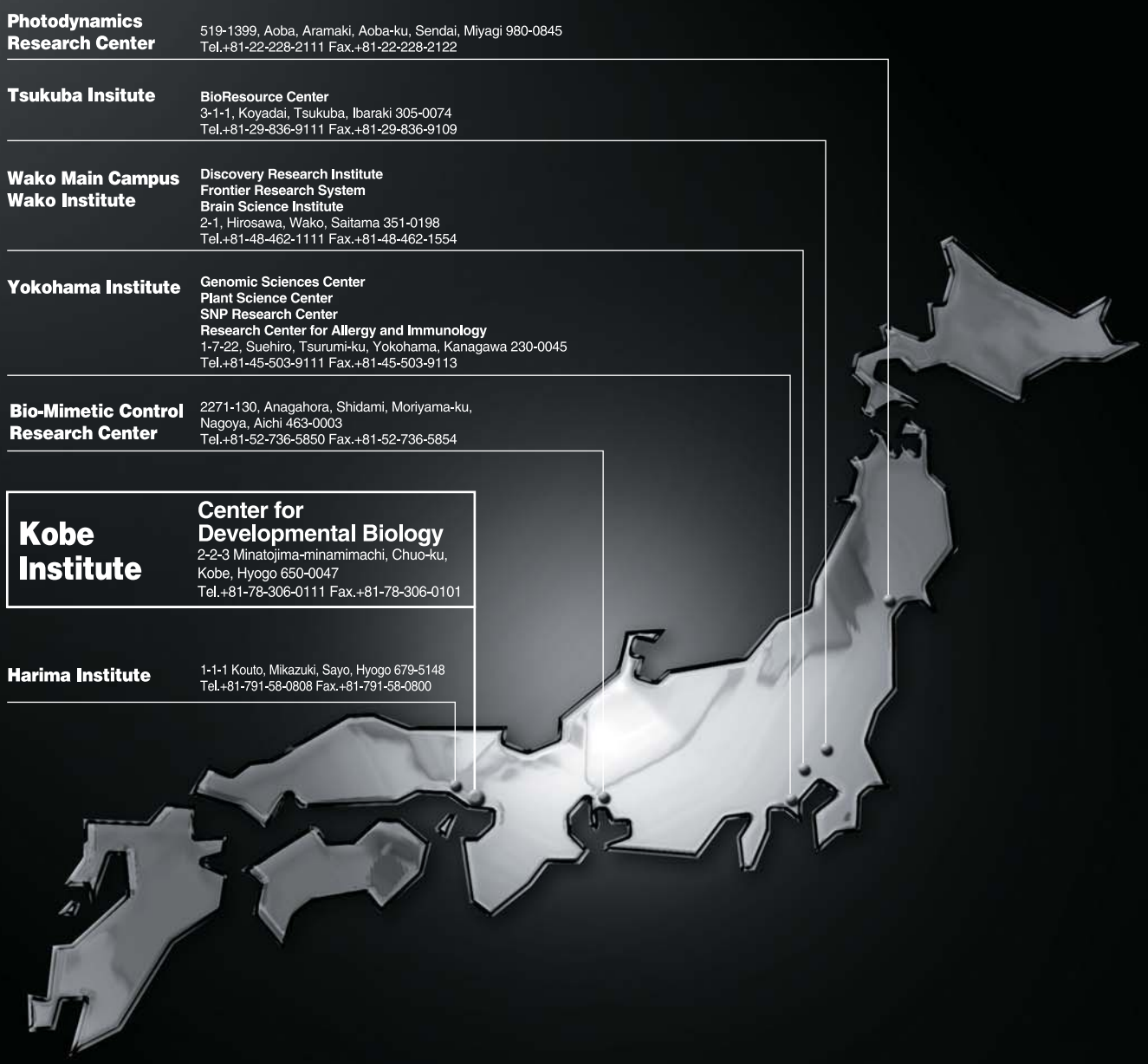
CDB laboratories are participating in a number of collaborative projects involving multiple RIKEN laboratories. Labs from the Brain Science Institute (Wako) and Genomics Sciences Center (Yokohama), in addition to the CDB, are engaged in a comparative genomic analysis of neurogenesis. Research staff from a number of CDB labs are also contributing to the FANTOM 3 mouse cDNA library project organized and led by RIKEN.

CDB Retreat

RIKEN Center for Developmental Biology scientists and staff gathered for the third annual CDB Retreat on October 18 and 19 on the island of Awaji in Japan's Inland Sea. Nearly 300 research and support staff traveled to the Yumebutai conference center for the opportunity to present and discuss their work with colleagues from throughout the Center.

This year's event, organized by Raj Ladher (Laboratory for Sensory Development), Tony Perry (Laboratory of Molecular Mammalian Embryology) and Miki Murase (Kobe Research Promotion Division), built on the success of previous retreats by introducing new scientific and social programs. Scott Gilbert (Swarthmore College, USA), as the first-ever invited speaker to a CDB Retreat, gave a thought-provoking opening lecture on the use of metaphor in describing concepts of developmental biology, and the potential risks that entails. "We all use metaphors when we talk about our science," he noted, "But we have to make sure that the ones we use don't lead to misunderstanding."

The remainder of the two-day program featured alternating sessions for poster and oral presentations, with eleven talks by postdocs and lab heads, and 150 posters describing studies from germline development in *Drosophila* to high-throughput systems biology. Speakers had additional opportunities to elaborate on and defend their work in Q&A sessions held after each talk. Poster presenters were even busier, discussing their findings with any and all comers. For many, the Retreat was the first experience of having to present their work in English, and made clear the importance of being able to express themselves clearly and convincingly to their peers.



RIKEN

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

Overexpression of DsRed-moesin fusion protein in *Xenopus* A6 cells induces filopodia-like structures. Actin filaments (green) are also shown.
PHOTO: Toshiyuki Watanabe

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