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On The Cover
Distribution of the ERM proteins ezrin (green) and moesin (red) in the forelimb bud of the mouse embryo.
Photo: Shigenobu Yonemura

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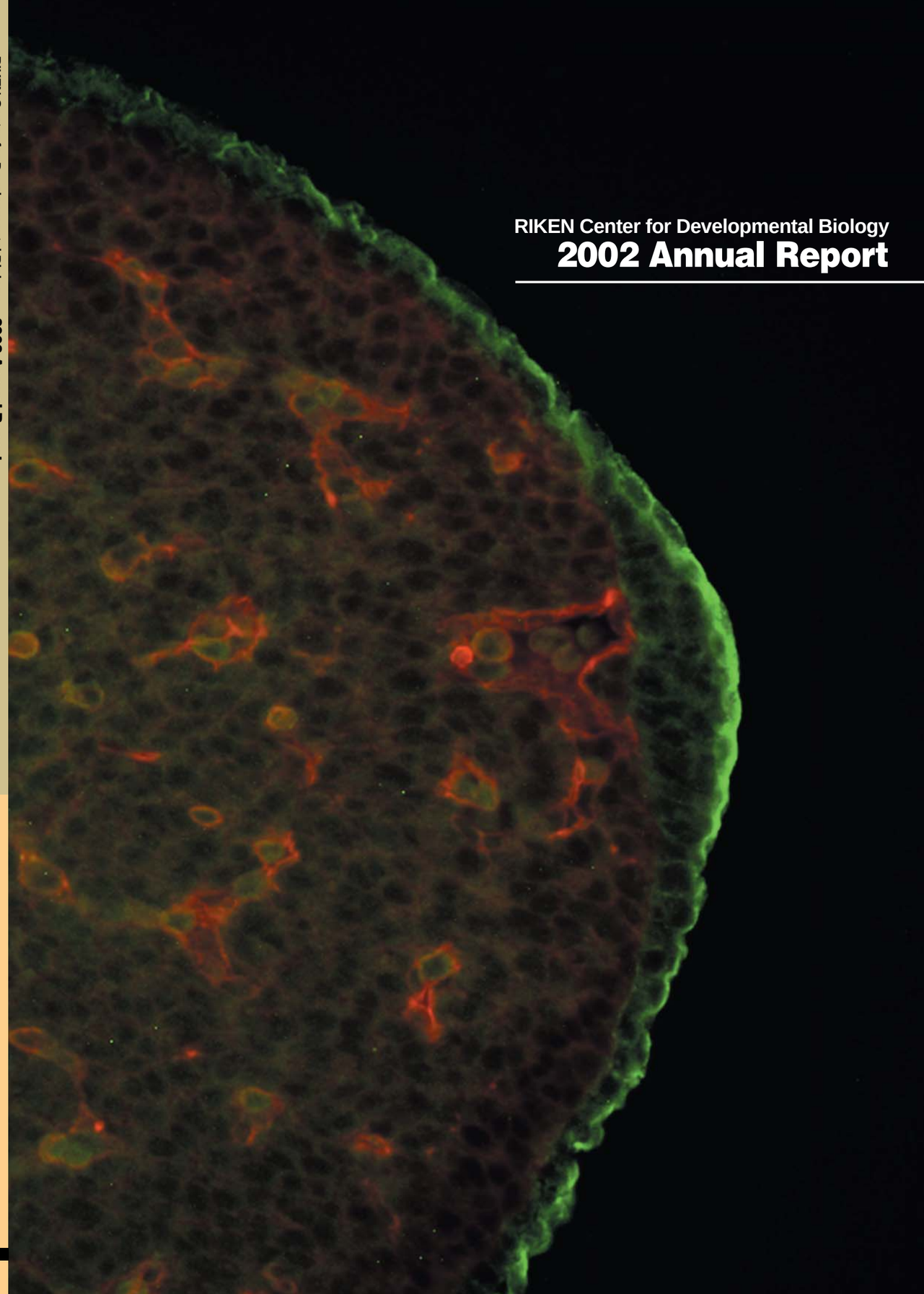
RIKEN Center for Developmental Biology 2002 Annual Report



RIKEN Center for Developmental Biology
2002 Annual Report

RIKEN Center for Developmental Biology

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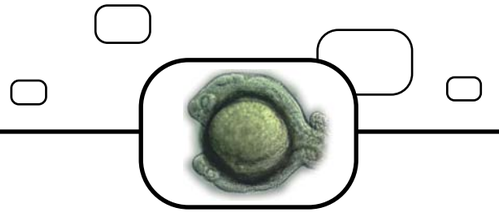
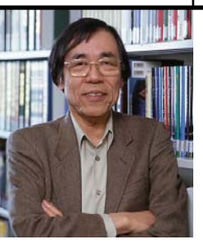


Center for Developmental Biology



Masatoshi Takeichi
Director, CDB

Message from the Director

2002 represented a landmark year in the history of the RIKEN Center for Developmental Biology, one which saw the completion of its main physical facilities, the addition of seven outstanding new laboratories, and the official opening of the Center itself in July. It has been a true pleasure to be able to play the dual roles of center director and head of the laboratory for Cell Adhesion and Tissue Patterning and to watch as the CDB grew from no more than an inspired idea in 2000 to become one of the largest developmental biology research centers in the world today.

The Center for Developmental Biology was launched 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 21th century. The drafters of this plan recognized the great potential for contributions by developmental and regeneration biologists in addressing the health challenges confronting an aging society, and so the concept of a national center for developmental biology was born. A vast array of details needed to be worked out in order to make this vision a reality, and the RIKEN research organization provided the organizational structure and support environment needed, giving initial form to what is now the CDB and helping to coordinate all aspects of the center's launch from construction planning to human resources to facility management.

During the process of its establishment, the decision was made to locate the CDB within a new and dynamically growing biomedical research park in Kobe, one of Japan's most attractive cities. This location situates the CDB within a world-class research setting, with the active support of local and national governments and participation by public, academic and corporate research organizations. A spirit of collegiality and international cooperation pervades the atmosphere here, and I look forward to seeing the results of collaborations between labs within the CDB with their colleagues at the Center, as well as their counterparts in the region, throughout Japan, and around the world.

It has been my goal as director of the Center for Developmental Biology to develop a new, open model for research organizations within Japan, with an emphasis on providing the freedom and independence to envision new directions of research, and the organizational and material support to make those visions real. Nearly three years have passed since the birth of the CDB as a concept, and the investment of time and money has already begun to bear fruit in the form of solid, innovative research into the mechanisms of development, regeneration and the scientific bases for regenerative medicine. The years ahead hold the promise of ushering in new conceptual insights regarding the biological processes of development, and of translating those insights into applications with the potential to revolutionize the way we think about aging, disease and medical therapy. I invite you to explore this report for a first glimpse into some of the challenges and mysteries that we have encountered, and a few of those that still await.

Why Study Development?

The study of developmental biology traces its roots back to Aristotle and the observations he made on the changes that take place in hen's eggs as the embryos grow from a formless mass into a highly organized and recognizable chick. Scientists today continue to ask many of the same questions about development that intrigued natural philosophers in the past, but at dramatically increased levels of sophistication and complexity. How do the organs of the body arise? How do cells differentiate? What enables animals to reproduce and transmit genetic traits to their offspring? What are the biological bases underlying evolution? The science of development now extends to and impacts upon nearly every field of biology in its quest to determine how the information stored in a fertilized egg can trigger and orchestrate the processes leading to the establishment of unique individuals and the endlessly diverse species they comprise.

Questions of how the body maintains and heals itself also drive research into regeneration. For centuries it has been known that different species have different self-healing powers, but the reasons for these differences and the mechanisms by which our own bodies replenish cells lost to aging, injury or disease are now coming into much sharper focus. With the emergence of improved techniques for studying regeneration at the cellular and molecular levels, the roles of biological systems governing the maintenance and restoration of the body's cells have grown clearer, spurring further interest into progenitor cell types known to be capable of generating appropriate new cells in response to damage and loss.

These progenitor (or 'stem') cells play fascinating and crucial roles in development and regeneration, and science now looks to harness their potential for medical application by discovering ways to induce and guide their differentiation to supply natural replacement cells to damaged organs and tissues. The emerging field of regenerative medicine looks to translate research findings from the laboratory bench to the hospital bed and to develop alternatives to the traditional medical approaches of pharmacological and mechanical intervention. Such therapies may someday make the physician's dream of using the body's own cells to heal itself a reality.

Laboratories at the RIKEN Center for Developmental Biology pursue research into the mechanisms of development and regeneration with a twofold mission: To make contributions to the betterment of human health and well-being by providing an academic foundation for regenerative medicine, and, equally important, to illuminate the laws of nature in the grand panoply of the living world. To shed light and offer hope – these are the goals of responsible scientists everywhere, goals that we share and hope will direct our work toward deciphering some of life's greatest mysteries.

Center for Developmental Biology

2002 Highlights

3 March 22

Completion of Research Building A and CDB Experimental Animal Facility

The CDB marked the completion of construction of Building A, adding 7 floors and 10,199 m^2 of laboratory space to the facility. The experimental animal facility (4,311 m^2) was officially opened on the same day, representing the launch of one of the world's most advanced facilities for the production and handling of experimental mice.

4 April 22-24

Opening Symposium and Meeting of the Advisory Council

The CDB held its first international symposium on "A New Paradigm in Developmental Biology," attended by developmental biology and regenerative medical researchers from around the world. Speakers included members of the CDB Advisory Council, which convened its second session in the two days immediately following the symposium.

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April 25

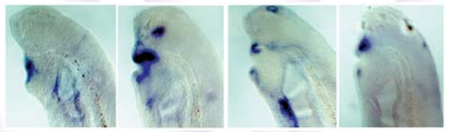
Publication of "Dominant role of the niche in melanocyte stem-cell fate determination"

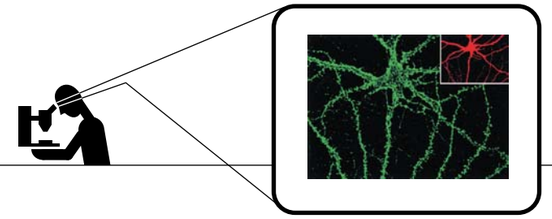
The work of the Nishikawa research group on the role of the microenvironment (or niche) in determining a stem cell's fate was featured on the cover of the April 25, 2002 issue of *Nature*.

5 May 17

Publication of "Heterotopic Shift of Epithelial-Mesenchymal Interactions in Vertebrate Jaw Evolution"

Research results from the Kuratani lab providing a concrete demonstration of the evo-devo concept of exaptation, in which a structure that originally evolved for one adaptive reason is converted to a different adaptive role in another species, were published in *Science* magazine.





7 July 3

Publication of "Cadherin Regulates Dendritic Spine Morphogenesis"

The July issue of *Neuron* featured an article from the Takeichi research group detailing a heretofore unknown function of cadherin cell-adhesion molecules in the formation of synapses between mammalian neurons.

July 8

Opening Ceremony and Public Science Forum

The CDB was officially inaugurated in an opening ceremony attended by members of the local and national government, including Kobe mayor Tatsuo Yada and MEXT Senior Vice-Minister Takashi Aoyama. A lecture session open to the public was held the same day at a nearby hall, with talks given by CDB group directors and team leaders to an audience of more than 500 people.

July 9

Visit by MEXT Minister Toyama

Atsuko Toyama, the Minister of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), visited the Center on its first full day of operation, chatting with scientists and observing experiments at a number of labs. RIKEN research is primarily funded through MEXT budget allocations.

9 September 27

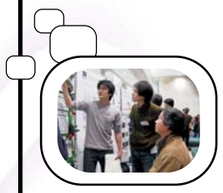
Completion of CDB Research Aquarium

The CDB's 455 m^2 aquarium for housing and breeding experimental aquatic species such as zebrafish and African clawed frogs officially opened on this day.

10 October 10

Publication of "FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians"

The publication of the Agata group's work in determining the gene responsible for restricting brain neural development to the head in planarians attracted news coverage in Japan and abroad.



11 November 25-26

First CDB Retreat

The CDB held its first annual retreat on the beautiful island of Awaji, located a short distance from Kobe in Japan's Inland Sea. The retreat gave CDB research staff the chance to relax, mingle and exchange research findings and opinions in a series of oral presentation and poster sessions. As with all CDB events, the retreat was held in English, allowing Japanese and non-Japanese scientists to communicate unimpeded by barriers of language or culture.

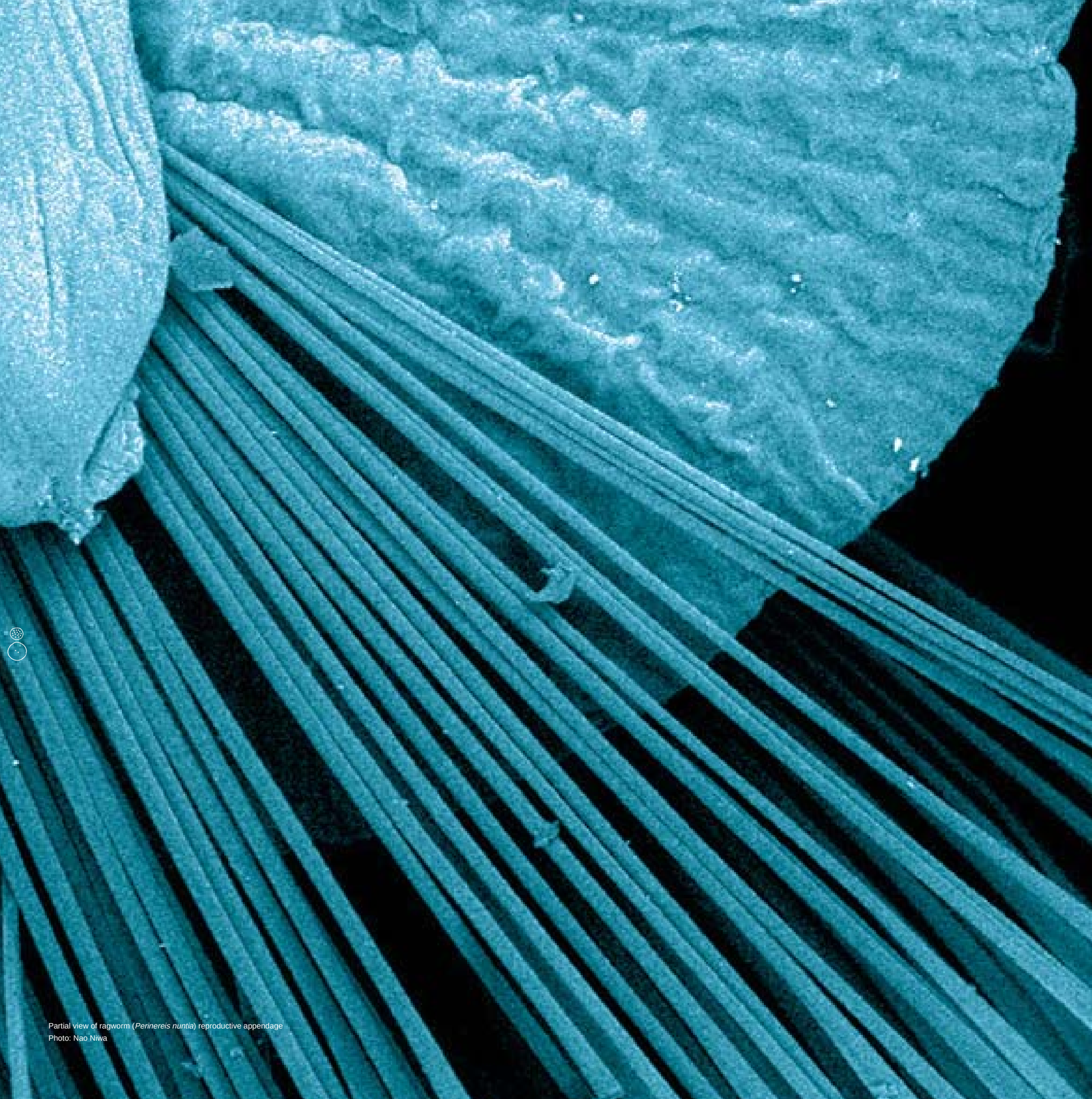
12 December 6

Completion of Research Building C

The opening of Building C (6 floors, 8,455 m^2) represented the completion of construction of the Center's physical facilities. A number of laboratories had relocated to their permanent locations in Building C by the end of 2002, and all current labs are scheduled to be in place by early spring of 2003.

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 three main themes of the CDB:
- Mechanisms of Development
- Mechanisms of Regeneration
- Scientific Bases of Regenerative Medicine
The Core Program consists of seven research groups, each lead by an eminent scientist in these fields. In addition to the group director, each group includes a number of research fellows and technical staff and in some cases a senior research fellow.



Partial view of ragworm (*Perinereis nuntia*) reproductive appendage
Photo: Nao Niwa

Laboratory for Cell Adhesion and Tissue Patterning

Cell Recognition

Masatoshi Takeichi
Ph. D.

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 his current positions at the CDB in 2000. Dr. Takeichi is best known for his discovery of cadherins, which are fundamental in the mechanisms of cell-cell adhesion.

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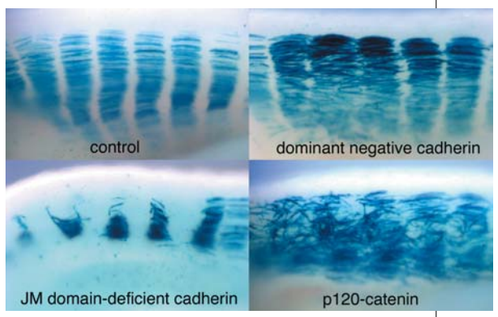


Myotome patterning in chicken embryos is perturbed by the overexpression of mutant cadherins or p120-catenin in different fashions.

Cell adhesion, recognition and morphogenesis

The formation of complex systems such as tissues and organs requires a high degree of communication and cooperation between their constituent cells. Since the discovery of cadherins, the role of the cadherin superfamily of molecules in regulating cell-cell adhesion has been studied extensively. These molecules form complexes with other intracellular factors to create bonds spanning membranes and intercellular spaces to join cells with other cells expressing similar cadherins. Such bonds are dynamic and regulable, acting more like Velcro tape than permanent glue. But recent work is revealing that the cadherins may serve a wider range of functions than as simple adhesives. Dr. Takeichi's research group is engaged in demonstrating expanded roles for these molecules in the formation of complex structures such as neural synapses.

Structure and function of cadherin complexes

Cadherins are the main regulators of calcium-dependent cell-cell adhesion, but they do not work alone. It has been shown that the function of cadherins relies on the formation of complexes with a group of molecules called catenins, which associate with the cytoplasmic tail of the cadherin molecule. The tail of the cadherin molecule comprises two main divisions. The innermost domain, to which the β- and α-catenin complexes bind, is known to be essential to cadherin function and mutations in these catenins have been implicated in some forms of cancer. One of Dr. Takeichi's recent areas of interest has been the role of the juxtamembrane (JM) domain, which is the binding site for another catenin, known as p120. He found that cells lacking the JM domain cannot undergo normal morphogenetic movement during the development of chicken somites, suggesting that this domain plays a critical role in allowing cells to relocate. These findings led Takeichi to propose the model that the JM domain is a signaling center involved in regulating cadherin activity, and may be important not only in embryonic morphogenesis, but in the processes of cancer invasion and metastasis as well.

The neural retina provides the Takeichi lab with another system useful for the study of developmental processes

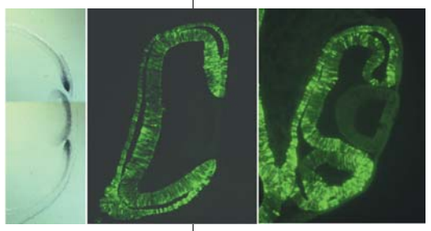
Synapse formation

The synapses formed between neurons are of central importance in neural signal transmission, but the processes by which synapses are formed remain poorly understood. The Takeichi research group previously discovered that cadherin complexes localize in interneuronal cell junctions in such a way that they do not interfere with signal transmission as carried out by synaptic vesicles and receptors on either side of the synaptic complex. The group has now reported that cadherins help to regulate the adhesion of dendritic branches known as 'spines' to axons, which is a crucial step in synapse formation. During neural network development, dendrites (protoplasmic processes that conduct impulses toward the nerve cell body) extend spines into the intercellular space. When a spine makes contact with an axon (which conducts impulses away from the neural cell body), a mushroom-shaped 'head' is formed, laying the foundation for a new synaptic site. The Takeichi lab conducted loss-of-function studies in hippocampal neurons with dominant negative mutations for N-cadherin, and in those obtained from αN-cadherin knockout mice, and found that both groups of neurons formed synapses with altered dendritic spines and synaptic organization. These findings indicate that cadherin/catenin complexes may be critical regulators of the morphogenetic processes underlying synaptic plasticity.

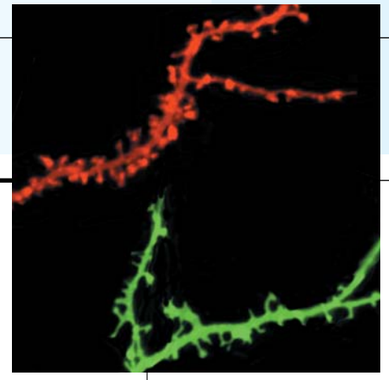
Organization and development of the neural retina

The retina, the innermost coat of the eyeball, is composed of neural and non-neural elements. The neural retina, which receives optical information from the lens and interfaces with the optic nerve to convey that information to the brain, provides the Takeichi lab with another system useful for the study of cellular organization and structure-specific developmental processes. The group's studies in this field have focused on the role of the gene *Wnt-2b* in retinal development in chick embryos. The developing retina gradually organizes into sheet-like laminated structures. Retinal cells in isolation, however, fail to form these structures and instead clump together in 'rosettes.' The molecular mechanism by which the laminated structures are generated was unknown. Members of the Takeichi group have now demonstrated that *Wnt-2b* produced in the ciliary margin, a small region located at the border of the retina with the iris, guides retinal cells to form laminar sheets. Using dissociated retinal cells in culture, the team found that cells exposed to Wnt-2b in culture organized into sheets, similar to the arrangement of cells in the normal retina. The same cells, when exposed to Frizzled, a Wnt-antagonist, clumped together in rosettes.

A second study of *Wnt-2b* function revealed its importance in maintaining undifferentiated progenitor cells in the ciliary marginal zone. Throughout embryonic development, the ciliary margin contains multipotent cells that serve as progenitors for the specialized, differentiated cell types that ultimately form the complex architecture of the retina. When *Wnt-2b* is overexpressed in developing retinas, normal cell differentiation is inhibited. Additional studies revealed that when the Wnt signaling pathway was blocked downstream, neuronal cells differentiated earlier than they normally would have. All of these findings suggest a role for *Wnt-2b* as a stem cell factor in the retina.



Wnt2b, expressed in the ciliary margin (left), controls the undifferentiated state of retinal cells. Overexpression of Wnt2b, coinjected with GFP (green), in retina induces the folded expansion of an undifferentiated cell layer (right). Middle, control, in which only GFP was injected.



Blockade of cadherins in hippocampal neurons alters dendritic spine shape. Red, a control dendrite; green, a dendrite expressing a dominant-negative cadherin.

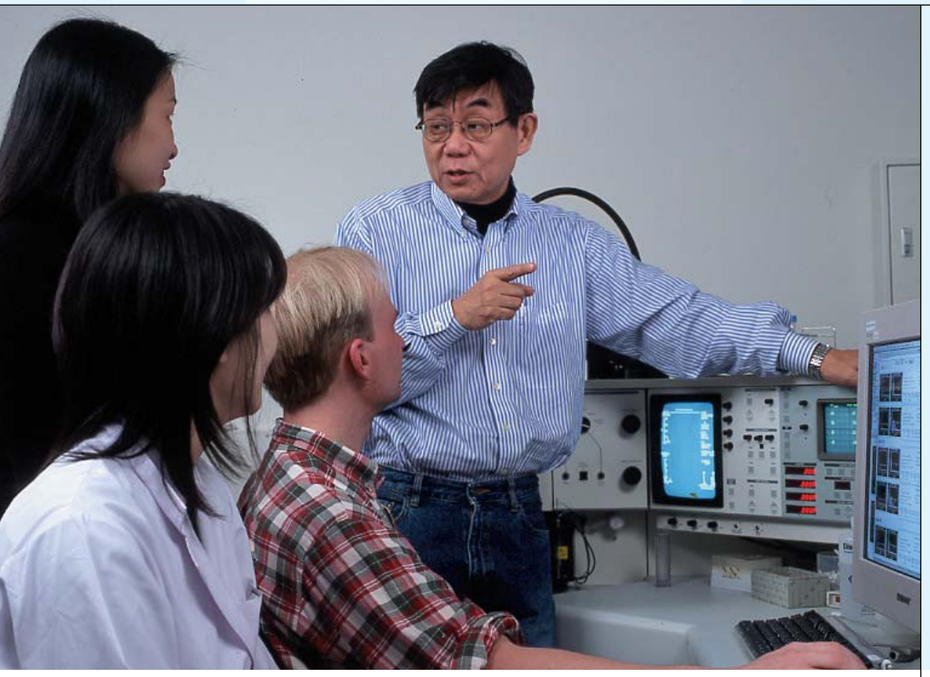
Publications

Kubo F, Takeichi M, and Nakagawa S. Wnt2b controls retinal cell differentiation at the ciliary marginal zone. *Development* (2003 in press).

Togashi H, Abe K, Mizoguchi A, Chisaka O, and Takeichi M. Cadherin regulates dendritic spine morphogenesis. *Neuron* 35:77-89 (2002).

Horikawa K, and Takeichi M. Requirement of juxtamembrane domain of the cadherin cytoplasmic tail for morphogenetic cell rearrangement during myotome development. *J Cell Biol* 155:1297-306 (2001).

Laboratory for 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 (Germany) 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.

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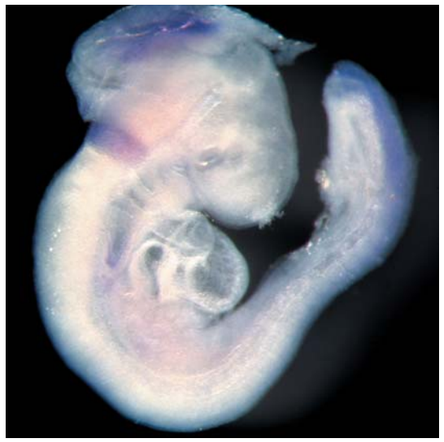


HoxB1 expression pattern in 9.5 d.p.c. mouse embryo

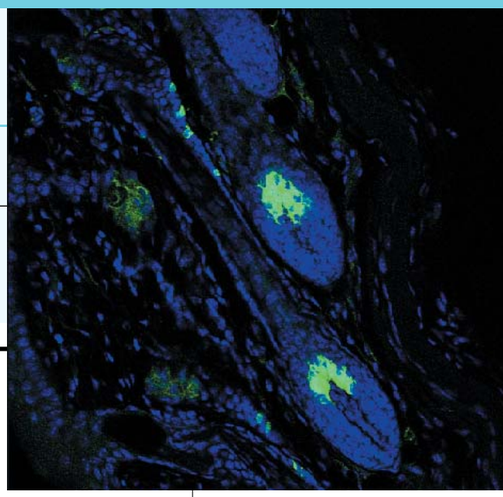
Stem cell biology

Stem cells are undifferentiated cells characterized by their capacity to self-renew and to generate specialized cells indefinitely. These cells provide the source for all of the myriad somatic cell types in the adult organism. The study of stem cells stands at the heart of many of the central problems in developmental biology, but many questions regarding the nature of these cells, such as the extent to which they can be reprogrammed to produce progeny of different fates and the identities of the intrinsic and extrinsic molecular factors that regulate a cell's 'stemness,' remain unanswered.

Shin-ichi Nishikawa believes that location is important in defining a stem cell's function

Shin-ichi Nishikawa believes that location is important in defining a stem cell's function. Recent findings that stem cells can arise in one tissue type but later be directed to give rise to another strongly suggest that a stem cell's role is at least partly determined by signals from its surrounding environment. By studying this microenvironment, known as the stem cell 'niche,' the Nishikawa research group seeks to gain insight into the identity and function of molecules involved in stem cell activities, and the potential of stem cell-based therapeutic applications.

Stem Cells



Distribution of melanocytes in mouse follicle

Melanocyte stem cell niches

One of the major challenges confronting stem cell researchers is the host of technical and biological problems associated with *in vivo* studies. Multipotent stem cells are responsible for the normal development of their progeny cells, which means that experimental manipulations of these cells can have far-reaching, even lethal, consequences. Nishikawa has chosen melanocyte stem cells as a research model as these cells share some common characteristics with the stem cells responsible for generating blood and sperm, but offer several experimental advantages over those stem cell types. Melanocytes, which produce skin and hair pigments, can be modified extensively, even lost, without causing any more serious effect than alterations in skin and hair color, and such alterations can themselves serve as visual confirmation of an experiment's result. Increased animal survival due to the absence of lethal phenotypes also makes the melanocyte system an ideal model from both the experimental and ethical perspectives.

In work published in 2002, Nishikawa and colleagues established a system for profiling melanocyte stem cells and tracking their locations in living hair follicles. Follicles are known to contain melanocyte stem cells, but the follicular microenvironment that sustains such cells is still poorly understood. In this study, the Nishikawa group identified cells with high expression levels of melanocyte stem cell markers, and then blocked the function of one of these molecules using marker-specific antibody. The results of these experiments indicated that melanocyte stem cells localize at two intrafollicular sites, the hair matrix and the bulge, each of which hosts a specific sub-population of stem cells. The matrix, where hair growth and pigmentation take place, provides an environment in which amplifying progeny cells become committed to a stem cell fate, while the bulge serves as a reservoir for stem cells at rest. Intermediate stem cells can migrate from the bulge to the matrix and even through the epidermis to neighboring follicles to replenish the actively proliferating population. Nishikawa hopes to characterize these niches in more detail to achieve a better understanding of the extrinsic factors responsible for maintaining stem cells in different states of activity.

Hematopoietic lineage maturation

In the classical view of lineage maturation, cell lines always develop toward increased specialization and away from differentiative potency. However, evidence exists to suggest that, in the case of hematopoietic stem cells at least, the process may follow a different route. Blood-forming cells are traditionally thought to originate in the lateral mesoderm, one of the three main developmental regions of the mesodermal germ layer. The Nishikawa group developed tools to distinguish cell types generated during early embryogenesis and identified several intermediate stages during the differentiation of blood cells from mesoderm. The group discovered two points of blood cell line divergence – either directly from lateral mesoderm cells or from vascular endothelial cells – a finding that contrasts with the widely-held view that blood cells and endothelial cells diverge from common precursors called hemangioblasts, and surprisingly indicates that an increase in potency occurs at the endothelial stage at which it would normally be expected to show a decline.

Regulating vascular architecture

Other recent work by the group has revealed a factor capable of maintaining the integrity of blood vessels derived in isolation from their natural environment. In the normal course of development, endothelial cells and perivascular mural cells work together to form a diverse vascular architecture from a relatively simple network. While the role played by mural cells in the structuring of vascular tissue remains unclear, their absence is a hallmark of diseases such as diabetic retinal disorders. Two proteins, angiopoietin-1 and PDGF-B, have been implicated in mediating the interaction between endothelial and mural cells. The Nishikawa group introduced a PDGF-B antagonist into neonatal mice, preventing mural cells from participating in the vascular formation process, and found that the resultant vascular system in the retina was poorly organized and leaky. However, when they introduced recombinant angiopoietin-1 to the eyes, they found that normal vascular architecture was restored, which suggests a potential application for angiopoietin in the treatment of mural cell disorders.

Publications

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Laboratory for 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 (US). 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, as well as team leader of the Laboratory for Animal Resources and genetic Engineering.

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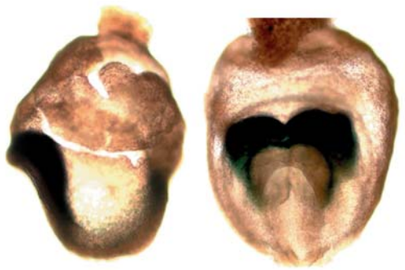
The vertebrate head-body boundary

The evolutionary development of the vertebrate head is the subject of a debate older than the concept of evolution itself. Beginning with Goethe's observation that the cranium of a sheep seemed to be formed from fused vertebrae, rival theories have been proposed in which it is asserted that the head is either the evolution of some preexisting structure, or that the emergence of the head was an evolutionarily novel event. What is certain is that the body plan of modern vertebrates is composed of three distinct regions characterized by the expression of different sets of regulatory genes – the trunk, the head, and the intervening hindbrain/pharyngeal region.

Shinichi Aizawa's research concentrates on the genetic and molecular characteristics of head development, particularly in the border between the fore-and hindbrain, which in vertebrates originates in segmented embryonic units known as 'rhombomeres.' Rhombomeres exhibit distinct gene expression and cell behavior profiles, and are situated at the developmentally interesting boundary between the trunk region, whose development is primarily regulated by homeotic Hox-family genes, and the rostral (anterior) head, which is governed by a different set of genes, predominantly the homologs of *Drosophila* head gap genes. Studying mutations in genes known to be responsible for body patterning in these regions, the Aizawa research group hopes to establish the factors that delimit and characterize the foremost rhombomeres, and thereby contribute to the understanding of the developmental criteria for the constitution of the anterior head as conserved across phyla.



Enhancer of *Otx2* expression in the anterior neuroectoderm

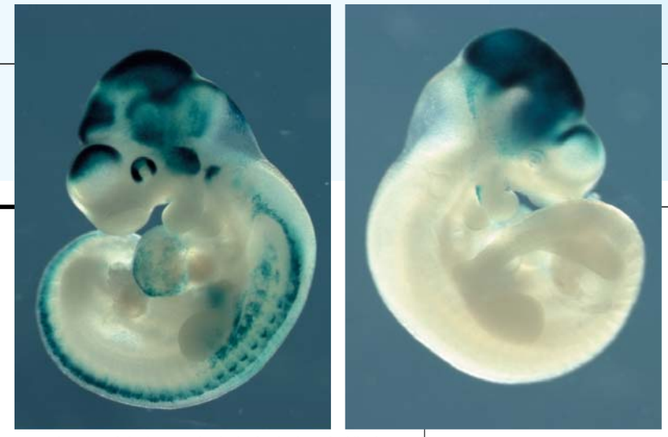
Head gap genes

Homologs of the *Drosophila orthodenticle* gene, which collectively form the *Otx* gene family, have been established as essential to the induction of the anterior head and the anterior-posterior body axis itself in both invertebrate and vertebrate species. In the mouse, *Otx2* homozygous mutants entirely fail to develop the fore- and midbrain. However, the mechanisms by which this gene induces normal anterior head development remain elusive. With the knowledge that signals from an embryonic neuroderm velopmental region called the anterior neural ridge are also essential in the induction and patterning of the forebrain, Aizawa set out to determine the precise role played by *Otx2* in mediating signals from this organizing center. Generating a series of allelic *Otx2* mutants resulted in the unexpected discovery of a hypomorphic allele characterized by arrested forebrain and anterior head development. Unlike homozygous *Otx2* mutants, however, these allelic embryos were able to form apparently normal anterior-posterior axes and progenitor fields. Explant assays were made by removing anterior neural plate sections from mutant embryos and transplanting them into wild type embryos, and vice-versa. It was found that while neural plates from *Otx* $^{frt\text{-}neo/-}$ mutants were able to respond normally to signals from the anterior neural ridge in wild type embryos, the reverse was not true, indicating that *Otx2* may be involved in the downstream mediation of forebrain-inducing signals, such as *Fgf8*, emanating from this region.

Locus mapping provides a means for identifying candidate genes of potential biomedical importance

Genetic determinants of craniofacial anomalies

In mice, embryos lacking a single copy of the *Otx2* gene develop a range of phenotypes in which craniofacial development is disturbed. These phenotypes, termed otocephaly or agnathia-holoprosencephaly, are also observed as human congenital anomalies and represent a failure of the head patterning program. However, in mice, the severity of the phenotype was linked to the genetic background of the strain in which the mutation was induced. By crossing strains exhibiting different phenotypes and performing genotypic analysis of the offspring, the Aizawa group was able to identify two loci linked to abnormalities in the lower jaw in these mutants. One of these loci, named *Otmf2*, may also be implicated in the development of holoprosencephaly, the most common congenital forebrain defect in humans, with an incidence of up to 1 in 250 early embryos. This study revealed the power of the mapping of loci responsible for determining phenotypic severity in naturally occurring variations in different strains of experimental animals, both as a tool for genotypic analysis and as a means of identifying candidate genes of potential biomedical importance.



Two enhancers of *Otx2* expression in the forebrain and midbrain

Body patterning in primitive species

Amphioxus, commonly referred to as the lancelet, is a small, marine organism thought to represent one of the earliest evolutionary ancestors of vertebrates as the lancelet embryo develops a number of structures common to chordate development, such as the notochord, during gastrulation. The polarized accumulation of **β**-catenin in the nuclei of specific cells is a crucial step in the early development of multicellular animals, playing roles in the formation of germ layers and signaling centers. The Aizawa lab studied the activity of **β**-catenin in the lancelet to investigate how its localization impacted on morphogenesis in these organisms. These studies, in which the asymmetric distribution of this protein was experimentally altered, indicated that embryos with non-typical **β**-catenin distributions are nonetheless able to establish dorsal-ventral axes, suggesting that dorsal morphogenesis is not dependent on early embryonic polarization in Amphioxus.

In experiments such as these, the Aizawa lab endeavors to characterize the evolutionary, genetic, and molecular bases of the vertebrate body plan, with a particular emphasis on the factors at work in inducing that crowning achievement of animal evolution, the forebrain.

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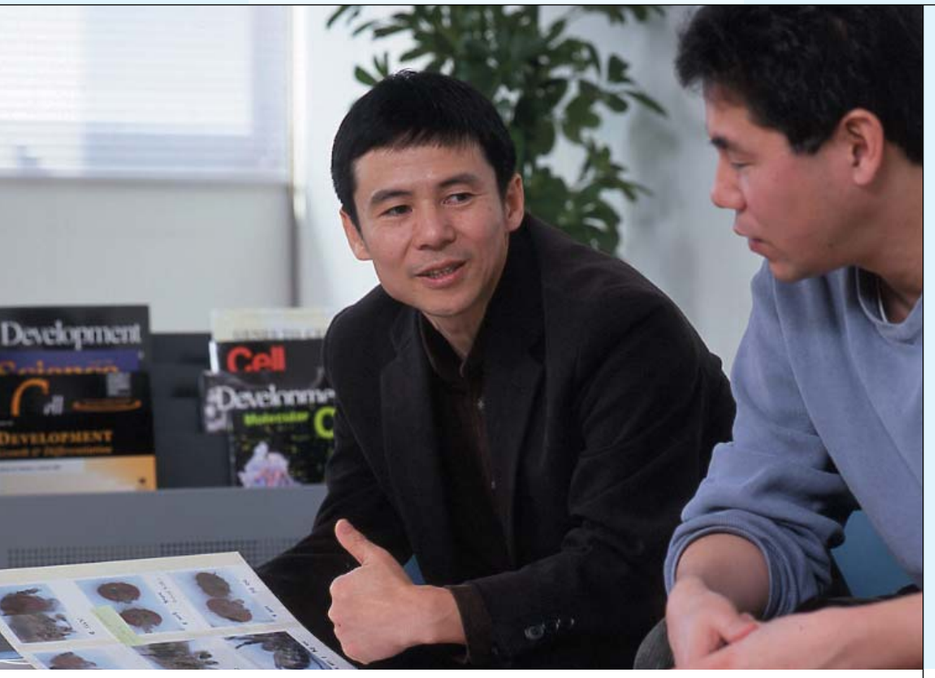
Laboratory for Morphgenetic Signaling



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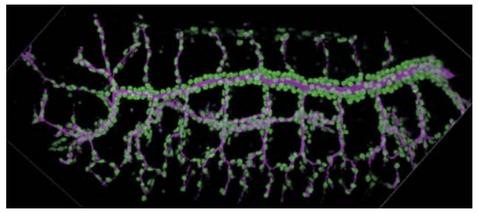


The tracheal system stained for nuclei (green) and luminal cavities (purple)

Shigeo Hayashi
Ph. D.

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

A society of cells

The metaphor in which cells are likened to individuals within a society is a common one in biology, and plays a particularly instructive role in understanding many of the mechanisms of early development. Just as students of social behavior seek to identify the factors responsible for human actions, developmental biologists ask similar questions about what drives a cell to divide, differentiate, migrate, or self-destruct. The answers to both sets of questions generally involve multiple factors. In animal development, the specification of cell fates and their behavior as individuals and in groups is determined by interactions between molecular factors intrinsic and extrinsic to the microscopic world of the cell – genes and their protein products, and signaling and transcription factors received from the world beyond the cell's membrane borders.

Questions of inter- and intracellular communication inform Shigeo Hayashi's research

Questions of inter- and intracellular communication inform Shigeo Hayashi's research. His work seeks to uncover genes and molecular factors that allow differentiated cells to communicate and coordinate with each other to form 'communities' of tissue, organ and anatomical structure within the 'society' of the organism as a whole. Hayashi has chosen to focus his studies on the systems of limb and tracheal development within the fruit fly *Drosophila* to outline the mechanisms of cellular behavior and interaction in organogenesis.

The adult *Drosophila melanogaster* fruit fly

Development of appendages

Like many insects, *Drosophila* is a highly competent flier, evolutionarily adapted to a life spent on the wing. Embryological studies in *Drosophila* have shown that both wings and legs share a common origin in developmental bodies known as limb primordia. In their studies of limb formation, Hayashi's group tracks the cellular growth, differentiation and migration characteristic of cells destined to become wings in the adult fly. Using time-lapse confocal microscopy of fluorescent-tagged cells, the group tracks three stages of the process of wing development: allocation, specification, and separation. In allocation, cells in the primordium undergo preliminary specialization from cells in the surrounding area. Specification involves further differentiation between cells that will remain in place and contribute to leg formation, and those that are marked for specification into wing tissue. The separation phase involves the migration of wing-specified cells to dorsal locations, from which the final wing structure arises. Non-migrating cells remain to form the leg primordium, which consists of proximal and distal domains. This simple organization is further elaborated by a series of segmentation processes.

Now, armed with the ability to make a clear visual record of the movements of wing and leg-specified cells to their ultimate destinations within the body, Hayashi and colleagues intend to pin down the molecular machinery that determines these outcomes using genome screening and molecular biology techniques. As the genetic factors involved in limb development tend to be highly conserved across species, findings from studies on the relatively simple fruit fly should offer insights into the fundamental mechanisms of organ and limb development in humans and other species.

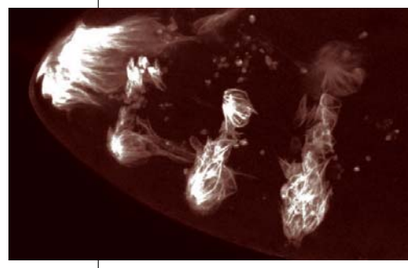
Tracheal development

The mechanisms of tracheal development in *Drosophila* are another focal area for research in Hayashi's lab. In fruit flies, the trachea is functionally analogous to the mammalian lung, and its development shares many characteristics with the development of many branched tubular systems including the lungs, kidneys, and blood vessels, which are all essential to the distribution and regulation of liquids and gases throughout the body in higher animals.

The development of the trachea in *Drosophila* originates with ten pairs of tracheal primordia, located in the contiguous thoracic and abdominal segments, T2 to A8. Cells from the primordium migrate, extend branches decorated with fine cellular extensions called filopodia, and respond to environmental cues to seek out and establish connections with other tubular cells. The trachea ultimately extends to every segment in the adult individual. Hence, the process of tracheal tubulogenesis involves both intercellular cooperation within individual segments, and coordination between cells throughout the entire body.

The migration of tracheal branches is controlled by a complex guidance mechanism involving several extracellular factors such as Dpp, Wingless and Fgf. Hayashi's group is investigating this guidance mechanism and the role of the Rac signaling pathway, which is known to participate in the mechanisms of cell adhesion, migration and guidance. The group, in collaboration with researchers from Tokyo Metropolitan University, uses selective overexpression of target genes to produce gain-of-function mutants as a preliminary screen for new gene functions required for tracheal patterning.

Gain-of-function studies serve to provide an initial glimpse of the roles of such genes. By regulating the expression of target genes and observing the phenotypes, it becomes possible to identify candidates for further investigation. To date, the Hayashi group has completed screening studies of approximately one-fourth of the *Drosophila* genome using randomly inserted UAS transposons, which serve to activate genes located downstream. Phenotypes of interest include mutants displaying absent tracheal branches, branch misrouting, changes in cell and tubule shape, and abnormal cell adhesion. The genes implicated in these mutations become candidates for follow-up studies to elucidate their involvement in tracheal development.



Drosophila embryo expressing tau-GFP fusion protein in the limb primordium and sense organs of the head

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Laboratory for Cell Asymmetry

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 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 Institute of Development, Aging and Cancer at Tohoku University and remained there until taking his current position as group director at the RIKEN CDB.

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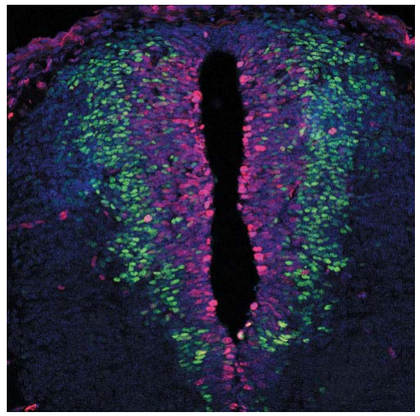
Intracellular asymmetry

All the cells in the body originate from a single progenitor, and nearly all share an identical DNA code. How then do the vast numbers of distinct cell types in complex organisms differentiate from one another? The process of asymmetric division, in which regulatory factors in a single mother cell are distributed unequally to daughter cells produced by cell division, is a key to answering that fundamental question. This asymmetric distribution of regulatory factors allows resultant cells to assume different roles from each other in a process called fate determination. This process relies on the establishment of intracellular gradients of factors regulating the expression of genes, which results in differential patterns of gene expression in daughter cells when the cell undergoes mitosis. As it is the expression of genes, and not their simple presence or absence, which is primarily responsible for determining cell identity, controlled asymmetric division is critical to ensuring the development of appropriate numbers and types of daughter cells, and so, to the development of the organism as a whole.

Neural cell fate determination in *Drosophila*

Fumio Matsuzaki has dedicated his research to explicating the role of asymmetric division in determining cell identity, using the *Drosophila melanogaster* nervous system as a model. This system provides an attractive research platform for studying the development of cell diversity, as the nervous system features more cell types than any other organ system, and the fruit fly is highly amenable to genetic manipulation. With the advent of techniques for molecular analysis, neurodevelopmental studies have shown that neural cells' fates are determined at a very early stage in the development of the organism. In previous research, Matsuzaki and others demonstrated that the fates of daughter neural cells are in large part determined by unequal distribution of fate-determining factors within the mother cell during the process of cell division. Two factors in particular, known as Prospero and Numb, have been shown to localize to the basal side of the neuroblast (the neural progenitor cell) prior to its division. This polar distribution results in two daughter cells exhibiting distinct gene expression patterns – a new neuroblast, and a smaller-sized ganglion mother cell (GMC) – in which Prospero and Numb are segregated in the GMC. In this process of asymmetric division, the neuroblast remains undifferentiated and retains its multipotency, while the GMC becomes committed to generating neurons and glial cells.

Further studies in the Matsuzaki and other labs showed that Prospero and Numb are tethered to the basal cortex of the neuroblast by the molecules Miranda and PON (for Partner Of Numb), respectively, and subsequently released into the newly-formed GMC. The molecular mechanisms involved in asymmetric distribution during cell polarization have further been shown to have homologous counterparts in vertebrates, providing strong evidence for the evolutionary conservation of intrinsic signaling in the process of asymmetric cell division. The Matsuzaki lab is now confronting questions regarding the roles of both extrinsic and intrinsic signals, and the mechanism by which cells of different sizes are generated in the process of mitotic division.

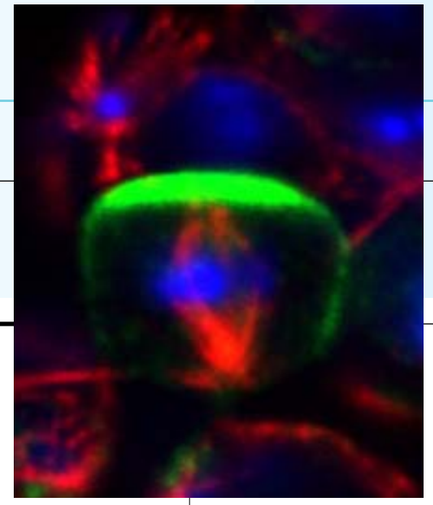


In the developing mouse spinal cord, Prospero (green) is transiently expressed in neurons immediately after their birth from mitotic neural progenitors (red).

Asymmetric cell sizes

This last question prompted investigations that led to the identification of a new regulatory function in a family of proteins, known as G proteins, in *Drosophila* neuroblast cell division, a process in which the resultant neuroblast is much larger than the GMC. During mitotic cell division, chromosomes replicate within the mother cell and attach to spindles that draw them in opposite directions to ensure that a full complement of chromosomes is available to each daughter cell. The chromatids are drawn to the spindle poles through the action of the cytoskeleton. In work carried out at the CDB, Matsuzaki's lab has found that Gβ protein signals are distributed unequally in neuroblasts and seem to restrict the development of microtubules, resulting in a shortening of the mitotic spindle on one side of the cell. These unequal spindle lengths cause the neuroblast to divide at a cleavage site that is off-center, and the sizes of the daughter cells reflect this imbalance, with the daughter neuroblast being more than twice the size of the GMC. Cells lacking the Gβ protein in question form a symmetrical mitotic spindle and produce daughter cells of equal size with the determinants being normally segregated. Normal gene expression and mitotic activity are gradually altered in neuroblasts undergoing such divisions, suggesting that asymmetrically-sized division is a mechanism that ensures the 'stemness' of neuroblasts by minimizing the reduction of cell volume during consecutive divisions. This is the first demonstration of a molecular mechanism responsible for asymmetric mitotic spindle formation, and provides a valuable platform for further studies in fields ranging from microtubule dynamics to animal morphogenesis.

Cells lacking the Gβ protein in question form a symmetrical mitotic spindle and produce daughter cells of equal size with the determinants being normally segregated.



In *Drosophila*, dividing neuroblasts localize the Miranda (green) / Prospero complex to be segregated into the daughter GMC.

Publications

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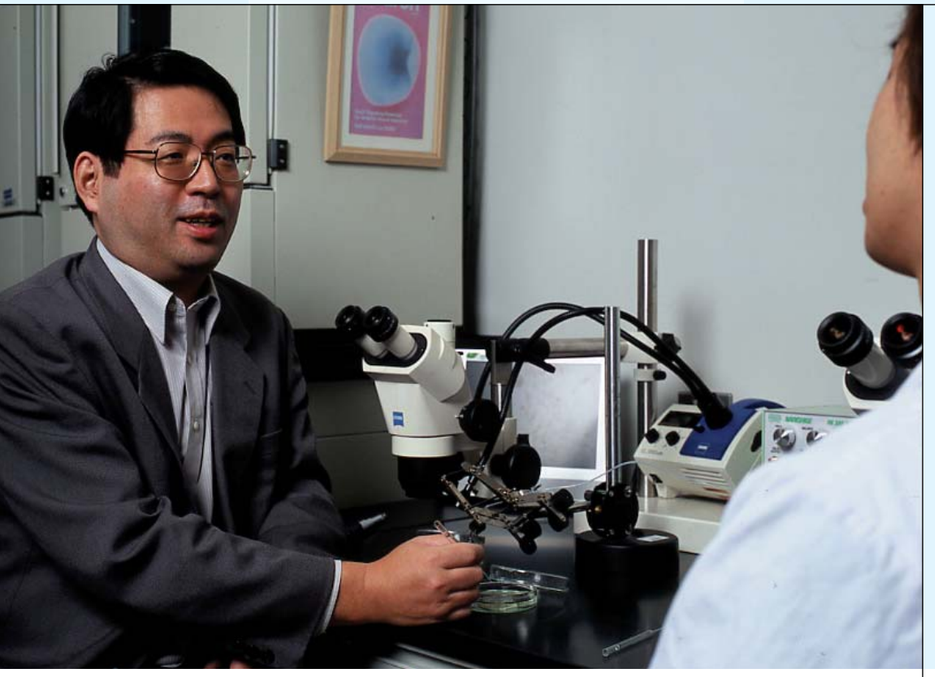
Laboratory for Organogenesis and Neurogenesis

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.*

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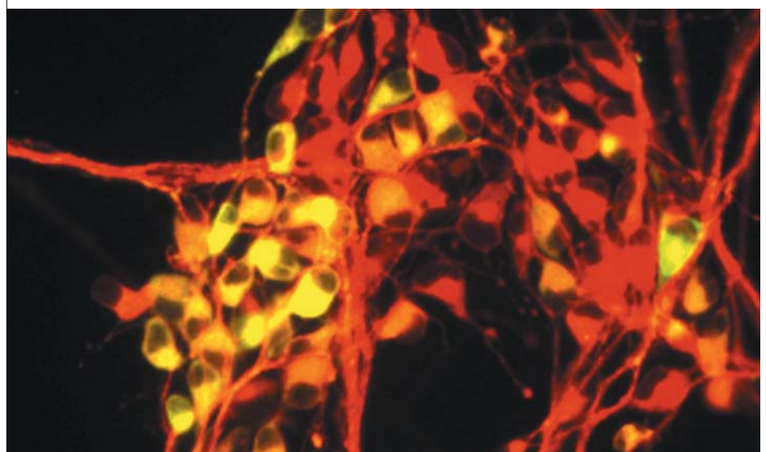
Induction of the dorsal nervous system

The vertebrate body plan can be construed as the distribution of three germ layers relative to three body axes. These interacting sets of patterns are established in early embryogenesis, and serve to determine the position and specification of cells as they differentiate and aggregate into tissues and organs. The specification of the dorsal-ventral (or back-belly) axis is significant in neural development: the central nervous system forms on the dorsal side of the body in all vertebrate species. 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.

Beginning with the identification of the neural inducing factor Chordin in the early 1990s, Yoshiki Sasai's research has focused on explicating the molecular signaling mechanisms that allow the early embryo to organize itself into a complex, mature organism. Using the African clawed frog, *Xenopus laevis*, as a model in molecular embryological studies, Sasai and his group are 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. Insights gained from studies in *Xenopus* are further tested in other organisms, such as zebrafish, chick and mouse, allowing for comparisons across species and evidence-based speculation into the evolutionary conservation of these signaling mechanisms. The group is also actively developing effective methods of inducing neuralization in mammals, work which has potential for application in the treatment of nerurodegenerative disorders, such as Parkinson's disease.



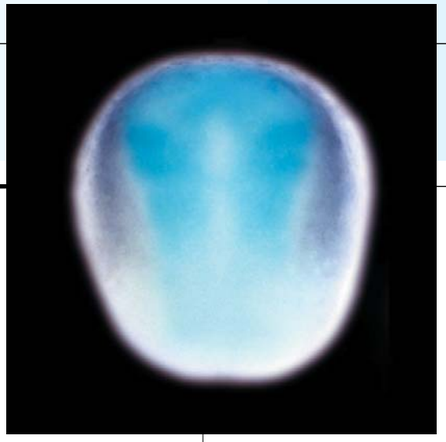
Dopaminergic neurons derived from primate ES cells by the SDIA method.

The Sasai group's work has potential for application in the treatment of nerurodegenerative disorders, such as Parkinson's disease

Tiarin, a novel dorsalization factor

In previous work, Sasai identified a number of neural differentiation factors that operate by blocking the effects of BMP4 (BMP for Bone Morphogenetic Protein), a molecule which instructs undifferentiated cells in the ectoderm to take up an epidermal fate. The conclusion of these studies was that anti-BMP factors, which include Chordin and its immediate and indirect downstream targets, actually serve to protect the 'default' neural status of ectodermal cells. Sasai has recently added a new member to the growing family of central nervous system dorsalizing factors with the identification of Tiarin (so named because its expression pattern in the *Xenopus* neurula resembles a tiara worn on the head).

With the goal of isolating genes that encode factors involved in the patterning of the anterior central nervous system, the Sasai group performed a cDNA library screen, yielding nine candidate genes, including *Tiarin*, which exhibited neural-specific expression patterns. Subsequent profiling of the *Tiarin* gene, which has homologs in chick and mouse, revealed that it is expressed in the non-neural ectoderm flanking the anterior neural plate, a developmental region which gives rise to the vertebrate forebrain. Misexpression of *Tiarin* induces dorsal neural markers and suppresses ventral markers, indicating its importance in establishing dorsal and neural identity. Although the molecular mechanisms by which this is achieved remain obscure, it is known that *Tiarin* operates independently of other major signaling networks known to influence body patterning, making it an important new factor in the patterning of the neural tube.



Expression of *Tiarin* (purple) in *Xenopus* neurula; Neural plate is stained with Sox2 cRNA probe (light blue).

Inducing neural differentiation in mammals

Over the past decade, the understanding of early neural differentiation in *Xenopus* has advanced rapidly, but that knowledge is not immediately transferable to the study of neural induction in other organisms. While the effects of anti-BMP signals are sufficient to induce neuralization in the frog, additional signals seem to be necessary in the mouse. Until quite recently, one of the main obstacles to the detailed study of mammalian neural differentiation *in vitro* has been the lack of an experimental system that offers the relative advantages of the animal cap assay used in *Xenopus*. The animal cap in the frog blastula is capable of producing a variety of tissues in response to appropriate signals, making it a valuable tool in the embryologist's experimental kit. Mouse embryonic stem (ES) cells promise similar differentiative potential, but difficulties in their guided induction have meant they have not seen widespread use in studies of early patterning.

The Sasai lab has developed a technique for inducing the differentiation of neural cells by culturing mouse ES cells on plates of connective cells, called stromal cells. These cells have a strong neuralizing effect, termed SDIA (for Stromal cell-Derived Inducing Activity), and induce ES cells to generate dopaminergic neurons and their precursors at high rates of efficiency. Such controlled neural induction represents an important step toward the development of an experimental platform amenable to the study of early neural differentiation in mammals. And, in work published in early 2002, Sasai and colleagues further demonstrated that the SDIA effect is preserved in the neural induction of primate ES cells as well, with dopamine-producing cells being generated at a frequency of about 35%. This method provides an immediate unlimited source for primate cells useful in experimental studies of neurodegenerative disease, and opens up avenues for developing stem cell-based therapies for such diseases in the future.

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Laboratory for 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 Prof. 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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Regeneration in planarians

Evolutionary regeneration

The process by which animals replace cells that have been lost due to aging, injury or disease is known as regeneration. All animals have some ability to regenerate, but during the course of evolution, the degree of that capacity has been distributed widely in different species. While some animals, such as humans, are quite limited in their regenerative capabilities, others demonstrate much greater potential to replenish lost cells. Research at Kiyokazu Agata's lab is focused at the intersection of two fields of study: the mechanisms of regeneration itself, and the evolutionary processes by which organisms have developed such diverse regenerative approaches. Stem cells are centrally important to both of these fields, and Agata's work seeks to identify molecules that maintain pluripotency in and present positional cues to these cells, as well to clarify their role in the evolution of complex cell systems.

Maintaining totipotency

Planarians, tiny flatworms only a few millimeters in length, are known for their remarkable regenerative abilities. A single planarian can be cut into dozens, even hundreds, of pieces, and each segment will regenerate into a complete individual organism. This is due to the presence throughout the planarian body of totipotent stem cells called neoblasts, which, given the proper signals, are capable of differentiating into any cell type in the animal's body. To study the mechanism by which neoblasts maintain their totipotency, Agata's research group concentrated on cells containing 'chromatoid bodies', structures within the cell marked by the presence of the gene *DjvlgA* (*Dugesia japonica* Vasa-Like Gene A, *D. japonica* being the name of one species of planaria). Chromatoid bodies are morphologically and compositionally similar to germ plasm, which is found in totipotent germ cells in *Drosophila* and *C. elegans* and is involved in germline specification in those animals.

Totipotent Stem Cells

Anti-synaptotagmin antibody staining showing axon bundles

Whole-mount *in situ* RNA staining with *DjPC2* probe, showing neural cell bodies

Planarians, flatworms only a few millimeters in length, are known for their remarkable regenerative abilities

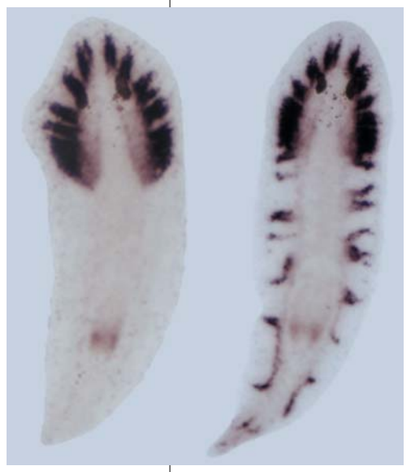
By comparing the compositions of chromatoid bodies in undifferentiated neoblasts and cells committed to, for example, neural or muscle cell fates, Agata's group discovered that the content of chromatoid bodies is heterogeneous, and dependent on the cell's designated fate. This finding runs counter to the classical view that only uncommitted, totipotent cells are capable of division in planaria, and suggests that certain stem cells migrate through the planarian body to the regenerating wound site, called the 'blastema.' These 'committed' stem cells exist in a state intermediate between the totipotency of uncommitted stem cells, and terminally committed cells that have lost the ability to divide. The group is now investigating the factors responsible for maintaining the 'stemness' of these cells prior to their arrival at the blastema.

Stem cell regulation

Although totipotent stem cells are distributed throughout the planarian body, these cells are able to generate specialized cell types appropriate to the site of injury when the animal is cut. This suggests that information regarding the position of the wound is conveyed to the stem cell, allowing it to make the necessary changes in gene expression to differentiate into the cell type that needs to be regenerated. This year, the Agata group announced the discovery of one gene involved in such positional cueing. The gene, named *nou-darake* (*ndk;* Japanese for 'brains everywhere'), ensures that brain neurons only develop in the head region; experiments in which the gene was knocked down by RNA interference resulted in animals that developed brain cells throughout their bodies. This gene seems to function by binding a second protein, FGF, to cells in the head region, causing them to adopt a brain neuron fate. Previous to this discovery it had been thought that the planarian brain produced some factor that inhibited brain growth in other parts of the body, but the *ndk* findings strongly indicate that the reverse is true; cells in the head (and those migrating to the wound site in decapitated animals) recruit a brain-inducing factor.

Evolutionary role of stem cells

In addition to the valuable insights they provide on the mechanisms of regeneration, stem cells can also shed light on the function of developmental processes in evolution. Indeed, in Agata's words, "Stem cells are key to understanding evolution." This is due to their unique ability to give rise to cells of different character than the antecedent cell. On the time scale of a single organism's life span, this leads to the development of complex cell systems within the organism. On the evolutionary time scale, however, it may be one of the primary factors leading to the divergence of species. For example, investigations into how planarian stem cells differentiate into the animal's simple eye could provide insights into how more complex eyes developed in more highly evolved animals. Agata's group seeks to trace diversification within families of conserved genes that are known to be linked to stem cell identity and function, in the hopes of clarifying the evolutionary contribution of stem cells to the development of more and more complex cellular systems.



Brain expansion in *ndk* knockdown planarian

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Creative Research Promoting Program

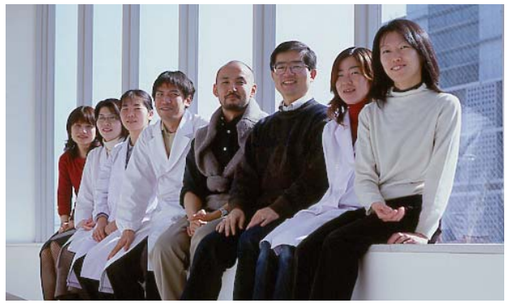
The Creative Research Promoting Program provides solid support to encourage relatively young researchers to carry out innovative and independent research plans. The teams are allowed a great deal of flexibility in regard to projects, budget, and lab size. The program also places great emphasis on cooperation and international participation. It is hoped that this unique system will help to cultivate a new generation of leading researchers by fostering the creativity and originality of investigators in a bottom-up fashion.

Undifferentiated mouse ES cells
Photo: Yayoi Toyooka

Laboratory for 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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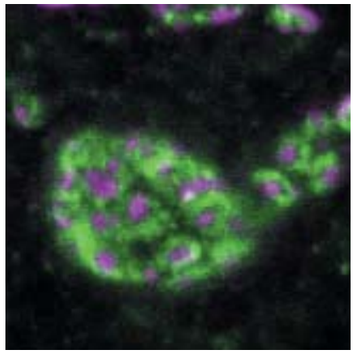
Neural networks

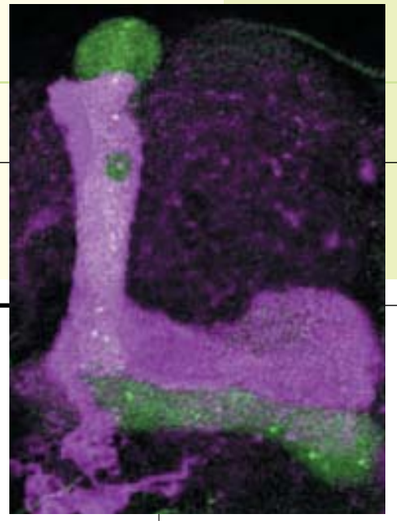
The formation of neural networks involves the orchestration of complex processes from neuronal migration, to axon growth to synaptic formation. In the human brain, which comprises on the order of 100 billion neurons, the mechanisms by which neuronal cells are able to identify and form synapses with appropriate partners, and the ways that circuits formed from interconnecting neurons are able to demonstrate adaptive plasticity are questions of staggering intricacy and depth. Chihiro Hama seeks to address these complex problems by investigations into the neural network of the *Drosophila* fly, a comparatively simply structured system which nonetheless demonstrates the capacity for both instinctive behaviors and learning. While there are pronounced differences in degrees of network sophistication and cell type diversity between *Drosophila* and more highly evolved organisms, they nonetheless share a large number of homologous genes important in neural development. Using a research strategy of large-scale mutant screening in the fly, the Hama research team seeks to open new windows into the genetic bases of neural circuitry.

GEF family genes

Screening for mutant phenotypes is an exacting process, involving methodical surveillance for anatomical or behavioral aberrations and requiring a thorough familiarity with the 'normal' characteristics of the experimental model. On observing reduced activity in mutant strains in previous screening studies, Hama and colleagues identified the gene still life *(sif)*, which participates in the maintenance of motor function in *Drosophila*. This gene, which codes for a GEF (Guanine-nucleotide Exchange Factor) that activates Rho family GTPases, has been shown to be involved in the processes of synaptic formation. Later, Trio, identified as a second GEF expressed in the nervous system, was revealed to be a pivotal factor for the regulation of axon extension. The Hama lab now plans to use RNAi gene silencing to perform functional analyses of the approximately 20 known GEF-family genes with the goal of identifying genes and molecular mechanisms contributing to neural development.



SIF (green) in the periactive zones of neuromuscular synapses



Trio (purple) in the mushroom body of *Drosophila* brain

Hama hopes to take advantage of the *Drosophila* olfactory system as an experimental model to shed light on the genetic bases of olfactory axon targeting

Olfactory network guidance

There are approximately one thousand different types of odorant receptors in the mammalian olfactory system, but it is believed that any given olfactory neuron expresses only a single receptor type. By mechanisms that remain largely unknown, the axons of olfactory neurons expressing a receptor of the same type converge into one of a thousand glomeruli, which are discrete structures composed of the axon terminals and the dendrites of the target neurons. Using a mutant screening approach incorporating gene knockout and GFP cell visualization techniques, Hama hopes to take advantage of the simpler *Drosophila* olfactory system as an experimental model to shed light on the genetic bases of olfactory axon targeting.

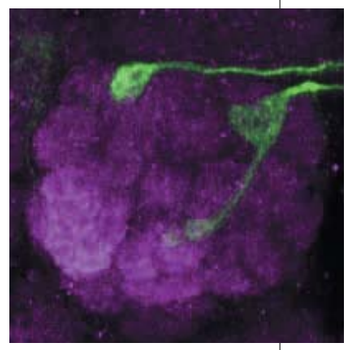
Vesicle transport in neural development

All cells contain cargo-bearing compartments known as vesicles. These vesicles function to deliver the macromolecules that serve the cells as membrane components or secreted signals, and the metabolite products of the cellular digestive process to the rest of the cell, where they are stored or used to fuel activity. Microtubules serve as 'railways' for vesicle transport; their ends anchor to sites on the cell cortex where the vesicles fuse. It is likely that the extrusion of cell membrane at given sites, such as takes place in axon extension, requires vesicles to be transported along microtubule in a specific orientation. To clarify how vesicle transport is involved in neural development, Hama plans to analyze *Drosophila* orthologs of genes encoding proteins known to be involved in the regulation of vesicular traffic in budding yeast. Hama hopes to elucidate the roles of these proteins in neurite extension, branching and guidance, and synapse formation as well, by conducting conditional RNAi studies using a DNA-based transgenic approach.

Development of brain structure

A fourth prospective direction for research in the Hama team lies in the functional analysis of a gene identified in a large-scale mutant screen as involved in the structural development of the brain. Aberrations in the brain structures can be caused by defects of many developmental events including neuronal proliferation, axon guidance and synapse formation. Mutations in the gene in question exhibit smaller than ordinary axon bundles and reduced numbers of cells in the brain tissue involved in olfactory learning and memory, which suggests that the gene regulates neuronal proliferation. The brain contains a variety of neuronal types, which can be generated serially from single neuronal precursors. Further analyses of the mutant may provide a hint about how switching of the neuronal types is regulated during brain development.

By adopting a broad-based, multi-focus approach, Hama seeks to illuminate the mechanisms by which cells that have followed the developmental route leading to neuronal differentiation are further able to organize into neural circuits, and ultimately to participate in the function of the brain.



Specific axonal targeting in the *Drosophila* olfactory system

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Laboratory for Vertebrate Axis Formation

Masahiko Hibi
M. D. , Ph. D.

Masahiko Hibi received his M. D. from Hiroshima University, School of Medicine in 1988, and his Ph. D. from the Osaka University Institute for Molecular and Cellular Biology in 1992. From 1992 to 1995, he worked as a postdoctoral fellow in Michael Karin's lab in the University of California, San Diego Department of Pharmacology, 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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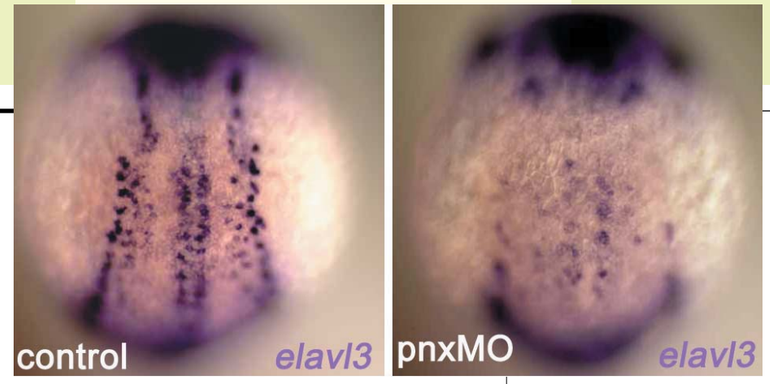
Body axis determination in zebrafish

In 1924, when Spemann and Mangold discovered the axis-organizing properties of a small section of the newt embryo located at the dorsal lip, it was the first demonstration that one group of cells can determine the developmental fates of neighboring uncommitted cells. This group of fate-determining cells, which came to be known as the Spemann organizer, is responsible for establishing the dorsal-ventral and anterior-posterior body axes in amphibians, and corresponding embryonic signaling centers have been shown to play similar roles in fish, birds and mammals. The discovery of the Spemann organizer raised questions that have challenged and intrigued developmental biologists ever since. The problems of how such organizing centers are formed, and how they function to steer the development of the body axes remain at the forefront of modern embryological research.

The research in Masahiko Hibi's lab seeks to address these problems, using the zebrafish as a model system for understanding dorsal organizer origins and function. A region of the zebrafish embryo called the 'embryonic shield' closely corresponds to the amphibian organizer. The high level of evolutionary conservation seen in the vertebrate organizer, combined with its amenability to genetic studies, its rapid embryological development, and extensive data regarding mutant phenotypes, makes the zebrafish an attractive platform for investigations into the genetic and molecular bases of organizer formation. Hibi focuses on the dynamic processes that take place in the first 24 hours of zebrafish development, a period in which the embryo grows and differentiates from a single cell into a recognizably vertebrate body organized along distinct axes.

Mutations in *ogon* lead to ventralization of the embryo.



Morpholino (MO)-mediated loss of *Pnx*, which contains an Eh1 repressor motif and a homeodomain, leads to reduction in primary neurons expressing *elavl3*.

Hibi focuses on the dynamic processes that take place in the first 24 hours of zebrafish development

Establishing the dorsal organizer

There is extensive evidence that an as-yet unknown dorsal determinant molecule in the vegetal pole of the very early zebrafish embryo is transported via the intracellular microtubule network to a dorsally located signaling center known as the Nieuwkoop center, which in turn produces signals that induce the dorsal organizer via interactions with the Wnt signaling pathway. Using both gain- and loss-of-function studies, Hibi has discovered a number of genes that participate in the induction of signaling centers and body axes, including *dharma/bozozok and ved.* His recent work has focused on determining the exact roles played by these genes in the signaling networks responsible for axis formation.

The *dharma/bozozok (dha/boz)* gene is known to play a central role in the establishment of the dorsal organizer in zebrafish, and is capable of inducing the organizer in a non-cell-autonomous manner. Hibi found that *dha/boz* is activated prior to gastrulation as part of a complex network of axis-determining factors that includes both the Wnt and BMP signaling pathways. However, several molecular steps in this proposed network remain unidentified, and the Hibi team is working to characterize the immediate and secondary downstream targets of the *dha/boz* gene product. One aspect that is becoming clear is that dha/boz works in concert with a group of ventrally-expressed proteins that inhibit other dorsalizing factors further downstream. The interplay between these dorsalizing and ventralizing factors is central to the formation of the anterior neuroectoderm, the developmental region that gives rise to the forebrain.

Posterior neurogenesis in the zebrafish

The nervous system is known to arise on the dorsal side of the vertebrate gastrula, but this system displays anterior-posterior (A-P) asymmetry as well. The fore-, mid- and hindbrain regions, and the spinal cord all form from precursive embryonic structures arranged along the A-P axis of the gastrula. In the Nieuwkoop model of neurogenesis, after a population of ectodermal cells is committed to a neural fate (the neuroectoderm), the population undergoes a patterning process in which the cells are further differentiated to form spatially distinct sub-populations that will develop into the various regions of the central nervous system, and, ultimately give rise to the primary and secondary neurons that comprise the nervous system proper. The Hibi research team has identified one gene, *pnx*, that is expressed in the posterior neuroectoderm, and regulates the formation of posterior neurons, such as those of the hindbrain and spinal cord. *pnx* encodes a homeodomain protein that mediates posteriorizing signals as part of a larger signaling network that functions in the patterning and later stages of neurogenesis.

The Hibi lab is also interested in the analysis of genes involved in ventralized zebrafish phenotypes. While there are many known dorsalized mutants in zebrafish, ventralized phenotypes are comparatively few. Hibi and colleagues are conducting analyses of one ventralizing gene, *ogon*, in the hopes of identifying its function in the dorsal-ventral axis signaling network. Hibi plans to continue to explore the formation and function of signaling centers in the zebrafish embryo, continuing on the genetic and molecular level the revolutionary work started by Spemann and Mangold nearly eighty years ago.

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Laboratory for Positional Information

Pattern Formation

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.

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Reaction-diffusion wave patterning

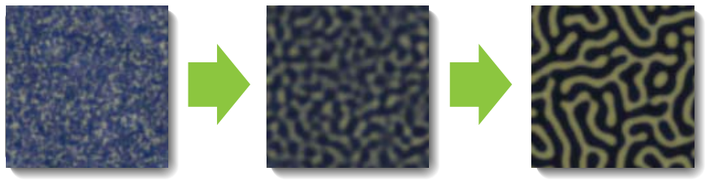
The natural world is filled with patterns that can be described by mathematical functions, such as the Fibonacci spiral of a nautilus shell, or the fractal arrangement of branches on some trees. In 1952, the British mathematician Alan Turing proposed a simple mathematical equation capable of generating a wide range of commonly observed patterns, such as stripes, spots and networks. 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. When the activator's diffusion rate exceeds that of the inhibitor, a 'moving wave' is formed, whereas a higher rate for the inhibitor results in a 'standing wave'. Both forms of wave can create periodic geometrical structures which exhibit the property of maintaining the size of the interval between structures as the overall pattern grows.

Shigeru Kondo 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. Research in the Kondo lab 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.

Zebrafish stripes and spots

As its name implies, the zebrafish is normally a striped animal. Similar stripes can be generated using the reaction-diffusion model, which inspired Kondo to seek the underlying biological mechanisms capable of fulfilling the model's mathematical conditions. Histological analysis of the zebrafish's pigmented regions revealed that the relative distributions of three types of pigmented bodies – melanophores, xanthophores and iridophores – near the surface of the skin corresponded to the overlying skin pattern. The existence of a spotted zebrafish leopard phenotype indicates that genetic factors are at work in determining the spatial distributions of the pigmented bodies; the reaction-diffusion model predicts that one or more activating and inhibiting factors interact to influence the formation of these pattern variants. His team is now engaged in a collaborative mutant screening search to identify the gene or genes responsible for maintaining regular stripes in these fish.

$$\frac{\partial u}{\partial t} = f(u,v) + D_u \nabla^2 u$$
$$\frac{\partial v}{\partial t} = g(u,v) + D_v \nabla^2 v$$



Autonomous formation of 2-D periodic pattern by Turing mechanism

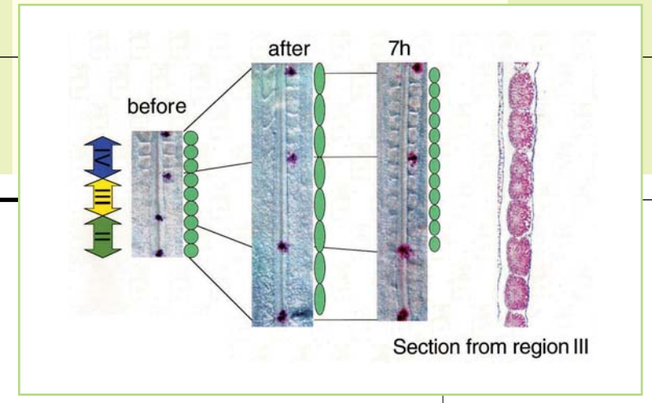
Stripe directionality

Angelfish present an unusual problem for the reaction-diffusion model, in that related species display patterns with similar striping, but in which the stripes are arranged in different directions. These fish, which are also unusual for the ability of females in male-less populations to change sex, develop stripes only in the process of acquiring male gender. In one species, the stripes are vertically arrayed, while in another closely related species, they form horizontally. While the stripes themselves conform to the predictions of the Turing equation, there is nothing inherent to the reaction diffusion model that would account for such directionality. While several mathematical explanations, such as boundary conditions or spatial gradients in reaction rate could conceivably produce differences in stripe direction, Kondo concluded that the developmental pattern suggested an inequality in distribution (anisotropy) was at work in the reaction-diffusion model. Comparisons of skin patterning in scaled and scaleless fish indicate that scale characteristics can cause marked changes in pattern formation, and computational modeling confirmed that even very small differences in a variable such as scale structure or arrangement could produce the changes in stripe direction observed in these fish. This work underscores the important point that minute perturbations in diffusion are capable of generating widespread and visible morphological effects.

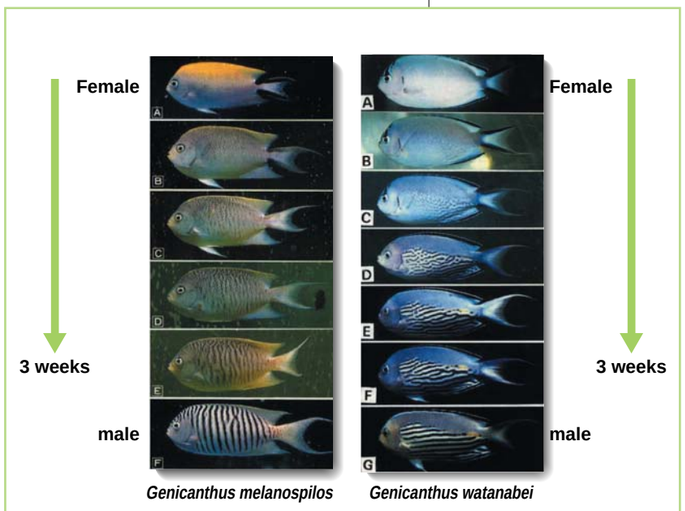
Minute perturbations in diffusion are capable of generating widespread and visible morphological effects

Periodicity in somitogenesis

The reaction-diffusion model is not only applicable to skin surface patterning, but to the formation of periodically repetitive internal structures as well. Using somitogenesis in the chick embryo as a model system, the Kondo team sought to demonstrate patterning consistent with the Turing model. In its embryonic development, the chick develops a regularly spaced series of bodies adjacent to the neural tube, called somites, which eventually give rise to the vertebrae, ribs and other structures. As regular periodicity is one of the features of Turing wave patterns, the Kondo team sought to determine whether somitogenesis could be explained by the reaction-diffusion model. Working with gastrulating embryos, the team stretched the region that somites arise from beyond its normal length, and found that somites in the elongated region divided to maintain their spacing, just as the model predicts. This experimental confirmation indicates that a balance between activating and inhibiting factors provides the basis for the pattern periodicity of somites, and the team is now working to identify the specific genes and proteins involved.



Regularly periodic formation of additional somites in extended chick presomitic mesoderm



Pattern alterations during sexual change in *Genicanthus*

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Laboratory for Evolutionary Morphology

Evolution of Structures

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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Evo-devo

Evolutionary developmental biology, sometimes referred to as 'evo-devo,' looks at the ways that changes in developmental genes and gene expression drive both the evolution of species and differences in morphology. Evolution has frequently been described as the result of the interplay between mutation and selection, but the advent of molecular developmental biology in the 1980s led to the increasingly widely accepted view that developmental constraints have a hand in shaping the directions, or 'trajectories,' that evolutionary processes are most likely to follow. This concept is perhaps best illustrated by the homeobox *(Hox)* genes, a developmentally important set of genes that shares a highly conserved domain (the 180-base-pair homeodomain) and plays a central role in regulating the morphological development of diverse organisms.

Combining experimental and analytic techniques from molecular biology, phylogenetics and comparative morphology, Shigeru Kuratani seeks to deepen the understanding of the part played by developmental biological mechanisms, such as the *Hox* genes, in the evolutionary divergence of species. His approach involves examining related genes in morphologically and phylogenetically distinct animals to uncover the ways in which context affects gene expression, and thereby influences body development. By comparing the molecular bases of evolutionarily novel structures, Kuratani hopes to illustrate the means by which developmental mechanisms mediate the translation of changes in the genome to changes in morphology.

Kuratani seeks to deepen the understanding of developmental biological mechanisms in the evolution of species

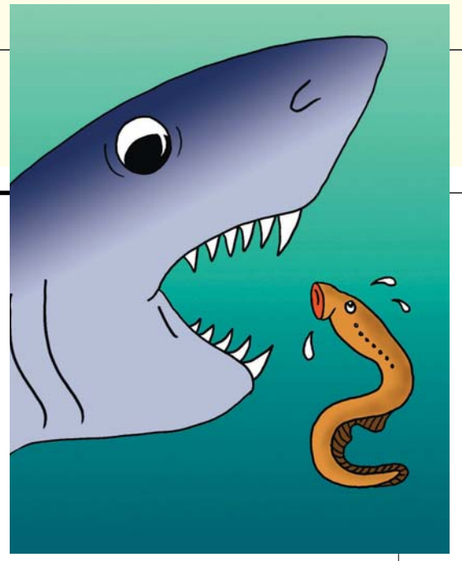
The turtle carapace

Evolution is generally considered to be a gradual process involving the accumulation of subtle changes over long spans of time. But marked changes occasionally do appear abruptly in the fossil record. This is the case for the advent of the turtle's shell, or 'carapace,' which appeared suddenly and with few recognizable precursors (although two now-extinct distant phylogenetic groups also evolved carapaces). Such a phenomenon would be difficult to explain by mutation and selection alone, as it is improbable that the genomic alterations necessary to produce such a dramatically new bodily structure would be achievable by the introduction of new genes in the short timeframe of the turtle's emergence. Evo-devo theory predicts instead that the genes responsible for carapace development actually belong to a set of genes shared by groups related to the turtle, such as lizards, dinosaurs and birds, but which function distinctly in the unique context of the turtle's molecular-genetic network. This would also explain the independent appearance of the carapace at other points in evolutionary history, as the underlying genetic elements are presumed to be conserved, making carapace formation one of the trajectories available to the process of morphological development.

The Kuratani lab is undertaking a systematic search to identify the genes and molecular mechanisms related to carapace development. This search involves differential screening of gene expression in chick and turtle. The chick is used as a basis for comparison, as birds are close evolutionary relatives of turtles, and share extremely similar genomes. By observing changes in gene expression in transgenic embryos, it is hoped that the developmental context that allowed the rapid emergence of the turtle's shell will become clear, and that the case for an evo-devo approach to morphological divergence will be strengthened.

Evolution of the jaw

The emergence of the jaw was an evolutionarily important step for gnathostomes (jawed vertebrates), as it conferred, among other things, the ability to grasp prey securely in the mouth, which was impossible for agnathan (jawless) species. However, the mouths of some agnathans, such as lampreys, feature upper and lower lips, which it was long believed were the evolutionary forerunners of the two-part, bony jaw. Kuratani tested this hypothesis by observing the expression patterns of the lamprey homologs of four genes known to be responsible for oral development in chicks. He found that, in the lamprey embryo, these genes, and the molecular cascades that their protein products participate in, do indeed serve to regulate the development of the mouth. However, interestingly, analysis showed that the lamprey mouth arises from different regions of the developing embryo than it does in the gnathostome chick. Thus, the vertebrate jaw and the lamprey mouth are morphologically somewhat similar and share certain gene expression patterns, but derive from distinct mesenchymal domains, which suggests that the molecular mechanisms common to oral development in these groups have been mobilized to form mouth structures at several discrete evolutionary moments, and not as part of an unbroken continuum. This nicely illustrates the evo-devo principle of exaptation, in which a structure or mechanism that originally evolved for one adaptive reason is co-opted as an autonomous adaptation in a different context. In experiments such as this, Kuratani hopes to bolster the already strong argument for the central role of molecular developmental biology in evolutionary processes.



The jaw conferred an evolutionary advantage

Ammocoete larvae of *Lampetra japonica*

Publications

Shigetani Y, Sugahara F, Kawakami Y, Murakami Y, Hirano S, and Kuratani S. Heterotopic shift of epithelial-mesenchymal interactions for vertebrate jaw evolution. *Science* 296:1319-21 (2002).

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Laboratory for Cell Migration

Cell Migration

Kiyoji Nishiwaki
Ph. D.

Kiyoji Nishiwaki received his B. Sc. from Osaka City University and M. Sc. from Osaka University where he worked on the molecular biology of the yeast *S. cerevisiae.* He joined NEC Corp. 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 development. He was awarded a Ph. D. by Osaka City University for work on the molecular analysis of *C. elegans* embryogenesis in 1994. He remained at NEC until receiving an appointment as team leader at the RIKEN CDB.

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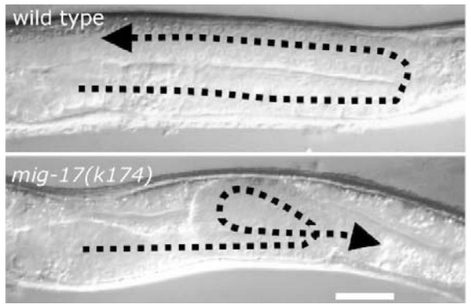


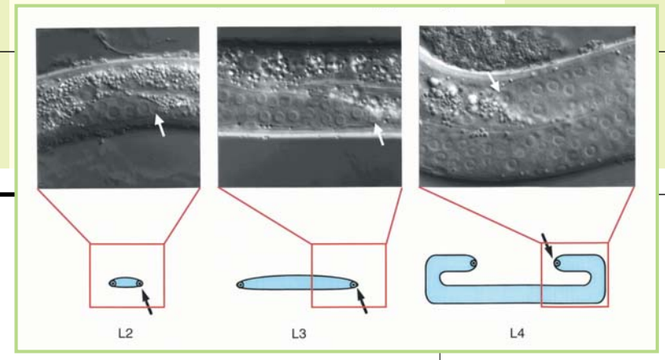
Distal tip cell migration. The path of the migrating DTC is shown by the dotted line.

Cell migration and organogenesis

In animal development, cell division, cell migration and changes in cell shape are coordinated to generate complex systems of tissue and organs. One aspect of this highly orchestrated process is the movement of cells in sheets, an activity orchestrated by mechanisms which remain poorly understood. Kiyoji Nishiwaki's research team studies gonadogenesis (the formation of reproductive organs) in the nematode *C. elegans* as a model system to improve the understanding of the molecular mechanisms that underlie cell migration during organogenesis. By developing a clearer picture of the mechanisms held in common across species, it is hoped that this research will lead to insights into cell migration and organ development in humans, and contribute to the understanding of human diseases involving disturbances in coordinated cellular movement.

Genetic control of gonad development

C. elegans has been a favorite model for geneticists and developmental biologists since its introduction by Sydney Brenner in the 1970s, as the lineage of each of the 959 cells can be traced precisely, its short reproductive cycle makes it amenable to breeding for genetic experiments, and its transparent body allows for direct observation of individual cells under a light microscope. In the nematode, the gonad develops through four larval stages (L1-L4) during which time the gonad-forming cells divide and move through the body cavity to form symmetrical gonad arms originating in a primordium on the ventral side of the body and terminating dorsally. The migratory path of the emerging gonad is determined by a pair of distal tip cells, one located at each end of the gonad primordium, but the mechanisms by which these cells initiate, steer and end migration at the correct stages in larval development are largely unknown.



Development of *C. elegans* gonad

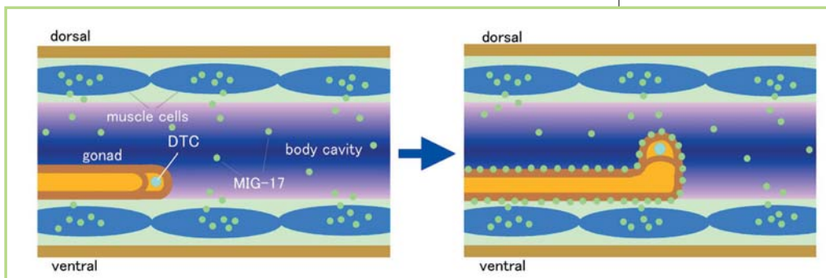
Nishiwaki sees the interactions between the basement membrane of the migrating gonad and that of the body wall as a key to the process of cell movement, and his team has conducted a large-scale screen for phenotypes that demonstrate distal tip cell migratory aberrance. One gene uncovered in this mutant hunt, *mig-17* (the *mig* prefix indicates a mutant phenotype with a migratory defect), has been found to encode a member of the ADAM (A Disintegrin And Metalloprotease) family of secreted proteins. Tracking studies using molecules genetically tagged with green fluorescent protein revealed that the *mig-17* product, MIG-17, is secreted into the body cavity by muscle cells in the body wall and binds to the gonadal basement membrane at the developmental stage when the distal tip cells make their initial turn dorsal-ward. The research team is now pursuing studies of other genes associated with migratory defects, with a particular focus on phenotypes in which the migratory mechanism is disturbed, but not abolished. These mutations allow for the tracking of molecular action and cell behavior throughout the migratory process, and to identify developmental checkpoints at which the distal tip cell's guidance mechanisms may go awry. Preliminary studies indicate that proteins associated with alterations in the glycosylation of MIG-17 or in its secretion may form a matrix that directs the localization of MIG-17 to the migrating gonad.

Protein interactions with MIG-17

To gain deeper insight into the role of cell migration in nematode gonadogenesis, Nishiwaki is interested in clarifying the network of proteins that interact with MIG-17. The team searches for mutations in mapping experiments designed to locate candidate genes. These mutations have been found to be capable of rescuing the meandering distal tip cell phenotype and restoring normal gonadogenesis, even in the complete absence of MIG-17. The biochemical analysis of these candidates is still ongoing, and it is hoped that the results of these efforts will provide better knowledge of the MIG-17 metalloprotease and the identification of molecules that directly interact with MIG-17.

Nishiwaki is interested in clarifying the network of proteins that interact with MIG-17

Nishiwaki's work to date has revealed that the basement membrane plays a far larger role in organogenesis than that of simple structural support. As his functional genetic studies of *C. elegans* have shown, basement membranes are integral in the control of cell migration as well. Judging from the high degree of homology with higher organisms, such as the mouse, these migratory mechanisms seem to be evolutionarily well-conserved. By focusing on the movements of a small group of cells in a tiny worm, Nishiwaki seeks to push back the frontiers of knowledge of how cells in the bodies of animals across species are able to get to where they need to go.



MIG-17 is produced in and secreted from muscle cells and localized on the gonad surface after the first turn of the DTC

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Suzuki N, Buechner M, Nishiwaki K, Hall DH, Nakanishi H, Takai Y, Hisamoto N, and Matsumoto K. A putative GDP-GTP exchange factor is required for development of the excretory cell in *C.elegans*. *EMBO Rep* 21:530-5 (2001).

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Laboratory for 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.

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Self-renewal and pluripotency

With their ability to self-renew indefinitely and to differentiate into cells of all three germ layer types (a differentiative capacity called pluripotency), embryonic stem (ES) cells represent one of the great prospects for regenerative medical applications, as well as an attractive platform for the study of a diverse spectrum of developmental processes. However, the mechanisms by which ES cells are able to maintain these functions are incompletely known, and a better understanding of the 'stemness' of these cells will be necessary in order to take optimal advantage of their remarkable properties. Two of the biggest challenges facing stem cell research are determining the factors that allow ES cells to generate limitlessly self-renewable progeny, and identifying molecules that direct the dividing ES cell to produce daughter cells with specific fates. Beginning with his work in identifying Oct-3/4 as a transcription factor important in ES cell maintenance, Hitoshi Niwa's research has addressed both of these challenges.

A better understanding of the 'stemness' of pluripotent cells will be necessary in order to take optimal advantage of their remarkable properties

Maintenance of pluripotency

The POU-family transcriptional regulator Oct-3/4 was the first factor found to elicit multiple differentiative outcomes dependent on its expression level. ES cells expressing normal levels of Oct-3/4 maintained their pluripotency, while its overexpression resulted in differentiation into primitive endoderm and mesoderm, and its inhibition caused the cells to take up a trophectodermal (the layer of extraembryonic cells that serves as the source of the placenta) fate. This established Oct-3/4 as a primary regulator of pluripotency in ES cells, but gave rise to new questions regarding the biochemically active domain in the Oct-3/4 protein. In work published in the spring of 2002, the Niwa lab showed that the function of Oct-3/4 in ES cell propagation depends on the combined presence of specific POU and transactivation domains. By refining the understanding of the molecular characteristics involved in the Oct-3/4-based ES cell maintenance in this way, it may be possible to pinpoint important target genes and thereby elucidate the regulatory pathways at work in the preservation of pluripotency.

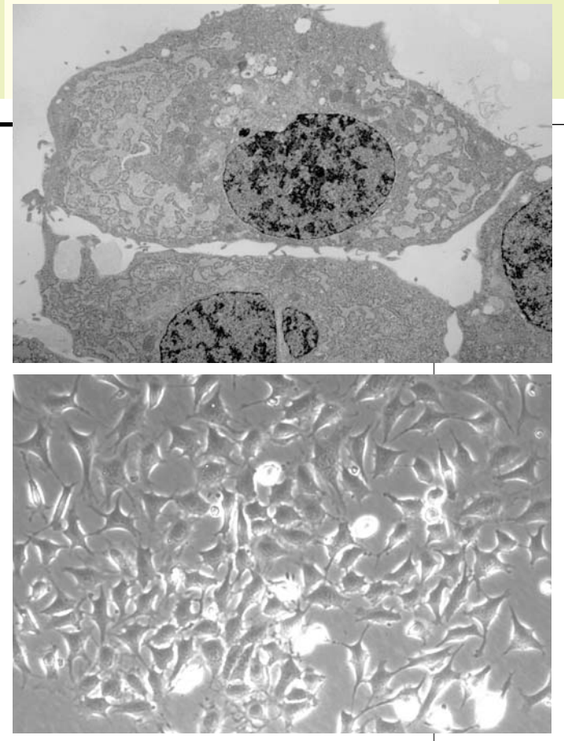
One aspect of this network that holds Niwa's interest is the identification of factors that interact with Oct-3/4 to govern ES cell fate. The tight regulation of Oct-3/4 is required to maintain pluripotency in ES cells, suggesting that a finely tuned feedback mechanism is at work in keeping Oct-3/4 transcription levels within the appropriate range. The resolution of the elements of this hypothetical feedback loops remains the subject of ongoing research and several candidate components have been identified.

Inducing differentiation

A second field of central importance to the stem cell research community is the development of methods by which undifferentiated ES cells can be prompted to commit to a specific cell lineage. Studying factors identified in published work on knockout mice, the Niwa research team analyzed transcription factors with potential roles in the development of extraembryonic and primitive endoderm lineages. (Primitive endoderm derivatives include the parietal and visceral endoderm, the extraembryonic regions that give rise to the yolk sac endoderm.) Two factors in particular, Gata-4 and Gata-6, known to be expressed in cells from these lineages, were studied to test whether their expression was sufficient to promote the differentiation of ES cells. Overexpression of the factors resulted in the specific conversion of undifferentiated ES cells into endodermal cells, and it was found that the induced expression of either of these GATA factors resulted in the expression of the other as well. This result was the first successful attempt to induce differentiation of ES cells by the misexpression of tissue-specific transcription factors, an important step toward the experimentally and clinically significant goal of controlling the differentiation of ES cells in culture.

ES cell growth in culture

This goal of controlling culture conditions to achieve specific outcomes is important to the growth of ES cells *in vitro* as well. The Niwa lab is working to develop a serum- and feeder-cell–free system for culturing mouse ES cells, which necessitates developing a detailed picture of both the extrinsic factors and the intrinsic networks that function in these cells in culture. It is known that a single ES cell in isolation will fail to proliferate, while colonies of such cells grow normally. It is also known that even isolated single cells can be induced to proliferate if the culture medium from a larger ES cell colony is transferred to the single cell's plate, suggesting that ES cells produce a growth-stimulating factor that acts by community effect. Niwa has developed an assay system to isolate candidate molecules for this hypothetical 'stem cell autocrine factor' (SAF), and is actively pursuing the characterization of the most promising candidates. The results of these analyses should improve the ability of researchers to grow ES cells in culture, facilitating the study of these fascinating and potentially revolutionarily important cells.



Parietal endoderm cells induced by Gata-6 overexpression in ES cells

Publications

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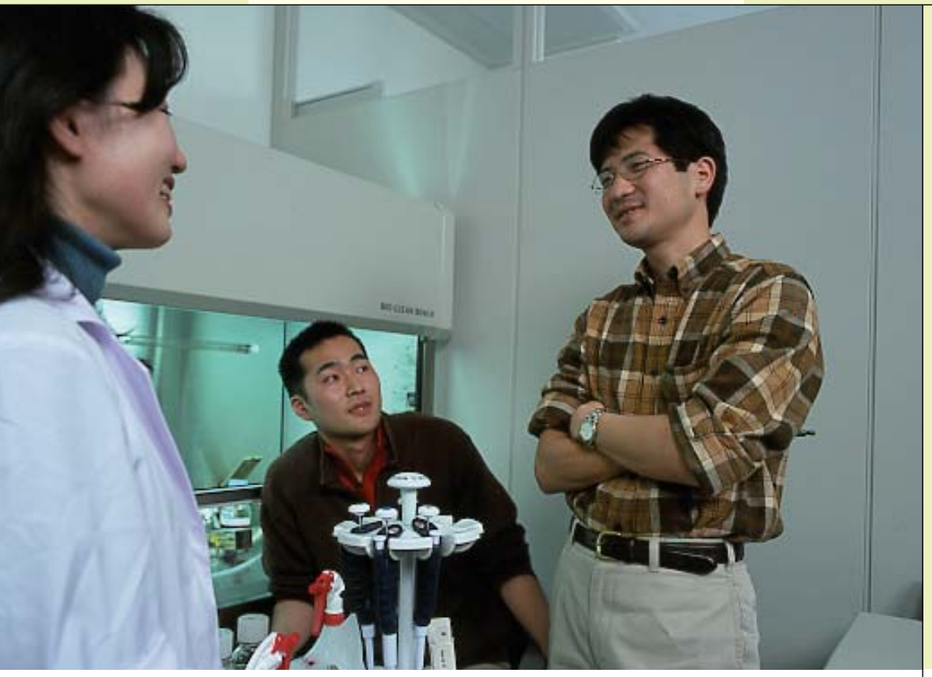
Laboratory for Mammalian Epigenetic Studies

Epigenetics



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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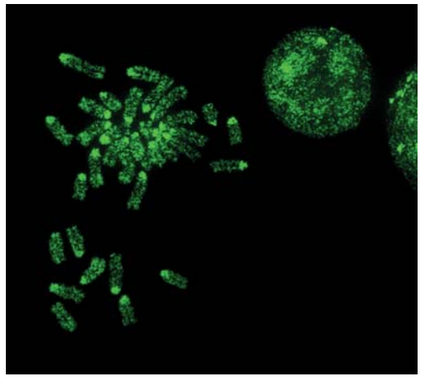
Akiko Tsumura



Distribution of methylcytosine in metaphase chromosomes of ES cells

Epigenetic instructions

One of the great questions remaining in the life sciences concerns how it is possible for the body's many cell types to express only the limited set of genes required for the performance of their individual functions when nearly every one of these cells is genetically identical. While a complete answer remains to be found, it is already evident that additions need to be made to the classical model of gene expression to account for a number of 'epigenetic' processes, such as X chromosome inactivation, parental imprinting, position effect variegation and gene silencing, which do not depend on DNA sequence. The concept of epigenetically determined differential gene expression can be likened to a corporation with a single comprehensive manual that provides instructions to every department, but which features a system whereby locally irrelevant sections of the manual can be labeled, bound up, and filed away unread. In this analogy, DNA methylation can be seen as one of the means by which unnecessary sets of DNA instructions are labeled and bound. Methylation targets cytosine bases in the genetic code, and genes methylated in this way are generally rendered inactive, or 'silenced.' Masaki Okano's research concentrates on the mechanisms by which DNA methylation is established and maintained throughout development.

De novo methylation

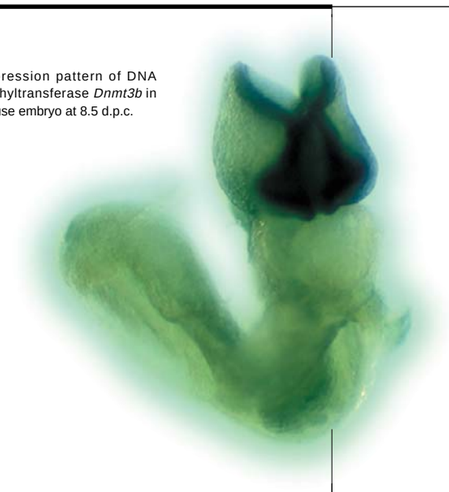
Methylation lays down developmentally significant gene expression patterns that are difficult to alter once established. This is important for the maintenance of consistent cellular function, but it is equally important for new individuals to have the ability to reset their methylation patterns. To use the corporate analogy, there needs to be a way for new companies spun off from a parent to remove old labels and bindings and to establish their own distinct sets of instructions. This process of establishing new instructions is called *de novo* methylation, and it seems to play a crucial role in the early development of many animals, including humans.

This process of establishing new epigenetic instructions is known as *de novo* methylation

In previous work, Okano showed that *de novo* methylation in mice required the expression of a pair of methyltransferase genes, *Dnmt3a* and *Dnmt3b*, at a very early stage of embryonic development, as well during gametogenesis. Prior to this finding, it was believed that a related gene, *Dnmt1*, was solely responsible for the methylation of DNA. Using *lacZ* reporter gene studies, Okano showed that these newly identified genes are highly expressed in germline (*Dnmt3a*), ES (*Dnmt3a* and *Dnmt3b*) and progenitor (*Dnmt3b*) cells, suggesting that they function in the epigenetic reprogramming of DNA methylation states. Based on these findings, it is now believed that *Dnmt1* functions primarily in methylation maintenance, which is necessary to the stable inheritance of tissue-specific methylation patterns, but that *Dnmt3a* and *Dnmt3b* are the principal determinants in initiating DNA methylation. Knockout mutations of *Dnmt3a* and *Dnmt3b* result in a variety of organic, neurological and spermatic defects, which indicates that the establishment of the DNA methylation state plays an important part in many aspects of development.

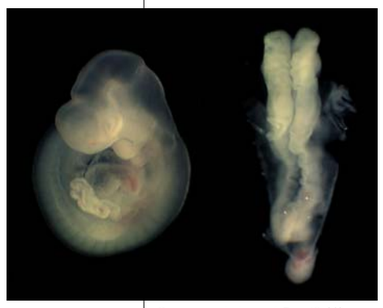
DNA methylation in maternal imprinting

In more recent work published in 2002, Okano and colleagues at the Cardiovascular Research Center at Massachusetts General Hospital demonstrated a new role for the *Dnmt3* family of genes, which they found to be essential to the process of maternal imprinting. In the mouse, the paternal and maternal genomes both contain small numbers of differentially expressed genes known as imprinted genes. The expression of such genes is determined by whether they are inherited from the mother or father, and they seem to function in a number of developmental processes. It is thought that DNA methylation is the epigenetic mechanism that governs genomic imprinting, and nearly all imprinted genes contain regions that are methylated differentially in maternal and paternal alleles, although it remains a mystery as to how methyltransferases such as the *Dnmt3* family recognize the sequences of imprinted genes. In a series of ovary transplantation studies, Okano showed that *Dnmt3a* and *Dnmt3b* function in the methylation of maternally imprinted genes, while colleagues demonstrated that a third gene, *Dnmt3L*, also participates in the same process. Although *Dnmt3L* shows no direct methyltransferase activity, it is thought that it may help to regulate maternal imprinting by interacting with the known methyltransferases *Dnmt3a* and *Dnmt3b*, with which it binds and colocalizes.



Expression pattern of DNA methyltransferase *Dnmt3b* in mouse embryo at 8.5 d.p.c.

In the future, the Okano lab will study how epigenetic reprogramming works at a molecular level. By identifying the genes involved in *de novo* DNA methylation, they hope to discover the means by which it is determined what genes will be methylated. Comparisons of the expression patterns of such genes across germ layers, or in normal mice against clones created from somatically derived cells should lead to new insights in the context-specificity of the DNA methylation process.



Gross morphology of *Dnmt3a*$^{(-/-)}$ *Dnmt3b*$^{(-/-)}$ mouse embryo at 9.5 d.p.c.

Publications

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Laboratory for Cell Fate Decision

Asymmetric Cell Division

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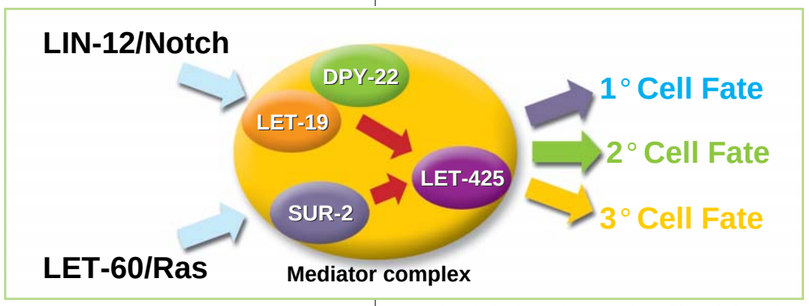
Hitoshi Sawa
Ph. D.

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.

Asymmetric cell division and cell diversity

Our bodies are made up of the myriad and multiform descendants of a single fertilized egg. The process of asymmetric cell division, in which a single mother cell splits to generate daughter cells with discrete characters, is widely accepted as the basis of cellular diversity but due to the difficulties associated with tracking the lineages produced by any single cell, the mechanisms by which this is achieved remain imperfectly understood. Utilizing the nematode *C. elegans* as a model, Hitoshi Sawa is engaged in the study of the molecular interactions that underlie this process. He seeks also to resolve the ways in which transcription-regulating complexes integrate the intracellular signaling networks that collaborate to help determine cell fate.



Ras and Notch signals set primary to tertiary cell fates via the Mediator complex in vulval development

Regulation of cell polarity

The nematode offers a number of advantages for use as an experimental model, including its rapid reproductive cycle, the complete accounting of its cell lineages, its amenability to growth in culture and genetic manipulation, and the ease with which individual cells in its transparent body can be observed *in vivo* under a low-power microscope. The Sawa research team studies the asymmetric division of *C. elegans* T cells, which give rise to pairs of daughter cells, one of which generates epithelial cells, while the other produces neural cell types. The Sawa research team uses molecular genetic techniques to probe the molecular mechanisms involved in cell fate specification. By cloning the gene *lin-17* (*lin* signifying lineage-defective) from a mutant strain exhibiting aberrant asymmetric cell division and analyzing its sequence, Sawa determined that *lin-17* is homologous to the *frizzled* gene in the fruit fly *Drosophila*, which is believed to serve as a receptor of Wnt-family ligands. Other experiments in which the Wnt-family protein LIN-44 was misexpressed ectopically resulted in the reversal of T cell lineage polarity, indicating that the Wnt/Frizzled signaling pathway is important in regulating the direction of polarization as occurs in the process of asymmetric mitosis and adding to its wide and well-characterized array of functions in such fundamental processes as cell proliferation and differentiation.

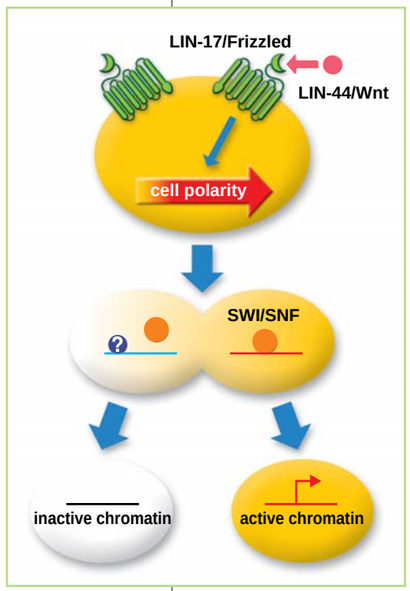
The Sawa team has also identified nearly forty genes in a *C. elegans* mutant screen

The Sawa team has also identified nearly forty genes from a screen of mutants in which both daughters of the T cells were found to produce only epithelial cells, rather than epithelial and neural cells. Of that group, the two genes *psa-1* and *psa-4* (*psa* for Phasmid Socket Absent) were found to encode proteins homologous to yeast SWI3 and SWI2/SNF2, respectively. These proteins are components of the SWI/SNF transcription regulatory complex, which is known to be involved in asymmetric cell division in yeast, a single-celled organism that nonetheless exhibits asymmetric mitosis. These results provide compelling evidence that conserved mechanisms may be at work in the asymmetric division of both one- and multi-celled organisms.

Integration of transcription-regulating networks

A second area of focus in Sawa's work is unraveling the ways in which complex intracellular signaling networks are integrated in the determination of cell fate, using vulva development in *C. elegans* as a model system. The nematode exists either as a male or a hermaphrodite animal. The hermaphrodite vulva is a small channel located near the midpoint of the ventral side, and functions both to receive sperm and release fertilized eggs during reproduction. This extensively-studied organ comprises only 22 cells, and its development is known to be mediated by both the Ras and Notch signal transduction pathways. Sawa is analyzing vulval mutants to gain a more detailed understanding of how these pathways function in the formation of this organ, a process that involves a rigidly stereotyped pattern of mitotic divisions. Such stereotyped cell division patterns are characteristic of nematode development, and are one of its most attractive features as a model system, allowing researchers to pinpoint phenotypic deviations at the level of the single cell. One result of this analysis of vulval mutants has been the identification of LET-19/TRAP240, components of a transcription-regulating complex Mediator, which operates downstream of Notch. The Mediator complex also incorporates another component, SUR-2, which functions downstream in the Ras pathway, strongly suggesting that that this complex plays a role in integrating the Notch and Ras pathways in vulva development.

Looking ahead, the Sawa research team intends to identify SWI/SNF activators and target genes, and to deepen further the understanding of the molecular mechanisms at work in asymmetric cells in the nematode, in the hopes of developing insights with application to research in mammalian cell asymmetry. He also plans to continue his research into the integration of signals in the process of cell fate determination.



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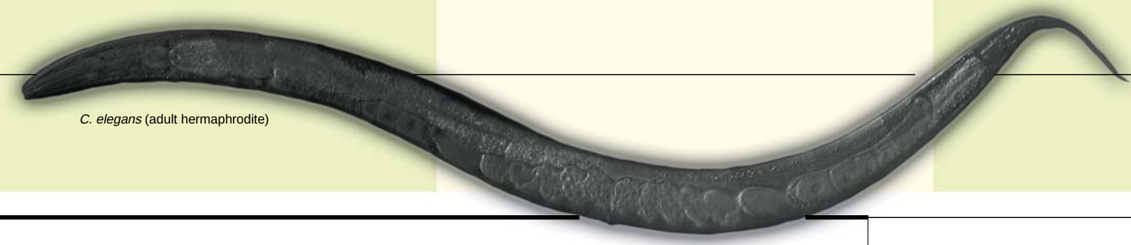
Laboratory for Developmental Genomics

Functional Genomics



C. elegans (adult hermaphrodite)

Asako Sugimoto
Ph. D.

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

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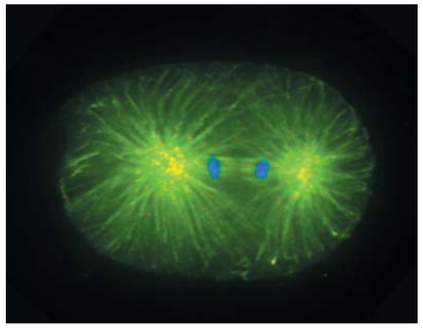


One-cell stage embryo. (Blue: chromosomes, Green: microtubules, Red: Protein Phosphatase 4)

The gene network

As recent findings of the limited numbers of genes in many genomes, including our own, have indicated, the interactions between genes at the network level seem increasingly significant in determining the intricate development and function of multicellular organisms. While the characterization of genomes at the level of the individual gene remains an important challenge, an analysis of how genes function in networks is now in equal demand. This represents a daunting task in highly evolved and complex species such as *Homo sapiens*, prompting scientists to seek more primitive and simpler models to gain fundamental insights in the hopes that a significant number of basic mechanisms are shared by many branches of the phylogenetic tree.

Asako Sugimoto has adopted as a model the nematode *C. elegans* for its tractability to the systematic functional analysis of its genome using unique high-throughput screening techniques. By studying the functional interaction of genes within *C. elegans*, whose genome has been completely sequenced, the Sugimoto research team seeks to identify the means by which genes working in combination help to establish and direct developmental processes. The lab also looks to take the findings from these studies as a base for advancing the understanding of developmentally important mechanisms such as programmed cell death (apoptosis) and microtubule dynamics in mitosis. Sugimoto hopes that by opening windows into the role of networked genes in guiding development in a simple worm, new light will be shed on universal mechanisms in the genetic regulation of the developmental program.

Sugimoto hopes to shed new light on universal mechanisms in the genetic regulation of the developmental program.

RNAi-based profiling of gene function

The wild-type nematode is made up of less than 1,000 cells, yet this simple organism exhibits a wide range of the specialized cell types, such as muscles and nerves, that characterize more highly evolved species. And, thanks to the complete knowledge of the lineage of every cell in the *C. elegans* body, the differentiative fate of every one of those cells can be traced from its origin in the fertilized egg to its role in the fully-grown adult. The amenability of this worm's sequenced genome to reverse-genetic techniques has also served to make it one of the preferred model organisms in the world of genetics research. The discovery in the late 1990s that the introduction of double stranded RNA (dsRNA) could be used to 'knock down' the expression of specific genes in the nematode has only added to its appeal, giving scientists the ability to inhibit gene function without disturbing its underlying DNA. Sugimoto has refined this technique of RNA interference (RNAi) by developing a method in which nematodes directly uptake dsRNA in solution. This process of 'RNAi by soaking' offers greater efficiency and ease-of-use than other RNAi methods and has made it possible to conduct systematic high-throughput studies of gene suppression more rapidly than ever before, achieving knock down analysis rates of up to 800 genes per month.

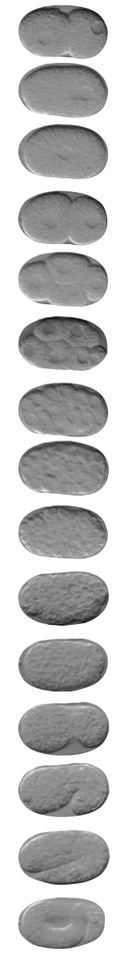
Starting with a cDNA library of approximately 10,000 genes expressed in developmental processes, the Sugimoto lab used RNAi to knock down each gene's function and built a database in which the resulting phenotypes were sorted by developmental outcome. To date, more than 4,000 phenotypes have been categorized, and it was found that loss of function in more than 25% of these genes resulted in lethal, morphologically altered or sterile phenotypes. Such developmentally important genes also seem to appear less frequently on the sex chromosome. The *C. elegans* male has only a single such chromosome, and selective pressure in the process of evolution may have determined that the most functionally important genes are located on chromosomes where the chances of a complete loss of function are reduced.

In addition to further phenotypic characterization and categorization of the embryonic lethal genes, Sugimoto next studied their post-embryonic functions using a conditional knockdown strategy. Approximately 60% of the genes studied played post-embryonic roles as well, indicating that many genes play multiple roles at different stages in development. It is anticipated that this comprehensive phenotype profiling will lead to the identification of gene networks that play crucial roles in developmental processes.

Programmed cell death

In addition to their work on system-level analysis of gene networks, the Sugimoto team is also engaged in detailed analysis of specific cellular functions, such as programmed cell death, in which cells self-destruct as part of the normal developmental process. Programmed cell death has been extensively studied in *C. elegans*, and it is known that exactly 131 cells undergo this programmed cell death in normal nematode development. It has been shown that a single genetic cascade is responsible for inducing cell death in *C. elegans*, and that this cascade has been evolutionarily conserved. By seeking to identify the molecular events that control the death of cells during the development of the nematode, Sugimoto hopes to uncover the regulatory foundations of this important mechanism.

The Sugimoto team is also engaged in investigating the molecular mechanisms underlying the formation and movement of mitotic spindles, and has identified a gene involved in spindle formation. Future research will employ live imaging of microtubule and centrosomes using GFP-tagged proteins in combination of RNAi knockdown analysis to provide a clearer picture of the role of specific genes in this process.



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Laboratory for Body Patterning

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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Pattern formation in the early embryo

Pattern formation, the process by which the boundaries that define germ layers and tissue types are established, occurs at highly specific sites and developmental stages in the early embryo. This closely coordinated regulation prevents cells from differentiating inappropriately, and enables the establishment of complex and specialized tissues and organs in the adult body. One type of pattern formation involves the regimented formation of periodically alternating bands of differentiated cells along the anterior-posterior body axis. The results of this process of segmentation can be observed in the orderly structures of the vertebrae and spinal ganglia. In vertebrates, segmentation takes place in the embryonic region known as the 'somitic mesoderm,' and involves the transient formation of segment organizing bodies called somites. Yoshiko Takahashi studies segmentation and somitogenesis in the chick embryo, and has made inroads into achieving a better knowledge of how groups of cells are able to cleave at specific boundary lines and re-organize into periodically segmented patterns. Investigations at the Takahashi lab seek to explain at the molecular level the mechanisms of pattern formation in development.

The segmenter and Notch signaling

In the early chick embryo, two bands of unsegmented mesoderm, collectively referred to as the 'segmental plate,' flank the neural tube, which runs along the anterior-posterior axis. Somites arise one by one from this segmental plate in a head-down direction. In this sense, if the segmentation of invertebrates such as the fruit fly can be described as an 'egg-slicer' effect in which all cuts are made simultaneously, then segmentation in vertebrates might be likened to a 'meat-slicer,' in which cuts are made sequentially, one after another.

In experiments designed to elucidate the molecular mechanisms that drive this vertebrate meat-slicer, Takahashi made sectional transplantations and locally targeted expression of various genes, the results of which led her to propose the existence of a region responsible for inducing segmentation, which she has named the 'segmenter.' Transplantation of this segmenter into the undifferentiated segmental plate triggers ectopic boundary formation, or segmentation. The segmenter region also specifically expresses a factor that is believed to regulate Notch signaling, a pathway which has been extensively studied for its diverse roles in the determination of cell fate and differentiation. Moreover, Takahashi found it was possible to mimic the effects of segmenter transplantation by modulating the expression of activated Notch in specific unsegmented mesodermal regions, suggesting a new function for the Notch pathway in boundary formation during mesodermal segmentation.

Epithelialization

After the segmenter has established the boundaries of a somite, the mesenchymal cells in the border region change to become epithelial cells. The reverse process, in which epithelial cells assume a mesenchymal fate, is also observed during development. The Takahashi lab would like to clarify the molecular mechanisms that regulate this mesenchymal-epithelial transition, and are concentrating in on the roles played by members of the Rho family of proteins in this process. Both the inhibition of Rac1 and the overexpression of Cdc42 were found to induce the conversion of epithelial cells to mesenchyme, while interference with Cdc42 function caused mesenchymal cells to take on epithelial characteristics. These suggestive findings led Takahashi to propose that the mesenchymal-epithelial transition is determined by relative Rac1 and Cdc42 activity levels, with higher levels of Rac1 inducing an epithelial fate, while higher levels of Cdc42 tip the balance in the mesenchymal direction.

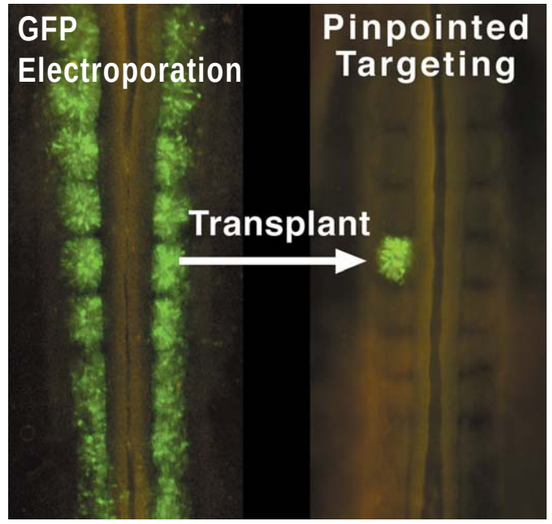
The simple egg develops into a marvelously complex organism

You can't tell an embryo what to do, only listen to what it can tell you

Periodicity

The fact that, despite the marked differences in the developmental mechanisms at work in invertebrates and vertebrates, organisms as diverse as the *Drosophila* fly and the chick exhibit patterns with similarly regular temporal and spatial intervals intrigues Takahashi. Her lab has been participating in collaborative research to determine whether the establishment and maintenance of such intervals can be explained by the reaction-diffusion model (see pp. 28-9).

The details of her current work aside, Takahashi emphasizes that what drew her to science and maintains her interest is the opportunities it offers to those willing and able to learn from natural phenomena. "You can't tell an embryo what to do, only listen to what it can tell you." But her range of interests is not limited to any single field; she prefers to pursue avenues that others may have overlooked. She studies molecules and genes for what they can teach her about somites, and somites for what they can teach her about pattern formation. And pattern formation, of course, has many lessons for those who wish to understand the bases of development itself.



Electroporation used in combination with classical explant techniques allows for pinpoint gene expression in target tissues

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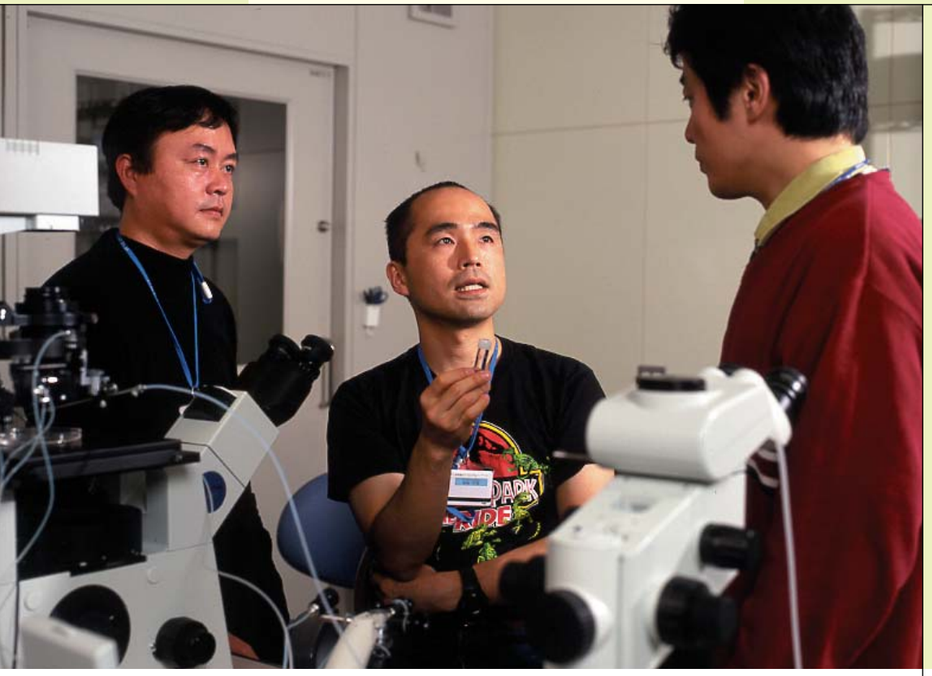
Laboratory for Genomic Reprogramming



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 three 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 Rockefeller University as a research assistant professor in 1999. He spent a year as a researcher at Advanced Cell Technology before returning to Japan to take his current position at the RIKEN CDB.

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Fertilization

In most animals, the sperm and the egg (collectively known as gametes) are highly specialized cells that enable the organism to reproduce in a way that commingles the genetic information from both partners in a sexual pairing. This can only be achieved by the fusion of gametes in the process known as fertilization. Under natural conditions, fertilization involves a complex chain of events and interactions, which in mammals begins with the entry of the sperm into the female reproductive tract. The environment within the uterus and oviduct produces changes in the sperm that allow it to dock with the outer surface of the unfertilized egg, disintegrate, and deliver its genetic contents. Penetration by the sperm also induces dramatic effects in the egg, including biochemical and structural reactions that prevent other sperm from entering, the transition from meiotic to mitotic cell division, and a series of intracellular changes that represents the first steps toward the construction of a new individual. Seen from this perspective, the fertilization of the egg is not merely the event by which two haploid genomes merge, but also the initiation of the entire developmental cascade.

Nuclear transfer by microinjection

Although he is perhaps best known for his pioneering work in the cloning of mice, Teruhiko Wakayama has dedicated his research to exploring the mysteries of fertilization. By observing the effects of varying experimental conditions on the interaction between the egg (or oocyte) and extrinsic genetic matter, Wakayama's research team seeks to understand the fundamental basis by which the fertilized egg is 'reprogrammed' from a highly differentiated state to become the totipotent progenitor of the myriad cell types in the adult body. Wakayama also strives to develop new laboratory techniques to make the difficult processes of manipulating eggs simpler and more efficient.

The fertilization of the egg is the initiation of the entire developmental cascade

Freeze-dried sperm

Sperm preserved for use in experiments and *in vitro* fertilization is traditionally frozen in liquid nitrogen at extremely low temperatures (around -190˚C). This cryopreservation allows the sperm to be maintained viably for very long periods, but requires expensive facilities and some degree of technical skill in handling. In the 1990s, Wakayama developed a method by which sperm could be freeze-dried and remain viable under low-cost, low-maintenance conditions. Sperm freeze-dried in such a way were later used in ICSI fertilization experiments, and although they were technically 'dead,' they demonstrated an effective 'fertilization shelf-life' of around thirty days when stored at room temperature and considerably longer when refrigerated at 4˚C. Wakayama continues to be interested in developing more efficient and economical techniques for sperm storage, and hopes to improve the storable duration and viability rates for sperm preserved in this manner.

Egg alterations

Wakayama is also interested in whether a partial egg is sufficient to produce a normally developed individual when fertilized. In a series of experiments in which he sequentially reduced the oocyte mass by factors of two and attempted sperm injection, he found that eggs of one-half normal size were competent to produce normal individuals, indicating a certain degree of functional redundancy in oocyte composition. In similar work, he investigated the competency of polar bodies (small satellite cells produced during the meiotic division of the egg) to produce offspring when fertilized, and found that these cells, too, are capable of developing into live-born, normal mice. Previously, it had been thought that polar bodies might be repositories for damaged DNA and, so, unlikely to develop normally if fertilized. The viability of polar bodies as surrogate oocytes has implications for the field of human *in vitro* fertilization, in which the supply of eggs is frequently a limiting factor.

Cloning

For many people, the concept of cloning conjures images of indistinguishably identical replicas of an individual. But Wakayama notes, "Clones are by no means perfect copies of the original." Experimentally cloned animals exhibit a range of embryonic and adult epigenetic defects. The process of cloning does, however, provide an excellent system for studying the mechanisms of fertilization, as it allows the specific control of factors such as nuclear source. In cloning experiments using nuclei from different sources, such as cumulus cells, somatic cells and embryonic stem cells, Wakayama has observed variations in patterns of live-birth rates and developmental abnormalities, which he hopes will fuel further investigations at the molecular level. In the future, he plans to continue to explore the secrets of the fertilized egg at the cellular level. In his own words, "Eggs are very beautiful and mysterious. I never get tired of watching them."

Wakame, the first mouse cloned at the RIKEN CDB

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Induction

Laboratory for Embryonic Induction

Signaling centers in mammalian development

The early mouse embryo is characterized by its dynamic transformation from a simple, nearly featureless cylinder of cells to a recognizable embryonic body in the space of a few days during gastrulation. This remarkably rapid transition is achieved by the highly coordinated differentiation of cells toward specific fates, a feat orchestrated by a number of cellular loci, collectively known as 'signaling centers.' These centers produce diffusible proteins that serve as regulators, guiding neighboring cell communities to take up the roles they will play in the adult animal, or steering them away from inappropriate fates.

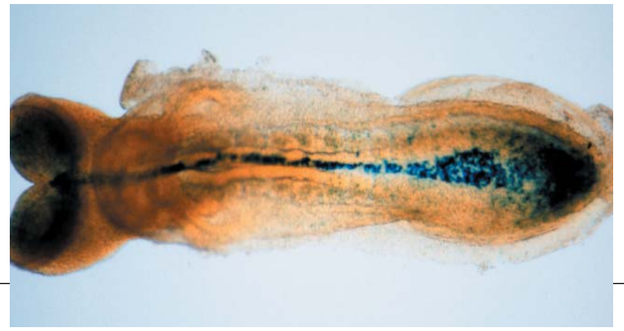
Hiroshi Sasaki's research team investigates the network of signaling centers that work to induce normal development in mammals. The node, the center responsible for induction of body parts in the mouse, is of particular interest, as it plays a role corresponding to the amphibian Spemann organizer, which first sparked Sasaki's fascination with signaling centers. Sasaki has identified a number of transcription factors involved in the formation and the function of the node in previous studies, and he continues to be acutely involved with questions of how these centers are formed, and how they regulate development.

the node and notochord. Using a marker gene to trace gene expression, his team identified the *HNF3β* enhancer, and has found that the key mechanism of its regulation is activation by a pair of transcription factors, functioning downstream of a known node-inducing signal, Wnt. The team will study the effects of the loss of function of these molecules in knockout mice, and search for potential co-factors using a yeast interaction-screening system.

The serendipitous discovery of a previously unknown mouse phenotype in which the head fails to develop has also spurred research in Sasaki's lab. It is rare for such 'headless' phenotypes to survive to later stages of embryonic development, and the known cases that do have all been linked to mutations in node-related genes. The phenotype under investigation develops normally in early embryogenesis, but exhibits a loss of anterior markers in late gastrulation, when the node normally induces the head. Sasaki is now performing a functional analysis of how one candidate gene interacts with other signaling molecules. In these and other studies, Sasaki seeks to identify molecules involved in signal center formation and function in the early embryonic development of the mouse.

Node-specific transcription factor networks

Hepatocyte Nuclear Factor 3β (HNF3β) is a highly conserved transcription factor expressed specifically in embryonic signaling centers. Sasaki is engaged in an analysis of molecules that activate *HNF3β* expression in



Enhancer of *HNF3β* drives gene expression in the signaling centers, node and notochord (blue).

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 lab 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 of the Laboratory for Embryonic Induction at the RIKEN Center for Developmental Biology.

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Publications

Nishizaki Y, Shimazu K, Kondoh H, Sasaki H. Identification of essential sequence motifs in the node/notochord enhancer of *Foxa2* (*Hnf3β*) gene that are conserved across vertebrate species. *Mech Dev* 102:57-66 (2001).

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Germ Cells

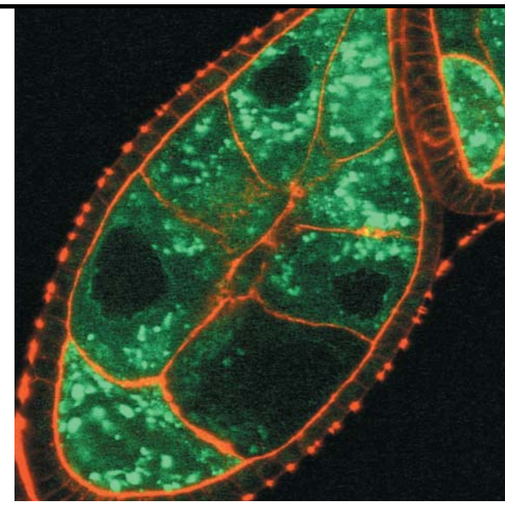
Laboratory for Germline Development

Germ cells

Germ cells are the only cell types capable of transmitting genetic information from generation to generation, and these cells are characterized by equally unique developmental processes as well. In many types of animals, the formation and differentiation of germ cells is controlled by mRNAs and proteins localized in the specific cytoplasmic region within eggs, called germ plasm. Germ plasm mRNAs are translated in a spatio-temporally regulated manner, but the means by which the germ plasm is formed and achieves this controlled translation remain obscure. Akira Nakamura studies the establishment of the *Drosophila* germ line as a model of the processes of germ plasm formation and differentiation, as well as for the insights this system can provide into the general mechanisms of mRNA localization and translation.

The *Drosophila* egg shares cytoplasm with neighboring nurse cells via an incomplete cell membrane, allowing mRNAs and proteins from the nurse cells to be transported to the egg in the form of ribonucleoproteins (RNPs). In order to ensure the proper function of these mRNAs, their translation must be prevented during their transport. Screening proteins with localization patterns similar to those of transported mRNAs, the Nakamura team identified Me31B, which is involved in the translational silencing of maternal mRNAs. The Me31B complex also includes a protein involved in directing the cytoplasmic transport of mRNAs, suggesting that RNP complex proteins cooperatively regulate both the translation and transport of mRNAs. The lab is now working on the functional characterization of other RNP proteins they have identified through several approaches.

In previous work, the Nakamura team identified a novel non-translatable RNA, *Pgc* (for Polar Granule Component), which is localized in the germ plasm. When



Maternal RNP particles shown by green fluorescent protein imaging

the function of *Pgc* is inhibited, germ cells fail to migrate to the embryonic gonads. Another maternal effect mutant, named *N14*, results in a similar phenotype, suggesting that these genes play roles in germ cell maintenance and migration, and possibly their programmed cell death. Their loss of function might correspond to a loss of 'germness' in these cells, a finding that provides a new potential link between germ cells and undifferentiated stem cells.

Future work in the Nakamura lab will focus on taking what has been learned from the study of germ line establishment in *Drosophila* and searching for patterns of conservation and divergence in chordates, using the sea squirt as a model.

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.

Staff

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Kazuko Hanyu-Nakamura
Keiji Sato
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Technical Staff
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Publications

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Development

Creative Research Promoting Program

Chromatin

Laboratory for 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 the Cold Spring Harbor Laboratory in Shiv Grewal's lab, before returning to Japan in December 2001 as a PRESTO researcher in the Japan Science and Technology Corporation (JST). He was appointed team leader in the RIKEN CDB Laboratory for Chromatin Dynamics in 2002.

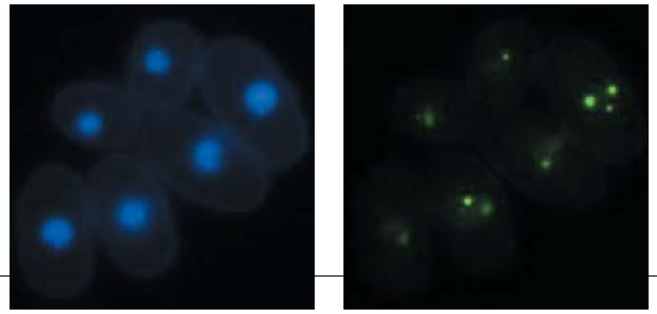
Epigenetic control of gene expression

In recent years, it has become increasingly evident that the controlled expression of genes cannot be accounted for by DNA sequences alone. Epigenetic (meaning 'heritable, but occurring independently of gene sequence') regulation of gene transcription must be maintained and propagated across cell division cycles and throughout the development of the organism proper in order for cells to establish and maintain their identities as specialized cell types. While many of the mechanisms by which such regulation is achieved remain unknown, it is clear that modifications to the nuclear DNA-protein chromatin complex are central to epigenetics. Chromatin occurs in highly-condensed and less condensed forms, which are known as heterochromatin and euchromatin, respectively. Jun-ichi Nakayama's research will concentrate on heterochromatin dynamics, modifications to the DNA-packing proteins called 'histones' in particular, and the molecular mechanisms that allow such chemical states to be heritably transmitted.

Heterochromatin proteins and gene silencing

The Nakayama research team uses the fission yeast *S. pombe* as a model system, as its chromatin structure is closely similar to that of higher organisms. In previous studies, Nakayama demonstrated that heterochromatin protein binding states play a role in the regulation of transcriptional gene silencing. Nakayama performed detailed analyses of the binding states of Swi6, a homolog of the mammalian HP-1 heterochromatin protein at silent mating type *(mat)* locus of the fission yeast. The results of that study showed that Swi6 protein is a dosage-critical component involved in imprinting the *mat* locus. This binding of Swi6 is maintained both across mitotic cell cycles and intergenerationally, as it is propagated through meiosis as well. Nakayama's study also showed that the deacetylation and subsequent methylation of a specific histone H3 lysine residue are essential to this process. Chemical modifications such as deacetylation, methylation, phosphorylation and ubiquitination are fundamental epigenetic processes that can act to switch the expression of a target gene on or off.

Through his work on this evolutionarily conserved heterochromatin protein, Nakayama has uncovered potentially important new roles for the molecule in the establishment, maintenance and transmission of epigenetic information. His findings suggest that the concept of a gene as a simple string of DNA nucleotides needs to be expanded to include the action of proteins in the functional genetic unit. In the future, Nakayama plans 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 yeasts stained by DAPI(left) or detected by GFP-Swi6(right)

Staff

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Publications

Nakagawa H, Lee J-K, Hurwitz J, Allshire RC, Nakayama J, Grewal SIS, Tanaka K, and Murakami Y. Fission yeast CENP-B homologs nucleate centromeric heterochromatin by prompting heterochromatin-specific histone tail modifications. *Genes Dev* 16:1766-78 (2002).

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Development

Regeneration

Regenerative Medicine

Creative Research Promoting Program

Stem Cell Organogenesis

Laboratory for 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 (USA). 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.

A new basis for regenerative medicine

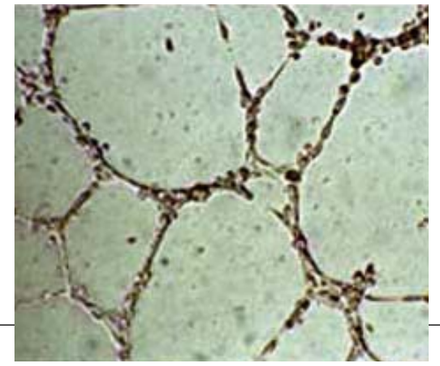
The use of transplanted adult stem cells to regenerate blood cells represents one of the first and, to date, most successful applications of regenerative medicine. But the developing field of stem cell-based therapy continues to receive attention as one of the most promising frontiers of medical science. Pluripotent cells, with their ability to produce both pluripotent and differentiated progeny on cell division, have enormous potential for clinical application, and are the subject of intense research in medical fields from autoimmune and neurodegenerative disease to disorders of the metabolism.

Takayuki Asahara brings a background in clinical cardiology to the CDB, and plans to use his experience at both the bedside and the research bench to investigate the potential for applications of post-natal pluripotent stem cells in regenerative therapies. Building on previous work in which he identified bone marrow-derived endothelial progenitor cells (EPCs) and demonstrated their role in the generation of new blood vessels, Asahara seeks to characterize stem cells in the adult body with even greater differentiative potential, and one day to translate that research into clinically relevant advances.

Blood vessel formation and organogenesis

One result of Asahara's and others' work in the 1990s was the realization that blood vessels are formed by two distinct physiological processes in the adult body. In angiogenesis, new blood vessels are generated from pre-existing, differentiated endothelial cells. Conversely, vasculogenesis involves the recruitment and differentiation of previously undifferentiated EPCs at the site of new blood vessel growth. These EPCs are themselves the progeny of more differentiatively potent adult stem cells known as hemangioblasts, which can be induced to demonstrate true pluripotency under appropriate culture conditions. Asahara's research at the CDB will hinge on developing gene expression profiles of post-natal pluripotent stem cells and performing functional assays of such cells at various stages of differentiation to elucidate the genetic and molecular mechanisms that underlie vasculo- and organogenesis.

In addition to their capacity for facultative tissue-specific differentiation, the ability of stem cells and their progenitor cell descendants to home to sites of active regeneration makes them attractive for use as vectors in targeted gene therapy. Adult stem cells also preferentially maintain a quiescent, non-differentiating state, and so they are believed to represent less of a tumorigenic risk than embryonic stem cells when transplanted into human patients, as, indeed, experience with hematopoietic stem cells has shown. With the goal of realizing truly translational research, Asahara also plans to study optimal methods for delivering stem cells to localized and system-wide targets in regenerative and gene therapy.



Vasculogenesis achieved by endothelial progenitor cells

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Publications

Asahara T, Kawamoto A, Kalka C, Masuda H. Therapeutic potential of bone marrow-derived endothelial progenitor cells for cardiovascular ischemic diseases *Gene Ther Reg* 1 (4):361-75 (2003)

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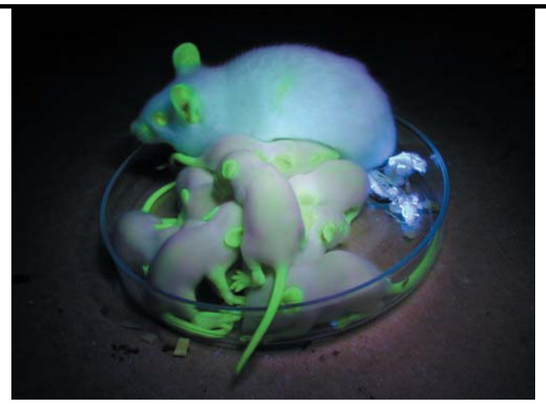
Embryology

Laboratory of Mammalian Molecular Embryology



Transgenic mouse and offspring expressing green fluorescent protein

The developmental program is initiated moments after the penetration of an egg by a fertilizing sperm. Although these initiation events are quintessential to the ontogeny of all mammals, they remain one of nature's most closely guarded secrets. Somehow, within hours of coming together, the two highly specialized cells that begin this process, produce a single cell that is utterly unspecialized: it can give rise to all cell types - in fact, it produces an individual. The single cell is, of course, a 1-cell embryo, and the two cells that must unite to produce the embryo are gametes - an egg (or oocyte) from the maternal side and a sperm from the paternal side.

Adopting the mouse as a model system, the laboratory of Tony Perry is trying to bring molecular and cellular techniques to dissect the magical events occurring within the earliest embryos. What are the key molecules involved, how do they relate to each other, and what influence do they have on developmental outcome? Central to these analyses is a novel method termed piezo-actuated micromanipulation. This allows scientists for the first time to manipulate mouse eggs and embryos, which are exquisitely sensitive to mechanical trauma.

Based on his work in this area, Perry has developed a model that seeks to explain how a sperm signals to the egg to initiate its dedifferentiation to a totipotent state. In this model, one or more sperm-borne oocyte activating factors act to trigger signaling networks in the moments that immediately follow fertilization. A growing body of evidence indicates that such networks have direct and far-reaching developmental consequences. The characterization of these activating factors at the molecular level is fundamental to an improved understanding of the process of fertilization, and knowledge of the mechanisms involved has the potential for application in novel methods of contraception and could open up new avenues in the treatment of some forms of infertility.

In addition to describing the earliest events of a new embryonic life, the Perry laboratory has an interest in exploiting these findings to develop novel methods of genome manipulation. This work includes the development of a new technique for introducing transgene DNA via co-injection with a nucleus into an egg. Since such eggs are typically arrested at the second meiotic metaphase, the method is called metaphase II (or 'mII') transgenesis. The mII transgenesis method enables the efficient introduction of large DNA segments to produce transgenic mice.

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 will work on mechanisms in mammalian preimplantation embryos.

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Publications

Perry ACF, Rothman A, de las Heras JI, Feinstein P, Mombaerts P, Cooke HJ, Wakayama T. Efficient metaphase II transgenesis with different transgene archetypes. *Nat Biotechnol* 19:1071-3 (2001).

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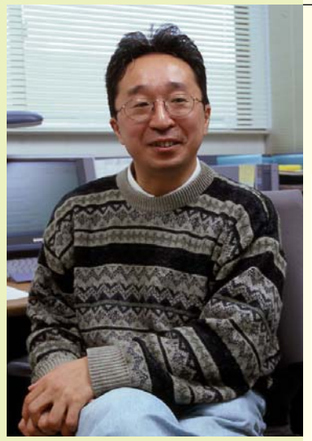
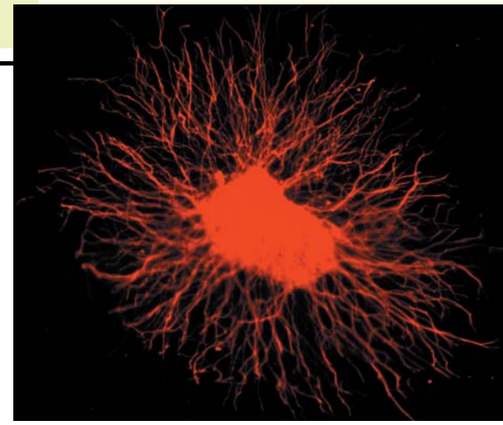
Neurotrophic Factors



Laboratory for Neuronal Differentiation and Regeneration



Explant culture of primitive sympathetic ganglia in mouse treated with Artemin, showing robust neurite outgrowth

Neurotrophic factors

Early neural network development is characterized by the growth of neurons in excess of the number that will ultimately populate any given region. Neurons that have formed synaptic connections with a target tissue need to establish what is known as a 'trophic interaction' in which the target provides the neuron with chemical signals necessary for its survival and continued function. The supply of such signals is limited and neurons which fail to compete successfully for these factors undergo the programmed cell death routine, apoptosis, a controlled die-off that establishes an appropriate balance in the neural population. The target-derived chemical signals in the trophic interaction are known as neurotrophic factors. The study of these signaling proteins dates back to the discovery of nerve growth factor (NGF) by Levi-Montalcini and colleagues in the 1950s. Subsequent research has revealed the diversity of neurotrophic factors, which are now thought to include neurotrophins (of which NGF is a member), neuropoietins, fibroblast growth factors, and transforming growth factors. Hideki Enomoto is primarily interested in this last group, and the GDNF (for Glial cell line Derived Neurotrophic Factor) Family Ligands (the GFLs) in particular. By pinpointing the extrinsic signals responsible for inducing normal neural development, he hopes to open up new avenues for ES cell-based therapies in the treatment of human neurological disorders.

The GFLs signal through a receptor complex comprising the RET receptor tyrosine kinase and the co-receptor GRFα. In genetic knockout studies, Enomoto discovered that the GFLs play a critical role in the development of enteric, sensory and autonomic neurons, establishing the GFLs as a neurotrophic family of equal importance to the better-known neurotrophins. Enomoto's work has shown, however, that the role of RET signaling is distinct from that of the neurotrophins in that it is involved in the migration, axonal growth and axon guidance of developing sympathetic nerve cells, not the survival of established neurons. This suggests that RET and TRK (the neurotrophin receptor) activate independent signaling pathways to perform their complementary functions.

The Enomoto research team plans to conduct systematic DNA chip analyses of enteric and autonomic neurons to achieve a better understanding of the molecular mechanisms involved in RET and TRK signaling. And, by gaining insights into the roles played by specific neurotrophic factor signaling pathways, Enomoto hopes to contribute to the development of stem cell-based neuroregenerative therapies.

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.

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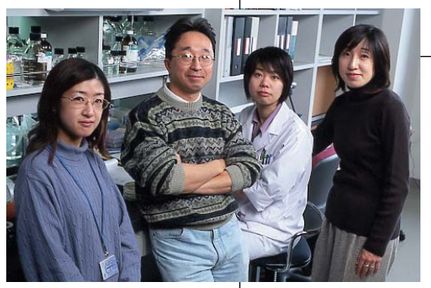

Publications

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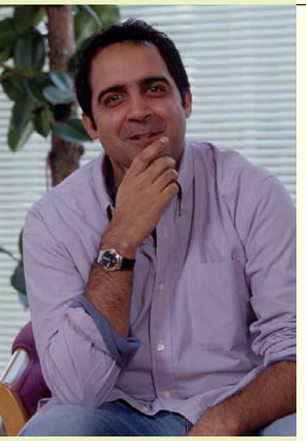
Sensory Organogenesis



Laboratory for 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*, under the supervsion of Jim Smith. 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.

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Sensory organogenesis

Sensory systems in vertebrates represent one of the pinnacles of evolutionary achievement, wedding intricate organic structures with specialized cellular function to permit the animal to apprehend its environment by processing a range of physical and chemical stimuli. The development of these systems involves an extraordinary level of coordination between cells and emergent tissues at very early stages of embryogenesis, and provides an ideal model for the study of such inductive interactions in general. Research in Raj Ladher's lab focuses on the induction of the inner ear in the chick embryo, a model system chosen for the chick's tractability to classical embryological techniques and the opportunities it affords for investigating the processes of developmental program initiation, cell differentiation and the integration of discrete cellular motifs in a structurally and neuronally specialized organ.

Inner ear induction

Sense organs originate in placodes, thickened regions within the early ectoderm. The inner ear arises from the otic placode, a cluster of cells which, in a process known as 'invagination,' recedes from the ectodermal surface and is folded over, resulting in the formation of a segregated hollow called the otocyst which ultimately gives rise to the mature inner ear. Raj is studying the inductive factors that dictate the location and timing of these developmental processes. In a series of tissue manipulation experiments conducted previously, he identified a trio of signaling factors – FGF-8, FGF-19, and Wnt-8c – each of which localizes in a separate germ layer, and which cooperate to induce inner ear development. In this network, it appears that FGF-8, expressed in the endodermal layer, induces the expression of FGF-19 in the immediately overlying mesoderm. FGF-19 in turn induces Wnt-8c in the neuroectoderm, triggering a complex regulatory loop in which FGF-19 and Wnt-8c maintain each other's expression for the duration of early ear development.

Sensory cellular differentiation

The operation of factors from non-ectodermal layers seems to be critical to cell differentiation in the inner ear as well. In a series of experiments in which he tested various tissue recombinations of sections from germ layers neighboring the site of inner ear development, Raj found that even though the mesodermal inducer FGF-19 could initiate inner ear formation, differentiation did not occur. He is currently testing the hypotheses that FGF-19 may have an inhibitory effect on differentiation at later stages, and that it may play a role in recruiting cells to the developing inner ear.

In the future, the Ladher lab looks to extend its research scope to other model systems, with the goal of determining whether some as-yet unidentified general mechanisms are at work in sensory organogenesis.

Expression patterns of *fgf-19* and *wnt-8c*. The gene products co-operate to induce the inner ear in non-neural ectoderm, adjacent to the *wnt-8c* neural domain and above the *fgf-19* mesodermal domain.

Publications

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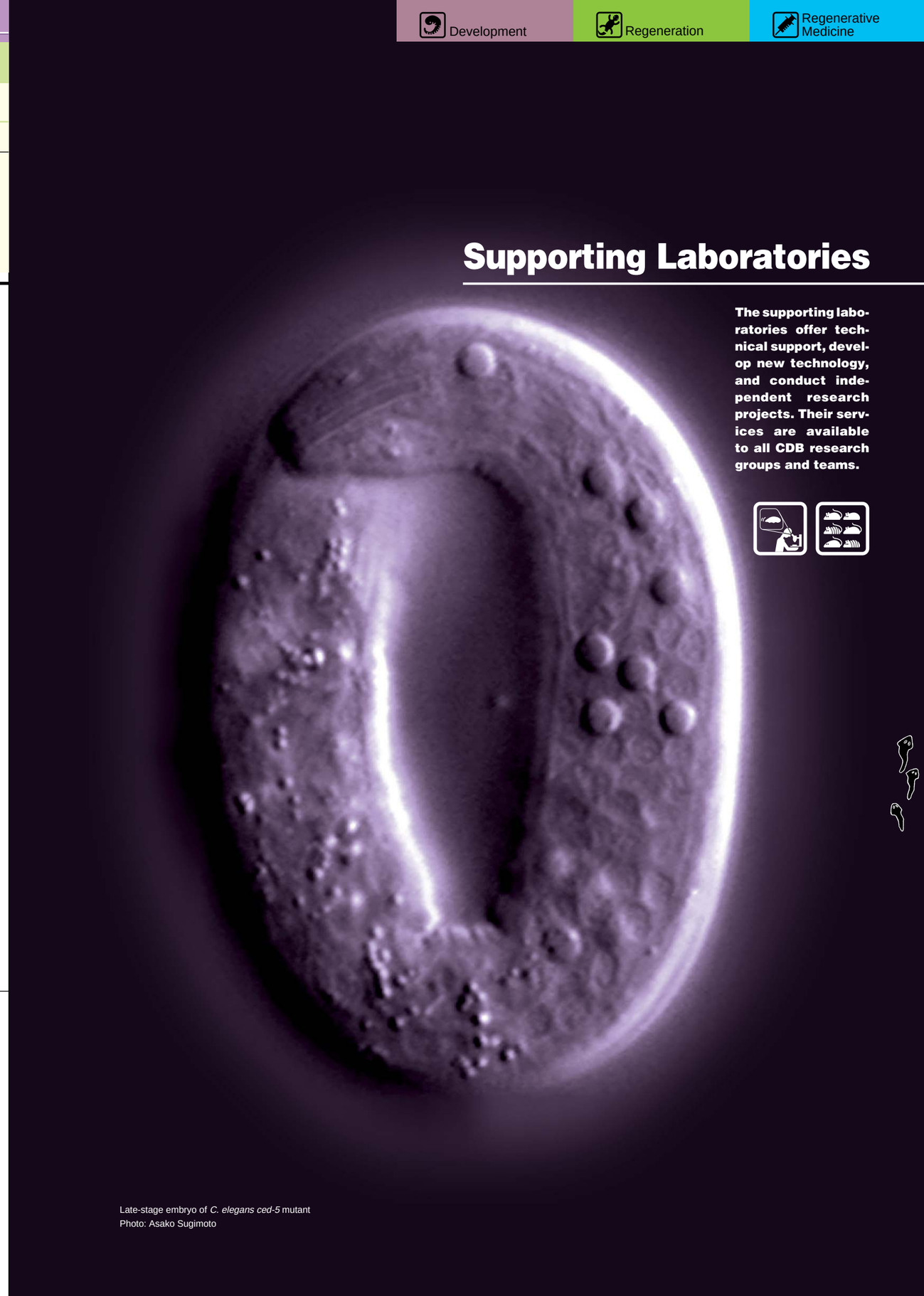
Supporting Laboratories

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.



Late-stage embryo of *C. elegans ced-5* mutant
Photo: Asako Sugimoto

Laboratory for 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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Cell morphology and imaging

Since the advent of the microscope in the 17th century, the development of novel methods of delineating and distinguishing biological structures at ever-greater resolutions has stood at the heart of progress in the life sciences. The most powerful electron microscopes now provide scientists with the ability to visualize biological objects as small as 2 nanometers in size, which has opened up new windows of insight into the relationship between form and function at the sub-cellular scale. In theory, even greater resolutions could be achieved, but the limits imposed by the materials under study – organic molecules with sometimes fragile structures – have kept such microscopes from realizing their full potential. One of the main goals in the field of microscopy is the development of new techniques in specimen preparation and image enhancement that will allow scientists to overcome these limitations and probe the hidden world even more deeply.

Shigenobu Yonemura brings a background in cell and molecular biology to bear on visualization methods and technology with applications in the study of animal development. He seeks to explore the links between cell shape, function and behavior by improving the means by which the cellular architecture can be brought to light. His special emphasis is on developing ways to localize with greater precision the molecular components of the cytoskeleton, a complex structure that determines cell morphology and participates in the movement and interactions of cells with their surroundings.

Visualizing Rho-family proteins

The Rho (for Ras Homologous) family of proteins – principally Cdc42, Rac and Rho – has been extensively studied for its effects on the structural activity of cells. These proteins serve as molecular switches capable of interacting with the actin cytoskeleton and inducing cells to form a range of surface structures, such as cylindrical microvilli projections and stress fibers that serve to anchor cells to the substratum. The Yonemura research team is studying the localization of Rho family members with the goal of gaining insight into how these molecules contribute to cellular morphogenesis.

Specimen preparation is considered to be as important as microscope power in the imaging of biological samples

One of the perennial technical challenges in the microscopic study of cells has been assuring that the specimen is not damaged or altered in the process of preparing it for examination. Indeed, specimen preparation is considered to be just as important as microscope power in the imaging of biological samples. In previous work on improving specimen preparation for immunofluorescence imaging, Yonemura introduced the denaturing agent trichloroacetic acid (TCA) for its fixative and preservative properties. TCA fixation produces good results in the visualization of Rho and Rac with few of the drawbacks of other traditional methods.

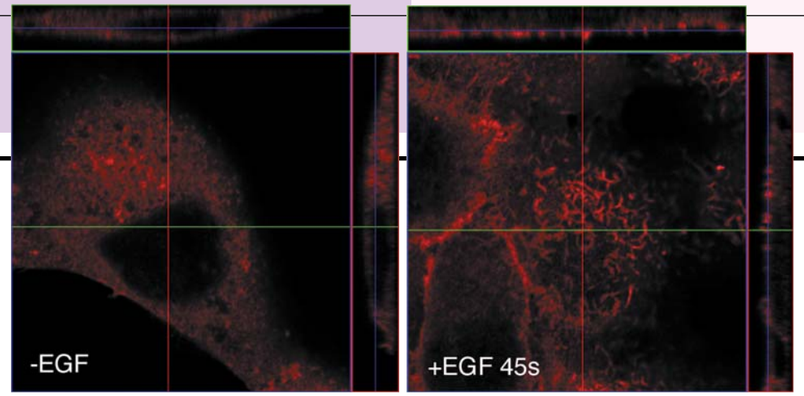
However, recent localization studies of the Rho family have produced inconsistent, sometimes contradictory, results. In studies using immunochemical and green fluorescent protein (GFP) tagging of the Rho protein, widely divergent distribution patterns were reported, calling into question the reliability of these visualization techniques. Yonemura has been developing monoclonal antibodies specific to each of the three Rho isoforms – RhoA, RhoB and RhoC. To ensure the validity of the staining of the endogenous target protein, Yonemura expressed an epitope-tagged target protein in cultured cells. These cells were processed for immunofluorescence imaging using antibodies against both endogenous and epitope-tagged target proteins. As antibodies to the endogenous protein should also bind to the epitope-tagged proteins, the failure of an imaging method to show a high degree of correspondence in the localization of the two indicates a flaw in the antibody-fixation protocol. By introducing such improvements to fixation and evaluation methodologies, Yonemura hopes to reinforce scientific rigor in the field of microscopic visualization and to be able to apply these techniques to the study of other members of the Rho family.

The actin cytoskeleton

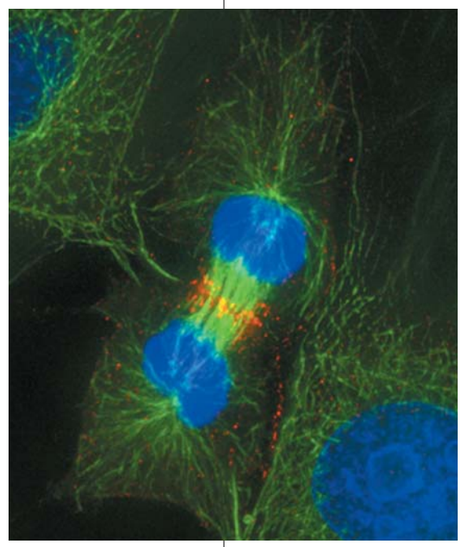
The skeletal framework of the cell is built up of an intricate assembly of filaments and accessory proteins, cooperating to stabilize the cell and give it shape. One of the main types of filaments is the actin filament (or microfilament), which, in addition to determining surface morphology, also play a critical role in driving cellular movement. As described above, the Rho-family proteins help to regulate the function of the actin cytoskeleton and, thereby, many aspects of cellular behavior as a whole. But the relationship between Rho expression and actin response is neither simple nor direct. Rather, Rho plays an integral part in an intracellular signaling network of great, but still unfathomed, complexity. Yonemura is working on one aspect of this network – the interactions between Rho, protein kinase C, and ERM, a cross-linker protein that joins actin to the cell membrane. Using the yeast two-hybrid system to identify proteins in this signaling network, Yonemura seeks to address some of the many as-yet unanswered questions of Rho function.

In addition to its full-time research efforts, the Yonemura team offers common-use microscopy services to other CDB laboratories. By providing fluorescence and electron microscopic imaging and instruction in techniques, the team hopes to help ensure the quality, fidelity and ease-of-interpretation of visualization data generated at the Center.

Morphology



Rho is rapidly recruited to elongating microvilli on EGF-stimulated A431 cells.



In dividing animal cells, Rho (red) is concentrated in the furrow region. Blue: nuclei, Green: microtubules.

Publications

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Yonemura S, Matsui T, Tsukita Sh, and Tsukita Sa. Rho-dependent and –independent activation mechanisms of ezrin/radixin/moesin proteins: an essential role for polyphosphoinositides *in vivo*. *J Cell Sci* 115:2569-80 (2002).

Experimental Mice

Laboratory for Animal Resources and Genetic Engineering

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, under CDB Deputy Director Shinichi Aizawa, 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 nuclear transfer cloning, isolating homologous recombinants and culturing embryonic stem cells, and works to develop improved methods of sample handling and storage, such as higher-efficiency modes of cryopreservation. 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.

In the coming year, the LARGE team plans to expand its services and initiate new programs, notably the generation of target vectors from sequence information, and the independent production of novel genetically-modified constructs, a drive that is anticipated to generate on the order of 80-100 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 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.

Staff

Team Leader
Shinichi Aizawa
Postdoctoral Researcher
Kenryo Furushima
Research Associate
Kazuki Nakao
Technical Staff
Hitoshi Miyachi
Rika Nakayama
Hiroshi Kiyonari
Technical Assistant
Chiaki Tamamura
Akemi Hara
Takashi Nomura
Tsunehisa Fujita
Assistant
Kaori Nasu

Publications

Ino M, Yoshinaga T, Wakamori M, Miyamoto N, Takahashi E, Sonoda J, Kagaya T, Oki T, Nagasu T, Nishizawa Y, Tanaka I, Imoto K, Aizawa S, Koch S, Schwartz A, Niidome T, Sawada K, Mori Y. Functional disorders of the sympathetic nervous system in mice lacking the α_{1B} subunit ($Ca_v2.2$) of N-type calcium channels. *Proc Natl Acad Sci* 98:5323-8 (2002).

Kim JM, Nakao K, Nakamura K, Saito I, Katsuki M, Arai K, and Masai H. Inactivation of Cdc7 kinase in mouse ES cells results in S-phase arrest and p53-dependent cell death. *EMBO J* 21:2168-21679 (2002).

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Nishinakamura R, Matsumoto Y, Nakao K, Nakamura K, Sato A, Copeland NG, Gilbert DJ, Jenkins NA, Scully S, Lacey DL, Katsuki M, Asashima M, and Yokota T. Murine homolog of SALL1 is essential for ureteric bud invasion in kidney development. *Development* 128:3105-15 (2001).

Sequencing Lab

The CDB sequencing lab provides DNA sequencing and analysis services for use by all laboratories at the Center. Equipment is available for small, medium and large-scale requests, with turnaround times from less than one week for smaller scale projects to one month for longer jobs, at rates of up to 1,920 samples per day. Smaller jobs are completed especially rapidly, and results can be delivered as soon as the following day when sequence-ready samples are submitted. Analysis services for editing, homology search, and assembling are also available. All work requests can be submitted and tracked online, and results are returned to the applicant's personal folder on the CDB intranet file server, making it possible for CDB research staff to sequence samples of interest without leaving their desktop computers.

Staff

Sequencing Lab
Research Scientist
Hiroshi Tarui
Technical Staff
Osamu Nishimura
Yukako Hirao
Takako Sano

Model Organisms

YEAST

Common name : fission yeast
Scientific name : *Schizosaccharomyces pombe*

Yeasts are known for their use as fermenting agents in the production of wine and beer, but they are also found in life sciences laboratories around the world, performing a very different but equally valuable service. The fission yeast has served as an experimental model for the past fifty years, thanks to it amenability to molecular genetic studies and the ease which it can be grown and manipulated in culture, and it is perhaps best known for its contributions to our knowledge of chromatin structure.

ROUNDWORM

Common name : nematode
Scientific name : *Caenorhabditis elegans*

With exactly 959 somatic cells in the body of a normal adult, the nematode offers perhaps the best-characterized model of a multicellular organism possessing simple nervous, muscle and reproductive systems. It is possible to track the fates of individual cells in its transparent body on a microscope slide glass, and the exact fate of every cell in every cell lineage is known. In 1998, geneticists added to that body of knowledge by sequencing the nematode genome, which was found to contain around 19,000 genes, many of which have human homologs. The discovery of the RNA interference mechanism that allows genes to be functionally 'knocked down' has further fueled interest in these tiny creatures as model organisms.

FLATWORM

Common name : planarian
Scientific name : *Dugesia japonica*

Found in freshwater streams and ponds, planarians are small worms only a few millimeters long which possess simple brains, nervous and digestive systems, and a strangely endearing pair of rudimentary eyes. Their most striking characteristic, however, is their regenerative ability; cut a single animal into dozens, even hundreds of pieces, and each fragment can develop into a new individual. This remarkable capacity has attracted the attention of biologists interested in the mechanisms of regeneration and much progress has been made in recent years in understanding how the planarian achieves its death-defying multiplication. The answer lies in the totipotent cells distributed throughout its tiny body, cells it is hoped will provide clues to the emerging field of regenerative medicine.

FLY

Common name : fruit fly
Scientific name : *Drosophila melanogaster*

The fruit fly came into its own as a laboratory model when scientists in the early twentieth century discovered *Drosophila* was easy to keep, feed and breed, and by the 1920s fruit flies were the subject of extensive experiments in heredity in the labs of Nobel Prize-winner T. H. Morgan and elsewhere. As its popularity as a model increased, data on all aspects of fly biology, from molecular genetics to learned behaviors, burgeoned. The discovery of homeotic genes in the fly, which determine segment identity and which were found to be conserved in animals from worms to humans, made *Drosophila* a favorite of developmental biologists as well.

Model Organisms

FISH

Common name : zebrafish
Scientific name : *Danio rerio*

The zebrafish, with its short reproductive cycle and high number of eggs per clutch, is one of the most popular vertebrate systems used in genetic analysis. Its rapid development has also made it popular in the developmental biology and embryology communities, as has the fact that its eggs are externally fertilized and translucent, making it possible to follow its developmental progress easily using a dissecting microscope. The tractability of this fish to gene knockdown methods and the existence of techniques for tracing the fates of individual cells have also helped to establish it as a favorite system in developmental genetics.

BIRD

Common name : chick (chicken)
Scientific name : *Gallus gallus*

The chick embryo has been in use as a model since the time of Aristotle, making it one of the most exhaustively studied systems in biology. The egg's extrauterine development made it attractive in the days before surgical techniques. The chicken's regular laying cycle and the fact that fertilized eggs can be cold-stored for several weeks, temporarily freezing their development, ensures constant access to embryos. Chick embryonic development shows some similarity to that of mammals, and the flat blastodisc of the chick is morphologically similar to the human embryo at the same stage of development. The introduction of techniques for embryonic sectional explantation and the production of chick-quail chimeras has only served to add to the popularity of the chick embryo as a model system.

FROG

Common name : African clawed frog
Scientific name : *Xenopus laevis*

The African clawed frog has a long history of use in the study of vertebrate embryology and development. As aquatic animals, they undergo external development, making it relatively easy to manipulate the fertilized egg as compared to animals in which the embryo grows within the uterus. Their eggs are also quite large, about 1 mm in diameter, which is about ten times the size of a mouse oocyte, allowing for various surgical manipulations and biochemical analyses. Ovulation can be induced relatively simply using luteinizing hormone, which eliminates some of the problems associated with extended reproductive cycles. And their rapid progress from fertilization to neurulation in less than 24 hours provides scientists with a dynamic picture of the first stages of life.

MOUSE

Common name : mouse
Scientific name : *Mus musculus*

For nearly one hundred years the mouse has been the animal of choice for modeling human genetics and disease. This application has been borne out by the recent completed sequencing of the mouse genome, which revealed an extremely high homology between the mouse and human genomes. As fellow mammals, humans and mice also share many physiological traits and susceptibilities, making the mouse an attractive model in biomedical studies. Its rapid breeding cycle and small size further add to its appeal, and it is little wonder that scientists have chosen this animal for developing or popularizing a wide range of genetic techniques from transgenesis to gene knockouts to cloning by nuclear transfer.

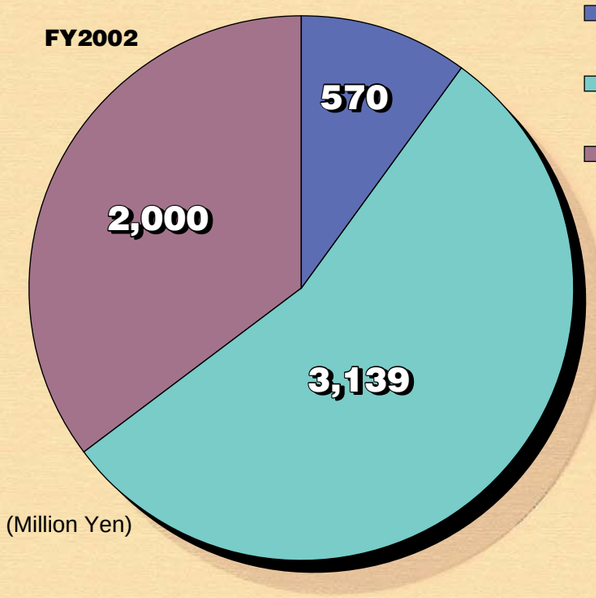
Administration and Activities

Kobe night skyline
Photo: Yutaka Doi

Administration

RIKEN (The Institute of Physical and Chemical Research) was established in 1917 to conduct comprehensive research in science and technology and to disseminate the results of its research and development efforts. RIKEN is organized as a public corporation under the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and conducts research in the fields of physics, chemistry, basic life sciences, engineering and medicine at laboratories located across Japan. The Center for Developmental Biology was established as the first research center within the RIKEN Kobe Institute in April 2000.

2002 CDB Budget



- Facility Expenses (energy expenses,maintenance,etc.)
- Research Management Expenses (personnel expenses,etc.)
- Research Expenses

CDB Staff (December 2002)

Group Directors/Team Leaders	26
Research Scientists	71
Research Associates	3
Technical Staff	82
Assistants	23
Visiting Researchers	9
Collaborative Scientists	19
Student Trainees	34
Part-time Staff	46
Research Promotion Division	38
Other	28
Total	379

Advisory Council

The CDB Advisory Council, 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 in related fields from Japan and around the world and acts as an unbiased review board for CDB research activities.

Institutional Review Board

The Institutional Review Board includes representatives from local academic, media 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.

Educational Activities

Encouraging young scientists to participate in advanced research projects as they prepare for careers in the life sciences is essential to ensuring the development of future generations of researchers. The CDB has collaborative educational programs with a number of graduate schools in Japan, providing students with opportunities for hands-on laboratory benchwork and lectures on topical issues in development and regeneration. The center also has also accepted 47 students from other universities as research assistants or trainees. In addition to regularly scheduled scientific seminars, students can attend events such as the CDB Forum research progress series and the Center's annual retreat. The Junior Research Associate program established by RIKEN also provides financial support to 4 graduate students working in CDB labs.

2002 Graduate School Affiliates

Graduate School of Biostudies, Kyoto University
CDB: Masatoshi Takeichi

Kobe University, Graduate School of Science and Technology Department of Life Science
CDB: Kiyokazu Agata, Shigeo Hayashi, Hitoshi Sawa

Department of Developmental and Regenerative Medicine
CDB: Hitoshi Niwa, Masahiko Hibi, Asako Sugimoto

2002 Seminars

The CDB hosts regular seminars by distinguished speakers from within Japan and abroad as part of its committed effort to promote the borderless exchange of scientific information. All CDB seminars are held in English. In addition to these seminars, the CDB also holds monthly Forums, in which CDB researchers share findings with their colleagues, and a range of informal lecture offerings in English and Japanese.

Date	Title	Speaker
2002.4.16	The rediscovery of the lymphatic system	Michael Detmar
2002.5.27	Germline stem cells and the niche in *Drosophila melanogaster*	Toshie Kai
2002.6.7	The evolution of homeobox gene cluster	P. W. Holland
2002.7.2	How the cavefish lost its eyes: A problem in development and evolution	Yoshiyuki Yamamoto
2002.7.23	Control of epithelial-mesenchymal transitions in the neural crest	Don Newgreen
2002.7.24	Cell polarity and asymmetric cell divisions in the *Drosophila* PNS	Francois Schweisguth
2002.8.8	The 1st CDB Meeting; Head development (I)	(multiple speakers)
2002.8.13	The 2nd CDB Meeting: Left-right determination in the lancelet embryo	Kinya Yasui
2002.8.13	The 2nd CDB Meeting: Molecular studies of hemichordate development: New insights into bilaterian evolution	Kumifumi Tagawa
2002.8.13	The 2nd CDB Meeting: Body plan of the sea urchin embryo	Koji Akasaka
2002.8.19	Transposon-mediated transgenesis and insertional mutagenesis in zebrafish	Kouichi Kawakami
2002.9.5	Construction of human artificial chromosome and its possible application for regenerative medicine	Mitsuo Oshimura
2002.9.12	CDB Meeting: Head Development (II)	(multiple speakers)
2002.9.23	Models for the generation of the primary body axes in higher organisms	Hans Meinhardt
2002.9.27	A 600 kDa microtubule-associated factor that contributes to maintaining genomic stability	Yoshihiro Nakatani
2002.10.7	Essential role of *Sall1* in kidney development	Ryuichi Nishinakamura
2002.10.22	Morphological asymmetry in dividing neuroepithelial cells:Its impact on brain histogenesis	Takaki Miyata
2002.10.24	Analysis of the function of *isthmin* in zebrafish embryos	Isato Araki
2002.10.24	Genetic regulation of mouse urogenital organogenesis	Richard R. Behringer
2002.10.30	Systems-biological analysis of dynamic and complex biological systems: Application to mammalian circadian rhythms	Hiroki Ueda
2002.10.30	Calcineurin, a component of G-protein coupled phosphorylation pathways, is involved in movement, fertility, egg laying, and growth in *C. elegans*	Joohong Ahnn
2002.11.5	Clonal identification of pluripotent stem cells in the developing liver and pancreas using flow cytometric cell sorting	Hideki Taniguchi
2002.11.14	Sensory signaling and neural modulation in the nematode *Caenorhabditis elegans*	Ikue Mori
2002.11.20	Distinct *Rac* regulators in cell migration, axon outgrowth and cell-corpse engulfment in *C. elegans*	Yi-Chun Wu
2002.11.21	Regulation of expression and function of cell differentiation factor Nkx2.2	Hirotaka Watada
2002.11.21	The tao of stem cells in the germline	Haifan Lin
2002.11.28	Transcriptional regulation of signalling competence in derivatives of the spemann organizer	Joshua M. Brickman
2002.12.5	Nuclear organization of chromosomes in fission yeast	Yasushi Hiraoka
2002.12.5	Dynamic organization of the nuclear envelope	Tokuko Haraguchi
2002.12.6	Cell signaling mechanisms that establish left-right asymmetry	H. Joe Yost
2002.12.6	Making and breaking the embryonic mouse heart: A transgenic approach	Simon J. Conway
2002.12.9	New insights into gastrulation and neural induction in the mouse embryo	Jerome Collignon
2002.12.9	Evolution of complex form and function in the developing mammalian heart	Richard Harvey
2002.12.11	What do we know of hematopoietic stem cells?	Hideo Ema
2002.12.16	Regulation of motor pool sorting by expression of Type II cadherins	Stephen Price
2002.12.17	Use of the *Cre/LoxP* system to study mouse development by a non-biased approach	Werner Muller
2002.12.24	Human central nervous system stem cells for cell transplantation	Nobuko Uchida
2002.12.25	Identification of novel genes important for pluripotency and tumorigenicity of mouse ES cells	Shinya Yamanaka

The CDB Opening Symposium

A New Paradigm in Developmental Biology 2002.4.22

Introduction to CDB	Shun-ichi Kobayashi (RIKEN President) Masatoshi Takeichi (CDB Director)
Novel Concepts of Development	Igor Dawid (NIH/NICHD) Peter Gruss (Max-Planck-Institute of Biophysical Chemistry)
Cellular Mechanisms	William Chia (MRC Centre for Developmental Neurobiology) Elaine Fuchs (The University of Chicago) Nobutaka Hirokawa (University of Tokyo)
Genetic Reprogramming and Regenerative Medicine	Austin Smith (University of Edinburgh) Yoshiki Sasai (CDB) Takayuki Asahara (St. Elizabeth's Medical Center)
Concluding Remarks	Shin-ichi Nishikawa (CDB)

Igor Dawid speaks at the Opening Symposium (April 22, 2002)

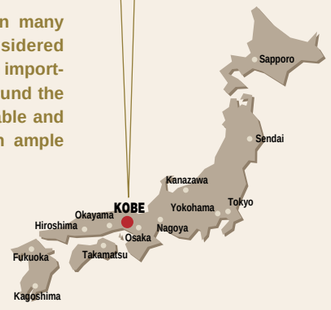
City of Kobe

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 a short drive or train ride away from Osaka and Kyoto, and sits nestled in the foothills of the Rokko Mountains facing out onto the Inland Sea. The climate is pleasant and features four distinct seasons similar to those in other temperate climes. 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.

Costs of living are comparable to those found in many Western cities. Kobe's shopping district is considered second-to-none in Japan, and shops specializing in imported goods make it easy to purchase items from around the world. Houses and apartments are clean, comfortable and modern, and hotels and convention space are in ample supply. The information infrastructure is well-developed, and access to broadband internet connections, mobile phone service and satellite TV is more prevalent than nearly anywhere else in the world.

Major national universities and cultural attractions are located both within the city and in the surrounding Kansai region, providing a stimulating intellectual and educational climate. And thanks to the advent of global communications and information technology, Kobe is securely connected to the worldwide network of ideas.



Exciting. Comfortable. Beautiful. Fun. These are some of the words our visitors from overseas have used to describe their experience in Kobe. The Center offers dedicated support to foreign research staff and guests, from assistance with accommodations and visas to short-term visitors, to renting apartments and help with setting up laboratories for newly arrived staff as they acclimate themselves to life in Japan. Whether as a traveler or a potential resident, we encourage you to visit the CDB and the city of Kobe to see for yourself how enjoyable life in Japan can be.

Medical Industry Development Project

In 1998, Kobe inaugurated its Medical Industry Development Project, marking the launch of one of Japan's first integrated biomedical research parks. The CDB plays a central role as a basic research facility in this growing international center of excellence. The CDB facilities are connected by an elevated walkway to the adjacent Institute for Biomedical Research and Innovation (IBRI) research labs, fostering communications and collaborations between researchers at both locations. And the completion of the IBRI clinical testing hospital (scheduled for April 2003) will further expand the translational research opportunities for CDB scientists and their collaborators.

The Medical Industry Development Project seeks to draw participants from academia, government research institutions and life sciences industries by providing world-class facilities and infrastructure, and a range of support services for basic and corporate research and development. Plans for 2003 include the construction of a Translational Research Informatics Center and a Biomedical Accelerator business incubation facility, and the continued recruitment of biomedical research firms from within Japan and abroad. CDB scientists are already working in close collaboration with colleagues at the IBRI and industry research labs in projects that promise to realize the potential of the bench-to-bedside translational research approach.