On The Cover
Terminal branch of the *Drosophila* trachea. A single terminal cell with a large polyploid nucleus (purple) spreads numerous cytoplasmic tubules with intracellular lumen (tracheoles) over the surface of the larval gut.
PHOTO: Shigeo Hayashi
The days leading up to the year’s end tend to be chaotic, but they also provide one with a valuable opportunity to look back on milestones passed and ahead to new objectives. As 2003 comes to a close, it’s good to be able to take stock of the achievements made by researchers at the RIKEN Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the Center for Developmental Biology, the challenges we face in the years ahead, and the state of Our first annual symposium on The Origin and Formation of Multicellular Systems, a three-day meeting of more than 200 distinguished speakers, poster presenters and attendees who gathered to discuss metazoan biology, from its single-celled forebears to its greatest evolutionary achievement in the formation of neural networks comprising billions of cells. We look forward to hosting more such meetings on a yearly basis, beginning with our next symposium on Developmental Remodeling, in March of 2004, which will be held on the CDB campus in an auditorium designed to increase the Center’s capacity for hosting scientific meetings and expand its ability to provide a venue for international exchange across fields.

Research advances from CDB labs have also provided a string of highlights this year. As you will see from the pages of this report, scientists at the CDB have made strides forward on many fronts, from uncovering new molecular mechanisms at work in the asymmetric division, signaling and guided migration of cells, to developing new technologies that may one day enable the targeted differentiation of embryonic stem cells toward clinically relevant ends. Studies in evolutionary development have also provided broadened contexts and perspectives, finding possible roots for the central nervous system in the genome of a tiny flatworm and challenging conventional wisdom about the regulatory origins of the vertebrate hindbrain.

Of course, this science did not take place in a vacuum. The central pillars of the Center’s research mission — developmental biology, regeneration and the scientific bases for regenerative medicine — are all vibrant, dynamic, truly global fields of endeavor, and the CDB is pledged to remain fully engaged with international colleagues at the organizational, laboratory and institutional levels. In 2003, this commitment was characterized by the establishment of scientific exchange relationships with the University of Texas Graduate School of Biomedical Sciences and the Temasek Life Sciences Laboratory Limited in Singapore, paving the way for new and better opportunities to visit and host researchers from these institutions. We looked forward to increasing the number and range of our inter-organizational exchange programs in the years ahead.

Domestically, Japan saw increased attention to a number of related issues and research fields. The national government unveiled plans for the Leading Projects, initiatives set up to fund mid-term research into a number of key areas identified as having the potential to improve public health, welfare and economic well-being. As the largest center for the study of developmental biology in Japan, the CDB received funding to carry out Leading Project research into potential applications for stem cells in regenerative medicine. Embryonic stem cells also featured in the national news this year, which saw the first establishment of a new line of human ES cells by a Japanese laboratory in Kyoto University. ES cells represent a biological resource of potentially revolutionary importance, but whose study demands careful consideration and ethical restraint. The use of human ES cells is for biomedical research is permitted in Japan, and in 2003 a number of CDB labs applied for permission to use the new Kyoto cell line. Approval of these research plans will allow scientists here to explore the secrets of cell differentiation and pluripotency, and perhaps one day to contribute to the development of cell-replacement therapies for intractable illnesses such as diabetes and Parkinson’s disease.

As a basic research institute, the CDB also appreciates the fundamental importance of maintaining ties with the world of academia, and we have pursued active affiliations with some of Japan’s top graduate school programs in the biosciences and regenerative medicine, allowing students from affiliated programs to conduct their academic research using the Center’s facilities. This year, we were proud to formalize relationships with the Kyoto University Graduate School of Medicine, the Nara Advanced Institute of Science and Technology Graduate School of Biological Sciences, and the Kwansei Gakuin University Bioscience Department, bringing the total number of such affiliations to six.

Public communications and outreach also featured prominently on the Center’s calendar this year. In May, the CDB opened its doors to the public for its first Open House, which was attended by more than 1,000 visitors who took advantage of the opportunity to talk in talks on stem cells and programmed cell death, in mock experiments, and sneak a peek at a day in the life of a developmental biology lab. The Center also joined in the public educational activities at the Kobe BioWeek event in August, a biotechnology convention that saw more than 8,000 attendees, including many who visited the CDB in a virtual sense via two-way video lectures and realistic software simulations of laboratory equipment.

In retrospect, the year has been a full one, and it has been gratifying to participate in the Center’s activities both as a scientist and an administrator. So it is a great pleasure for me to introduce our 2003 Annual Report, which illustrates the range and depth of research at the CDB and serves both as a spark for happy memories and as a source of inspiration as we enter a new year, with its promises of new discoveries and change.
Center for Developmental Biology

Message from the Director

Masatoshi Takeichi
Director, CDB

The days leading up to the year’s end tend to be chaotic, but they also provide one with a valuable stimulus to look back on milestones passed and ahead to new objectives. As 2003 comes to a close, it’s good to be able to take stock of the achievements made by researchers at the RIKEN Center for Developmental Biology, the challenges we face in the years ahead, and the state of our rapidly evolving science. This year has seen the emergence of news and civic issues that have placed the fields of developmental biology, regeneration and regenerative medicine frequently in the spotlight and sometimes at the center of controversy on the world stage. A position accompanied by all the advantages and drawbacks that public prominence entails.

For us at the CDB, the year began in March with the unqualified (and uncontroversial) success of our first annual symposium on The Origin and Formation of Multicellular Systems, a three-day meeting of more than 200 distinguished speakers, poster presenters and attendees who gathered to discuss metazoan biology, from its single-celled forebears to its greatest evolutionary achievement in the formation of neural networks comprising billions of cells. Our efforts to host more such meetings on a yearly basis, beginning with our next symposium, on Developmental Remodeling, in March of 2004, which will be held on the CDB campus in an auditorium designed to increase the Center’s capacity for hosting scientific meetings and expand its ability to provide a venue for international exchange across fields.

Research advances from CDB labs have also provided a string of highlights this year. As you will see from the pages of this report, scientists at the CDB have made strides forward on many fronts, from uncovering new molecular mechanisms at work in the asymmetric division, signaling and guided migration of cells, to developing new technologies that may one day enable the targeted differentiation of embryonic stem cells toward clinically relevant ends. Studies in evolutionary development have also provided broadened contexts and perspectives, finding possible roots for the central nervous system in the genome of a tiny flatworm and challenging conventional wisdom about the regulatory origins of the vertebrate hindbrain.

Of course, this science did not take place in a vacuum. The central pillars of the Center’s research mission — developmental biology, regeneration and the scientific bases for regenerative medicine — are all vibrant fields, truly global fields of endeavor, and the CDB is pledged to remain fully engaged with international colleagues at the organizational, laboratory and institutional levels. In 2003, this commitment was characterized by the establishment of scientific exchange relationships with the University of Texas Graduate School of Biomedical Sciences and the Tama Life Sciences Laboratory Limited in Singapore, paving the way for new and better opportunities to visit and host researchers from these institutions. We look forward to increasing the number and range of our inter-organizational exchange programs in the years ahead.

Domestically, Japan saw increased attention to a number of related issues and research fields. The national government unveiled plans for the Leading Projects, initiatives set up to fund mid-term research into a number of key areas identified as having the potential to improve public health, welfare and economic well-being. As the largest center for the study of developmental biology in Japan, the CDB received funding to carry out Leading Project research into potential applications for stem cells in regenerative medicine. Embryonic stem cells also featured in the national news this year, which saw the first establishment of a new line of human ES cells by a Japanese laboratory in Kyoto University. ES cells represent a biological resource of potentially revolutionary importance, but whose study demands careful consideration and ethical restraint. The use of human ES cells is for biomedical research is permitted in Japan, and in 2003 a number of CDB labs applied for permission to use the new Kyoto cell line. Approval of these research plans will allow scientists here to explore the secrets of cell differentiation and pluripotency, and perhaps one day to contribute to the development of cell-replacement therapies for intractable illnesses such as diabetes and Parkinson’s disease.

As a basic research institute, the CDB also appreciates the fundamental importance of maintaining ties with the world of academia, and we have pursued active affiliations with some of Japan’s top graduate school programs in the biosciences and regenerative medicine, allowing students from affiliated programs to conduct their academic research using the Center’s facilities. This year, we were proud to formalize relationships with the Kyoto University Graduate School of Medicine, the Nara Advanced Institute of Science and Technology Graduate School of Biological Sciences, and the Kwansei Gakuin University Bioscience Department, bringing the total number of such affiliations to six.

Public communications and outreach also featured prominently on the Center’s calendar this year. In May, the CDB opened its doors to the public for its first Open House, which was attended by more than 1,000 visitors who took advantage of the opportunity to take in talks on stem cells and programmed cell death, join in mock experiments, and sneak a peek at a day in the life of a developmental biology lab. The Center also joined in the public educational activities at the Kobe BioWeek event in August, a biotechnology convention that saw more than 8,000 attendees, including many who visited the CDB in a virtual sense via two-way video lectures and realistic software simulations of laboratory equipment.

In retrospect, the year has been a full one, and it has been gratifying to participate in the Center’s activities both as a scientist and an administrator. So it is a great pleasure for me to introduce our 2003 Annual Report, which illustrates the range and depth of research at the CDB and serves both as a spark for happy memories and as a source of inspiration as we enter a new year, with its promises of new discoveries and change.
April 1
New Graduate School Affiliations
The CDB formalized its affiliations with the Kyoto University Graduate School of Medicine and the NAIST Graduate School of Biological Sciences, allowing doctoral students from those schools to conduct their thesis research in CDB laboratories under the guidance of lab heads designated as visiting professors.

May 13
Publication of “Generation of neural crest-derived peripheral neurons and floor plate cells from mouse and primate embryonic stem cells”
In a study published in the May 13 issue of the Proceedings of the National Academy of Sciences (PNAS), the Sasai research group demonstrated the application of somal cell derived inducing activity (SDIA) in the high-efficiency induction of peripheral neurons from embryonic stem cells.

May 24 - 26
First CDB Symposium: Origin and Formation of Multicellular Systems
The CDB held its first annual symposium at the nearby Portopia Hotel. The meeting drew more than 200 speakers and attendees from around the world, who presented and discussed the fundamental processes of development and regeneration of higher order structures, such as neural networks.

May 31
CDB Open House
The CDB opened its doors to the public for a one-day event featuring walk-through presentations and lively discussion on the origins of metazoan biology, the fundamental processes of development and regeneration and the formation of higher order structures, such as neural networks.

June 24
Scientific exchange agreement with University of Texas Graduate School of Biomedical Sciences
As part of its ongoing efforts to develop organizational ties with the international developmental biology research community, the CDB formalized an agreement for active scientific exchange with the University of Texas Graduate School of Biomedical Sciences at Houston. The agreement facilitates the hosting and exchange of research staff and the joint sponsorship of scientific meetings with one of the foremost academic centers for developmental biology research in the United States.

June 26
Publications of “Origin and evolutionary process of the CNS illustrated by comparative genomics analysis of planarian ESTs”
On June 24, PNAS published this study by the Laboratory for Evolutionary Regeneration Biology providing evidence of genetic roots for the central nervous system in a primitive bilaterian, the flatworm Dugesia japonica.

July 2
Scientific exchange agreement with Temasek Life Sciences Laboratory Limited
The CDB established formal ties with the Temasek Life Sciences Laboratory in Singapore, a non-profit organization with affiliations to the National University of Singapore and Nanyang Technological University established to conduct molecular biology and genetics research.

July 10
Production of “An NDPase links ADAM protease glycosylation with organ morphogenesis in C. elegans”
In a study published in the online edition of Nature Cell Biology, the Laboratory for Cell Migration, under team leader Kiyoji Nishiwaki, reported the role of a protein modification known as glycosylation in guiding the migration of gonadal cells in the nematode, C. elegans.

August 19
Publication of “Traveling stripes on the skin of a mutant mouse”
The August 19 issue of PNAS featured a cover image taken from a research article from Shigeru Kondo’s lab describing the mathematical properties of a strikingly patterned mutant mouse strain.

August 27-9
Kobe BioWeek
The city of Kobe played host to more than 8,000 visitors participating in Kobe BioWeek, a series of meetings and events focusing on the growth and future directions of the biotechnology sector in the region and around Japan. The CDB joined in the public education activities, with two-way videoconference lectures and Q&A sessions, hands-on simulations of lab equipment and a virtual lab tour program using web cameras that allowed the public to watch CDB labs at work in real-time.

October 1
RIKEN Becomes Independent Administrative Institution
As part of a restructuring of Japanese public corporations, RIKEN was officially re-designated as an independent administrative institution, a move intended to simultaneously increase the Institute’s autonomy and accountability. As part of this transition, the Nobel Prize-winning chemist Ryoji Noyori was named the new president, ushering in a new era for RIKEN, which was first established in 1917.

October 20-1
2nd CDB Retreat
The CDB held its second annual retreat on the 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 two days of oral presentation and poster sessions.
HIGHLIGHTS

April 1
New Graduate School Affiliations
The CDB formalized its affiliations with the Kyoto University Graduate School of Medicine and the NAIST Graduate School of Biological Sciences, allowing doctoral students from those schools to conduct their thesis research in CDB laboratories under the guidance of lab heads designated as visiting professors.

May 13
Publication of “Generation of neural crest-derived peripheral neurons from mouse and primate embryonic stem cells.”
In a study published in the May 13 issue of the Proceedings of the National Academy of Sciences (PNAS), the Sasai research group demonstrated the application of stromal cell derived inducing activity (SDIA) in the high-efficiency induction of peripheral neurons from embryonic stem cells.

May 24 - 26
First CDB Symposium: Origin and Formation of Multicellular Systems
The CDB held its first annual symposium at the nearby Pompia Hotel. The meeting drew more than 200 speakers and attendees from around the world, with plenary presentations and lively discussion on the origins of metazoan biology, the fundamental processes of development and regeneration and the formation of higher-order structures, such as neural networks.

June 24
Publication of “Origin and evolutionary process of the CNS illustrated by comparative genomics analysis of planarian ESTs.”
On June 24, PNAS published this study by the Laboratory for Evolutionary Regeneration Biology providing evidence of genetic roots for the central nervous system in a primitive bilaterian, the freshwater Dugesia japonica.

July 2
Scientific exchange agreement with Temasek Life Sciences Laboratory Limited
The CDB established formal ties with the Temasek Life Sciences Laboratory in Singapore, a non-profit organization with affiliations to the National University of Singapore and Nanyang Technological University established to conduct molecular biology and genetics research.

July 13
Publication of “Traveling stripes on the skin of a mutant mouse.”
The August 19 issue of PNAS featured a cover image taken from a research article from Shigeru Kondo’s lab describing the mathematical properties of a strikingly patterned mutant mouse strain.

August 27 - 9
Kobe BioWeek
The city of Kobe played host to more than 8,000 visitors participating in Kobe BioWeek, a series of meetings and events focusing on the growth and future directions of the biotechnology sector in the region and around Japan. The CDB joined in the public education activities, with two-way videoconference lectures and Q&A sessions, hands-on simulations of lab equipment and a virtual lab tour program using web cameras that allowed the public to watch CDB labs at work in real time.

October 1
RIKEN Becomes Independent Administrative Institution
As part of a restructuring of Japanese public corporations, RIKEN was officially re-designated as an independent administrative institution, a move intended to simultaneously increase the Institute’s autonomy and accountability. As part of this transition, the Nobel Prize-winning chemist Yuji Noyori was named the new president, ushering in a new era for RIKEN, which was first established in 1917.

October 20 -1
2nd CDB Retreat
The CDB held its second annual retreat on the 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 two days of oral presentation and poster sessions.

December 21
Publication of “An NDPase links ADAM protease glycosylation with organ morphogenesis in C. elegans.”
In work published in the online edition of Nature Cell Biology, the Laboratory for Cell Migration, under team leader Hiroyi Nishita, reported the role of a protein modification known as glycosylation in guiding the migration of gonadal cells in the nematode, C. elegans.
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 led by an eminent scientist. 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.
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 led by an eminent scientist. 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.
Evolutionary regeneration biology

Its position on an important but scientifically under-explored branch of the evolutionary tree and its remarkable biology combine to make the planarian flatworm a fascinating and invaluable model for basic research in fields from evolutionary development to regenerative medicine. Kiyokazu Agata has adopted the freshwater planarian, Dugesia japonica, as a model species in his study of the origins and properties of stem cells, which are prevalent and experimentally accessible in these worms and which he believes hold the key to understanding the development, organization and maintenance of the cellular diversity that characterizes metazoan life.

Isolating planarian stem cells
Agata’s laboratory has developed a method for identifying subsets of planarian stem cells, also called neoblasts. These somatic stem cells are the only mitotically active cells in the planarian body, making them susceptible to X-ray irradiation. Examination of cell populations shown to be vulnerable to elimination by X-rays revealed that planarid neoblasts, like stem cells in other species, seem to exist in proliferating and resting states. Real-time PCR analysis indicates that each of these sub-populations expresses discrete sets of genes. One fraction, X1, expresses genes specific to actively proliferating cells, while a second fraction, X2, lacks this expression profile and is thought to comprise stem cells in a state of quiescence. Interestingly, many X1 cells also express signal receptor molecules which are switched off in the X2 fraction, while the corresponding ligands are expressed in a variety of differentiated cells resistant to X-ray irradiation.

Brain regeneration by stages
A planarian can re-grow a fully functional brain within five days following the amputation of its entire head, an extraordinary feat of self-healing that involves recapitulating the development of the worm’s entire nervous system. Researchers in the Agata group studied this process and identified patterns of gene expression that indicate the regeneration of the brain comprises five distinct stages. In the first stage, about eight hours after wound closure, a noggin-like gene (Dpgg4) is activated in the stump prior to the formation of a blastema, a mass of proliferating undifferentiated cells that serves as the frontline of regeneration. Soon thereafter, the brain-specifying gene nou-darake is switched on in the anterior fringe of the blastema, allowing the brain rudiment to be formed. These first two steps occur within 24 hours of decapitation, and set the stage for the third phase in which brain patterning is established by the expression of a set of three otd/otd-related genes (relatives of which also function to pattern brain development in many other taxa, including vertebrates) followed by that of the planarian homolog of Wnt that of the planarian homolog of Wnt.

In the fourth step, which occurs by day four of regeneration, a homolog of the axon-guidance gene netrin is triggered. At this stage the discrete components of the regenerating brain begin to form connections and higher-order organizations; chiasmatasia cross-linking eyes and brain develop, and connections are set up between the brain and the ventral nerve cords, which serve as a rudimentary peripheral nervous system. Thus, the basic components and connections of the planarian neural network are in place by the fourth day, but the functional recovery of the brain is not completed until a final stage in which two newly-identified genes are expressed is entered.

A parallel study revealed that these two genes, H202DD and ey53, are necessary for planarians to regain their normal light avoidance behavior, known as negative phototaxis. Knockdown of these genes by RNAi had no discernible effects on brain or eye morphology, but the worms failed to respond to light stimuli in the normal manner, even after five days of regeneration, when the structure of the brain has been fully reinstated.

Evolution of the central nervous system
The planarian is one of the lowliest forms of animal known to possess a central nervous system, making it an apt model for the study of the evolution of this most complex and elaborately organized biological system. Using clones of over 10,000 expressed sequence tags (ESTS) isolated from the planarian head region, the team was able to identify 116 clones with significant similarities to genes closely linked to the nervous system in other species, including genes involved in neurotransmission, the neural network, brain morphogenesis and neural differentiation. These results point toward a shared evolutionary origin for many nervous system genes, indicating that the nervous system is likely to have arisen in a single common ancestor of bilaterian animals. Intriguingly, nearly 30% of the genes identified also have homologous sequences in yeast and the plant species, Arabidopsis thaliana, both of which entirely lack neural development, which suggests that a significant number of genes that predate the advent of the nervous system have been co-opted to perform functions specific to neurobiology.

The Agata lab is also now participating in an international collaboration established to sequence and annotate a set of more than 40,000 planarian ESTs, which promises to provide an invaluable resource for the study of evolutionary biology, comparative genomics, and the genetics underlying the unique characteristics of these organisms. The availability of an annotated database of planarian cDNAs will provide keys to the understanding of stem cell biology, tissue plasticity and maintenance and other fundamentally important processes.

Performances

Some image elements and references are not included in the text.

---

**Evolutionarily conserved expression patterns of planarian (right) and Xenopus (left) planaria (Takahiro Murakami)**

**Expression of various neuron-related genes**

**Genes involved in brain differentiation**

**Functional regionalization of the planarian brain**

---

**Publications**

Evolutionary regeneration biology

**Isolating planarian stem cells**

Agata's laboratory has developed a method for identifying subsets of planarian stem cells, which are capable of regenerating the whole body. These cells are characterized by their expression of specific genes and their ability to form new tissues and organs.

**Brain regeneration by stages**

A planarian can re-grow a fully functional brain with five stages following the amputation of its entire head, an extraordinary feat of self-healing that involves recapitulating the development of the worm's entire nervous system. Researchers in the Agata group studied this process and identified patterns of gene expression that indicate the regeneration of the brain comprises five distinct stages. In the first stage, at about eight hours after wound closure, a noggin-like gene (Djog) is activated in the stump prior to the formation of a blastema, a mass of proliferating undifferentiated cells that serves as the front line of regeneration. Soon thereafter, the brain-specific gene noggin-daruble is activated on the anterior fringe of the blastema, allowing the brain rudiment to be formed. The neural differentiation occurs within 24 hours of decapitation, and the stage for the third phase in which brain patterning is established by the expression of a set of three otd/Cdx-related genes (relatives of which also function to pattern brain development in many other taxa, including vertebrates) followed by that of the planarian homolog of Wnt, which two newly-identified genes are expressed is place by the fourth day, but the functional recovery of the brain is not completed until a final stage in which two newly-identified genes are expressed is entered.

A parallel study revealed that these two genes, 1020HH and ey53, are necessary for planarians to regain their normal light avoidance behavior, known as negative phototaxis. Knockdown of these genes by RNAi had no discernible effects on brain or eye morphology, but the worms failed to respond to light stimuli in the normal manner, even after five days of regeneration, when the structure of the brain has been fully remodeled.

**Evolution of the central nervous system**

The planarian is one of the low-est forms of animal known to possess a central nervous system, making it an apt model for the study of the evolution of this most complex and elaborately organized biological system. Using clones of over 3,000 expressed sequence tags (ESTs) isolated from the planarian head region, the team was able to identify 116 clones with significant similarities to genes closely linked to the nervous system in other species, including genes involved in neurotransmission, the neural network, brain morphogenesis and neural differentiation. These results point toward a shared evolutionary origin for many nervous system genes, indicating that the nervous system is likely to have arisen in a single common ancestor of bilaterian animals, intriguingly, nearly 30% of the genes identified also have homologous sequences in yeast and the plant species, Arabidopsis thaliana, both of which entirely lack neural development, which suggests that a significant number of genes that predate the advent of the nervous system have been co-opted to perform functions specific to neurobiology.

**A planarian can re-grow a fully functional brain within five days following the amputation of its head**

The Agata lab is also now participating in an international collaboration established to sequence and annotate a set of more than 10,000 planarian ESTs, which promises to provide an invaluable resource for the study of evolutionary biology, comparative genomics, and the genetics underlying the unique characteristics of these organisms. The availability of an annotated database of planarian cDNAs may provide keys to the understanding of stem cell biology, tissue plasticity and maintenance and other fundamentally important processes.

**Functional regionalization of the planarian brain**

Evolutionarily conserved expression patterns of planarian (right) and Xenopus (left) dorsal forebrain (DvFR) (right)

Expression of various neural-related genes
Despite their many differences, taxa as diverse as fish, amphibians, reptiles, birds and mammals share a common body plan comprising three regions: the trunk, the hindbrain/brainpan region, and the rostral head. Shin Aizawa is interested in the molecular bases for and phylogenetic origins of this regionalization, concentrating primarily on the genetic activity and molecular attributes of head development.

All animals develop from the head. The formation of inductive head organizer precedes that of the trunk organizer, as was first demonstrated by Spemann and Mangold in their studies of amphibian embryogenesis. Gene knockout studies in mice, have demonstrated that the rhombomeres r12 is the ground state in the mammalian body plan. The head organizer induces the rostral head anteriorly to this rhombencephalon, while the trunk organizer guides trunk development caudally to the same r12 landmark. Studying mutations in genes responsible for body patterning in this region, the Aizawa research group hopes to reveal the genetic cascades for the constitution of the anterior head as conserved across vertebrate phyla.

The development of the head traces back to before gastrulation, and is inseparably linked to the formation of the anterior-posterior axis. In mice, this process begins prior to gastrulation when the cells of the distal ventral endoderm migrate to the region that will become the animal’s anterior, forming the anterior visceral endoderm (AVE), which suppresses posteriorizing signals in the adjacent epithelium. In previous work, members of the Aizawa lab demonstrated that Otx2 plays an essential role in triggering this anterior migration. Following gastrulation, organizing centers in the epiblast induce the formation of the anterior neuroectoderm, which subsequently regionalizes into multiple primordia structures destined to form the areas of the brain. In this process, the isthmus and anterior neural ridge act as local organizing centers for mid- and forebrain development.

Otx2 functions as a master gene in vertebrate head development, and the molecular bases for the processes of anterior-posterior axis formation, head induction, brain regionalization and cortical development.

**Roles of Otx2 in head development**

Otx2 plays central roles in each step of head development in vertebrates, but the regulatory mechanisms by which this gene’s activity is mediated have remained largely unknown. The development of the head traces back to before gastrulation, and is inseparably linked to the formation of the anterior-posterior axis. In mice, this process begins prior to gastrulation when the cells of the distal ventral endoderm migrate to the region that will become the animal’s anterior, forming the anterior visceral endoderm (AVE), which suppresses posteriorizing signals in the adjacent epithelium. In previous work, members of the Aizawa lab demonstrated that Otx2 plays an essential role in triggering this anterior migration. Following gastrulation, organizing centers in the epiblast induce the formation of the anterior neuroectoderm, which subsequently regionalizes into multiple primordia structures destined to form the areas of the brain. In this process, the isthmus and anterior neural ridge act as local organizing centers for mid- and forebrain development.

Otx2 functions as a master gene in each phase of head ontogeny. Aizawa and colleagues have been working to identify and characterize regulatory factors that control the gene’s expression in specific sites and stages of development. In the past, the lab found enhancers responsible for promoting Otx2 expression in the visceral endoderm, definitive anterior mesendoderm and the cecal neural crest cells. These cis-regulatory elements were all located relatively near the transcriptional start site for the gene. More recently, the group has identified and analyzed enhancers that guide Otx2 expression in epiblast, anterior neuroectoderm and fore- and midbrain, which they named the EP, AN and FM enhancers, respectively. All of these elements are located more remotely from the coding region (more than 80 kb upstream) than the previously identified Otx2 enhancers.

**The development of the head is inseparably linked to the formation of the anterior-posterior axis**

The activity of the EP enhancer is independent of that of the EP enhancer, and plays an essential role in maintaining the anterior neuroectoderm through Otx2 expression, once that region has been induced. The Aizawa group’s studies have also indicated a phylogenetic relationship between these elements, in which FM is the most deeply conserved across the gnathostome (jawed vertebrate) lineage, while the epiblast enhancer appears to have been acquired later, perhaps after the ascendance of amphi- and, to include the anterior neuroectoderm enhancer as an essential component. Using these results as a springboard, Aizawa and colleagues have begun to investigate the significance of this phylogenetic specialization of enhancers in terms of the evolution of the vertebrate head, and to continue the search for upstream factors at work in the regulation of Otx2.

---

**Emx genes in early cortical development**

In the mouse, the development of the cerebral cortex is immediately preceded by the closure of the anterior neural plate at around E8.5 in the presumptive forebrain/midbrain junction. In the earliest stages of corticogenesis, the structures of the archipallium, the non-neuronal components of the choroid plexus, the hippocampal complex and the fimbria, are generated. It has been suggested that the last of these structures, the fimbria, are located at the border of the cerebral cortex and the choroid plexus, as a local signaling center in cortical development. One model of archipallial patterning involves the expression of ligands, receptors, transcriptional factors and inhibitor molecules to form morphogenetic gradients that direct the differentiation of areas on either side of the cortical hem, but the actions of specific players in this model remain to be worked out. The Aizawa group’s studies of Em1 and Em2, mouse homologs of the Drosophila head gap gene, have shown that these two genes cooperate in two phases of cortical development. Previous work demonstrated that Em1 and Em2 work together to generate Cajal-Retzius cells and subplate neurons. Recent work now indicates that the two genes also play a combinatorial role in establishing the archipallium as distinct from the roof plate, immediately following the closure of the neural tube.
Despite their many differences, taxa as diverse as fish, amphibians, reptiles, birds and mammals share a common body plan comprising three regions: the trunk, the hindbrain/brainyglial region, and the rostral head. Shin Aizawa is interested in the molecular bases for and phylogenetic origins of this regionalization, concentrating primarily on the genetic activity and molecular attributes of head development.

All animals develop from the head. The formation of inductive head organizer precedes that of the trunk and gut, and is first demonstrated by Spemann and Mangold in their studies of amphibian embryogenesis. Genetic knockout studies in mice, have demonstrated that the rhombomeres r1-2 is the ground state in the mammalian body plan. The head organizer induces the rostral head anteriorly to this rhombomere, while the trunk organizer guides trunk development caudally to the same r1/2 landmark. Studying mutations in genes responsible for body patterning in this region, the Aizawa research group hopes to reveal the genetic cascades for the constitution of the anterior-posterior axis. In mice, this process begins prior to gastrulation when the cells of the distal ventral endoderm migrate to the region that will become the animal’s anterior, forming the anterior visceral endoderm (AVE), which suppresses posteriorizing signals in the adjacent epiblast. In previous work, members of the Aizawa lab demonstrated that Otx2 plays an essential role in triggering this anterior migration. Following gastrulation, organizing centers in the epiblast induce the formation of the anterior neuroectoderm, which subsequently regionalizes into multiple primitive structures destined to form the areas of the brain. In this process, the isthmus and anterior neural ridge act as local organizing centers for mid- and forebrain development.

Otx2 functions as a master gene in vertebrate head development, and the molecular bases for the processes of anterior-posterior axis formation, head induction, brain regionalization and cortical development.

**Roles of Otx2 in head development**

Otx2 plays central roles in each step of head development in vertebrate, but the regulatory mechanisms by which this gene’s activity is mediated have remained largely unknown. The development of the head traces back to before gastrulation, and is inseparably linked to the formation of the anterior-posterior axis. In mice, this process begins prior to gastrulation when the cells of the distal ventral endoderm migrate to the region that will become the animal’s anterior, forming the anterior visceral endoderm (AVE), which suppresses posteriorizing signals in the adjacent epiblast. In previous work, members of the Aizawa lab demonstrated that Otx2 plays an essential role in triggering this anterior migration. Following gastrulation, organizing centers in the epiblast induce the formation of the anterior neuroectoderm, which subsequently regionalizes into multiple primitive structures destined to form the areas of the brain. In this process, the isthmus and anterior neural ridge act as local organizing centers for mid- and forebrain development.

Otx2 functions as a master gene in each phase of head ontogeny. Aizawa and colleagues have been working to identify and characterize regulatory factors that control the gene’s expression in specific sites and stages of development. In the past, the lab found enhancers responsible for promoting Otx2 expression in the visceral endoderm, definitive anterior mesendoderm and the cephalic neural crest cells. These cis-regulatory elements were all located relatively near the transcriptional start site for the gene. More recently, the group has identified and analyzed enhancers that guide Otx2 expression in epiblast, anterior neuroectoderm and fore- and midbrain, which they have named the EP, AN and FM enhancers, respectively. All of these elements are located more remotely from the coding region (more than 80 kb upstream) than are the previously identified Otx2 enhancers.

**The development of the head is inseparably linked to the formation of the anterior-posterior axis**

The activity of the EP enhancer is independent of that of the EP enhancer, and plays an essential role in maintaining the anterior neuroectoderm through Otx2 expression, once that region has been induced. The Aizawa group’s studies have also indicated a phylogenetic relationship between these elements, in which FM is the most deeply conserved in the mouse, while EP has been acquired later, perhaps after the ascension of amphibians, and to include the anterior neuroectoderm enhancer as an essential component. Using these results as a springboard, Aizawa and colleagues next plan to investigate the significance of this phylogenetic specialization of enhancers in terms of the evolution of the vertebrate head, and to continue the search for upstream factors at work in the regulation of Otx2.

Emx genes in early cortical development

In the mouse, the development of the cerebral cortex is immediately preceded by the closure of the anterior neural plate at around E8.5. The pre- and post-midbrain-fornix junction. In the earlier stages of corticogenesis, the structures of the archipallium, the non-neuronal components of the choroid plexus, the hippocampal complex and the fimbria, are generated. It has been suggested that the last of these structures, the fimbria, which are located at the border of the cerebral cortex and the choroid plexus, as a local signaling center in cortical development. One model of archipallial patterning involves the expression of ligands, receptors, transcriptional factors and inhibitor molecules to form morphogenetic gradients that direct the differentiation of areas on either side of the cortical hem, but the actions of specific players in this model remains to be worked out. The Aizawa group’s studies of Em1 and Em2, mouse homologs of the Drosophila head gap genes, have shown that these two genes cooperate in two phases of cortical development. Previous work demonstrated that Em1 and Em2 work together to generate Cajal-Retzius cells and subplate neurons. Recent work now indicates that the two genes also play a combinatory role in establishing the archipallium as distinct from the roof plate, immediately following the closure of the neural tube.
Japan for Genetic Studies of the Genetics Society of received the incentive prize in 1999. Also in 1999, he became an associate professor of Genetics. He University of Colorado Matthew Scott’s lab at the three years as a postdoctoral fellow. His research focus changed in 1987 for his work on the chicken delta crystallin matrix. The means by which cells are able to aggregate and work together to build a body are central to the study of morphogenesis, the focus of Hayashi’s research in the Hayashi laboratory.

The regulation of epithelial adhesion

Epithelial tissues are constructed from sheets of cells with distinct apical-basal (top-bottom) polarities, which form junctions with each other and interact with the extracellular matrix, the macromolecular environment in the spaces between cells. A range of vital developmental processes, including the abilities to build complex structures through selective cell adhesion, to move through the body, and to form epithelial tissues rely on interactions between cells and other cells, and with the extracellular matrix. The means by which cells are able to achieve these feats as they communicate, congregate and work together to build a body are central to the study of morphogenesis, the focus of research in the Hayashi laboratory.

The group found that reductions in Rac activity led to increases in the amount and range of cadherin cell adhesion molecules in both embryonic epidermis and tracheal epithelia, which they attribute to a post-transcriptional mechanism and changes in epithelial structural organization. This strengthening of intercellular adhesion was accompanied by a reciprocal decrease in the ability of epithelial cells to rearrange dynamically. Conversely, when they overexpressed the constitutively active form of Rac in tracheal cells, the researchers found that cadherins decreased and eventually disappeared from cell junction regions, resulting in the loss of tracheal cell adhesion and the detachment of these cells from the epithelium. These complementary findings strongly suggest that a strictly maintained balance of Rac activity is essential in determining the proper junctional assembly and disassembly of cadherins in the Drosophila tracheal epithelium, a role it is thought to fulfill by regulating cell adhesion and motility to ensure the appropriate remodeling of epithelial sheets into tubules.

A number of other signaling molecules that function in the tracheal system are known to cause gain-of-function phenotypes when overexpressed. In work related to the Rac study, the Hayashi lab has conducted a gain-of-function screen for genes that disrupt the tracheal epithelium on overexpression. The lab has identified several candidate genes whose hyperactivation leads to disruptions in epithelial integrity, including genes encoding regulators of actin dynamics, cell adhesion and cell motility. The functions of these candidate genes are now being analyzed, with the goal of developing an improved understanding of the genetic control of epithelial morphogenesis.

Leg and wing specification

The insect leg is an evolutionary innovation that is thought to have arisen as a derivative of the leg during an early stage in the evolution of the insect body plan. The close relationship between the wing and leg can be observed in the embryogenesis of Drosophila, which is a highly derived winged insect. It has been shown that wing and leg both derive from a common precursor structure called the limb primordium. This structure gives rise to both the limb and wing imaginal discs, pouches of undifferentiated cells in the larva that form the leg and wing in the adult.

The Hayashi lab has investigated the role of Wingless signaling in the wing and leg primordia. The Wg protein, which mediates a wide range of inductive interactions in Drosophila, is known to be required for the induction of imaginal discs during embryonic development. In the leg imaginal disc, Wg serves as an axis determinant, triggering dorsal-ventral pattern formation by specifying the ventral region of the leg, and by activating the Distal-less (Dl) gene in the dorsal region. However, Wg’s roles in leg development have not been fully explored.

In a study published this year, the Hayashi group determined that Wg signaling is required for proximal-distal patterning during embryonic leg development, a role it performs by helping to recruit cell types specific to the proximal and distal domains. This finding counters previous work that suggested Wg plays no role in late embryonic leg disc development, and led Hayashi to propose a new model for leg specification in which dorsal-ventral, proximal-distal and cell types are distinguished by differences in the expression domains of specific genes, including Wg. The group also found that ectopic Wg signaling inhibits wing disc development; ectopic activation of the Wg signal in the wing disc causes a reduction in the number of wing cells that develop. These results indicate that Wg determines leg cell specification while inhibiting that of the wing, thereby playing the role of a crucial binary switch in the establishment of wing and leg identities.

Time course of tracheal development. Time lapse pictures of an embryonic expressing GFP in the tracheal system.

Ventral view of the thoracic region of a Drosophila embryo. Expression of Wingless (purple) expression overlay the leg primordia (green) and specify the proximal leg identity.
**Core Program**

**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 institute in 1987 for his work on a semi-specific regulation of the chicken alpha crystallin gene. Inspired by the discovery of the homeobox genes, he moved to the laboratory of Matthew Scott at the Institute of Genetics of the University of Berne in Switzerland, where he changed his research focus to the developmental genetics of Drosophila and spent three years as a postdoctoral researcher in Matthew Scott’s lab at the University of Colorado. Before returning to Japan to join the RIKEN CDB in May 1994, he was a research associate in Matthew Scott’s lab at the RIKEN CDB in Japan for Genetic Studies.

In 1999, he received the incentive prize of the Genetics Society of Japan. In 1994, and professor in 1999, and professor at the same Institute of Genetics. He was named group leader in the developmental genetics research group in the Hayashi laboratory.

**morphogenetic signaling**

Epithelial tissues are constructed from sheets of cells with distinct apical-basal (top-bottom) polarities, which form junctions with each other and interact with the extracellular matrix, the macromolecular environment in the spaces between cells. A range of vital developmental processes, including the abilities to build complex structures through selective cell adhesion, to move through the body, and to form epithelial tissues rely on interactions between cells and other cells, and with the extracellular matrix. The means by which cells are able to achieve these feats as they communicate, congregate and work together to build a body are central to the study of morphogenesis, the focus of research in the Hayashi laboratory.

**The degradation of epithelial adhesion**

The ability of epithelial cells to attach to and desengage from their neighbors is closely linked to the activity of cadherin family molecules, which span the cell membranes to regulate this dynamic cell-cell adhesion. A number of studies have also indicated roles for the small GTPase Rac as another key regulator of cell adhesion, but these roles have yet to be confirmed in the context of embryonic development. In work published in 2003, members of the Hayashi research group reported the results of their investigations into Rac’s role in epithelial cell rearrangement in the development of the Drosophila tracheal system, a tubular network formed from epithelium.

The group found that reductions in Rac activity led to increases in the amount and range of cadherin cell adhesion molecules in both embryonic epidermis and tracheal epithelia, which they attribute to a post-transcriptional mechanism and changes in epithelial structural organization. This strengthening of intercellular adhesion was accompanied by a reciprocal decrease in the ability of epithelial cells to rearrange dynamically. Conversely, when they overexpressed the constitutively active form of Rac in tracheal cells, the researchers found that cadherins decreased and eventually disappeared from cell junction regions, resulting in the loss of tracheal cell adhesion and the detachment of these cells from the epithelium. These complementary findings strongly suggest that a strictly maintained balance of Rac activity is essential in determining the proper junctional assembly and disassembly of cadherins in the Drosophila tracheal epithelium, a role it is thought to fulfill by regulating cell adhesion and motility to ensure the appropriate remodeling of epithelial sheets into tubules.

A number of other signaling molecules that function in the tracheal system are known to cause gain-of-function phenotypes when overexpressed. In work related to the Rac study, the Hayashi lab has conducted a gain-of-function screen for genes that disrupt the tracheal epithelium on overexpression. The lab has identified several candidate genes whose hyperactivation leads to disruptions in epithelial integrity, including genes encoding regulators of act dynamics, cell adhesion and cell motility. The functions of these candidate genes are now being analyzed, with the goal of developing an improved understanding of the genetic control of epithelial morphogenesis.

**Leg and wing specification**

The insect wing is an evolutionary innovation that is thought to have arisen as a derivative of the leg during an early stage in the evolution of the insect body plan. The close relationship between the wing and the leg can be observed in the embryogenesis of Drosophila, which is a highly derived winged insect. It has been shown that wing and leg both derive from a common precursor structure called the limb primordium. This structure gives rise to both the limb and wing imaginal discs, pouches of undifferentiated cells in the larna that form the leg and wing in the adult.

The Hayashi lab has investigated the role of Wingless (Wg) signaling in leg and wing primordia. The Wg protein, which mediates a wide range of developmental modes and lesion reactions of Drosophila. The Wg signaling is required for the induction of imaginal discs during embryonic development. The Wg ligand and Wg receptor are expressed in the developing limb and wing imaginal discs, which they attribute to a post-transcriptional mechanism and changes in epithelial structural organization. This strengthening of intercellular adhesion was accompanied by a reciprocal decrease in the ability of epithelial cells to rearrange dynamically. Conversely, when they overexpressed the constitutively active form of Rac in tracheal cells, the researchers found that cadherins decreased and eventually disappeared from cell.

The Wg ligand has been shown to act in a trans-signaling role, affecting the development of the leg and wing through the reception of Wg signaling. The Wg signaling is also required for the induction of imaginal discs during embryonic development, and is thought to have arisen as a derivative of the leg during an early stage in the evolution of the insect body plan. The close relationship between the wing and the leg can be observed in the embryogenesis of Drosophila, which is a highly derived winged insect. It has been shown that wing and leg both derive from a common precursor structure called the limb primordium. This structure gives rise to both the limb and wing imaginal discs, pouches of undifferentiated cells in the larna that form the leg and wing in the adult.

Leg and wing specification is an evolutionary innovation that is thought to have arisen as a derivative of the leg during an early stage in the evolution of the insect body plan. The close relationship between the wing and the leg can be observed in the embryogenesis of Drosophila, which is a highly derived winged insect. It has been shown that wing and leg both derive from a common precursor structure called the limb primordium. This structure gives rise to both the limb and wing imaginal discs, pouches of undifferentiated cells in the larna that form the leg and wing in the adult.

**Ventral view of the Drosophila embryo.** Stained with Wg and HRP to show the leg primordium (green) and specify the proximal leg identity.

In a study published this year, the Hayashi group determined that Wg signaling is required for proximal-distal patterning during embryonic leg development, a role it performs by helping to recruit cell types specific to the proximal and distal domains. This finding counters previous work that suggested Wg plays no role in late embryonic leg disc development, and led Hayashi to propose a new model for leg specification in which dorsal-proximal, ventral-distal and proximal-distal domains are distinguished by differences in the expression domains of specific genes, including Wg. The group also found that ectopic Wg signaling inhibits wing disc development; ectopic activation of the Wg signal in the wing disc causes a reduction in the number of wing cells that develop. These results indicate that Wg determines leg cell specification while inhibiting that of the wing, thereby playing the role of a crucial binary switch in the establishment of wing and leg identities.

**Time course of tracheal development.** Time lapse pictures of an embryo expressing GFP in the tracheal system.

**Publications**

<table>
<thead>
<tr>
<th>Publication</th>
<th>Title</th>
</tr>
</thead>
</table>
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.

Neural cell fate determination in Drosophila

Fumio Matsuzaki has dedicated his research to exploring 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, Matsuzaki and others demonstrated that the fates of daughter neural cells are in large part determined by unequal distribution of 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 localization, 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.

Neural cell fate determination in Drosophila

Fumio Matsuzaki has dedicated his research to exploring 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, Matsuzaki and others demonstrated that the fates of daughter neural cells are in large part determined by unequal distribution of 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 localization, 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.

Neural cell fate determination in Drosophila

Fumio Matsuzaki has dedicated his research to exploring 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, Matsuzaki and others demonstrated that the fates of daughter neural cells are in large part determined by unequal distribution of 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 localization, 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.

Neural cell fate determination in Drosophila

Fumio Matsuzaki has dedicated his research to exploring 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, Matsuzaki and others demonstrated that the fates of daughter neural cells are in large part determined by unequal distribution of 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 localization, 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.

Asymmetric cell sizes

This last question prompted investigations that led to the identification of a new regulatory function for 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 chromosomes are drawn to the spindle poles through the action of molecular motors known as microtubules. In work carried out at the CDB, Matsuzaki’s lab has found that G protein-signaling pathways 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 G protein in question form a symmetrical mitotic spindle and produce daughter cells of equal size with the determinants being normally segregated. This provides a valuable platform for further studies in fields ranging from microtubule dynamics to animal morphogenesis.

The fates of daughter neural cells are in large part determined by unequal distribution of factors within the mother cell during the process of cell division.

In the developing mouse spinal cord, Prospero and Numb (green) are transiently expressed in newborn neurons immediately after birth from the mitotic neural progenitor cell.
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.

Neural cell fate determination in Drosophila

Fumio Matsuzaki has dedicated his research to exploring 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, Matsuzaki and others demonstrated that the fates of daughter neural cells are in large part determined by unequal distribution of 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 cortex of the neuroblast (the neural progenitor) and promote the asymmetric division of neurons. This protein complex, which includes the aPKC and heterotrimeric G protein, plays a critical role in orienting the mitotic spindle. Thus multiple intracellular signals operate coordinately and differentially to create intracellular asymmetry, and promote the asymmetric division of neural cells. The Matsuzaki lab is now confronting questions regarding the roles of extrinsic signals and intrinsic players, and the mechanism by which cells of different sizes are generated in the process of mitosis.

Coordinating asymmetric division

The basal localization of determinants is by itself not sufficient for the parental neuroblast to segregate cell fate determinants to the daughter (GMC). It is also necessary for the division to occur along the axis of polar distribution of the Miranda-Prospero complex. This is achieved by the coordinated alignment of the mitotic spindle along the apicobasal axis. In the neuroblast, both the asymmetric localization of cell fate determinants and spindle orientation are governed by a multi-protein complex that creates intracellular asymmetry. This protein complex is known as the “apical complex” for the site of its localization in the neuroblast, includes two different signaling complexes: atypical protein kinase C (aPKC) and heterotrimeric G protein. The Matsuzaki lab recently demonstrated that the two signaling pathways included in the apical complex play differential roles in the asymmetric segregation of the determinants. aPKC signaling is essential for the localization of the basal Miranda-Prospero complex, while the Pir protein, known to be associated with an alpha subunit of the G protein in the apical complex, plays a critical role in orienting the mitotic spindle. Thus multiple intracellular signals operate coordinately and differentially to create intracellular asymmetry, and promote the asymmetric division of neural cells. The Matsuzaki lab is now confronting questions regarding the roles of extrinsic signals and intrinsic players, and the mechanism by which cells of different sizes are generated in the process of mitosis.
stem cell biology

Stem cell biology

Stem cell types, characterized by their abilities to self-renew and to generate more highly specialized cells indefinitely, are the key to replenishing the body’s cells. The study of these cells stands at the heart of some of the central questions in development, but many aspects of stem cell biology, such as the limits (if any) of the ability to reprogram them to produce progeny cells of diverse lineages, and the identities of the intrinsic and extrinsic molecular factors that regulate a cell’s decisions whether to maintain stemness or to commit to differentiation have yet to be resolved.

A stem cell’s location and immediate environment can be important factors in defining its fate. Recent findings that stem cells may arise from one tissue type and then later be directed to give rise to another strongly substantiate the model in which a stem cell’s behavior and properties are influenced by external signals. By studying the microenvironments, known as ‘niches,’ in which stem cells originate, abide and proliferate, the Nishikawa research group seeks to gain insight into the identity and function of molecules involved in stem cell activity, and the potential of stem cell-based therapeutic applications.

Characterizing the stem cell niche

Working to isolate the defining characteristics of specific stem cell niches, the Nishikawa group has chosen melanocyte stem cells as one of the main model systems for their research, as melanocytes are readily identified by the presence of a melanocyte-specific promoter, Dct, and are amenable to experimental manipulations that allow specific cell types to be isolated from mixed populations. As the existence of niche cells and the identification of their characteristics can only be determined by first isolating the stem cells they support, Nishikawa’s lab has developed an approach in which stem cells are dissected from their surroundings, dissociated and used to construct single cell libraries, a process involving the technically challenging initial step of isolating single intact hair follicles from the skin. Targeted GFP expression is next used to label the follicle’s resting stem cells, making them excel lent sites for studies of niche properties. Each of these areas hosts a specific sub-population of stem cells. The niche region, the site of active hair growth and pigmentation, provides an environment in which amplying progeny become committed to a stem cell fate, while the bulge serves as a reservoir for stem cells in their resting, or quiescent, state. Subtractive comparisons of cDNAs from stem cell, differentiated cell and ‘niche-like’ (keratinocyte) libraries have been performed to identify genes whose expression is switched on or off, depending on the pathway a cell has followed and the stage it has reached. Analysis of the stages in this stepwise process has also made it possible to develop a system for guiding differentiation from ES cell to endodermal, mesenchymal or mesodermal (paraxial and lateral) fates in vitro using combinations of extrinsic factors. By characterizing the identities of cells at each step on each branch of the lineage tree and collecting samples to produce DNA arrays, the Nishikawa lab is working to build a database of cDNA profiles of the intermediate stages in ES cell differentiation in culture, which can be queried and visualized using software developed in the lab. Analysis of this cDNA chip database promises to lead to a better understanding of the process of endodermal differentiation, and of stem cell specification in general. This powerful new DNA analysis software application, which promises to make a great contribution to the harmonization of multiple DNA expression databases, is being developed in collaboration with the Institute of Biomedical Research and Innovation, located adjacent to the CDB in the Kobe Bio-medical Industry Park.

Profiling gene expression in melanocytes and resting stem cells promises to provide a new resource for scientists investigating stem cells’ genetic signatures

Characterizing the stem cell niche provides an environment in which amplifying progeny become committed to a stem cell fate, while the bulge serves as a reservoir for stem cells in their resting, or quiescent, state. Subtractive comparisons of cDNAs from stem cell, differentiated cell and ‘niche-like’ (keratinocyte) libraries have been performed to identify genes whose expression is switched on or off, depending on the pathway a cell has followed and the stage it has reached. Analysis of the stages in this stepwise process has also made it possible to develop a system for guiding differentiation from ES cell to endodermal, mesenchymal or mesodermal (paraxial and lateral) fates in vitro using combinations of extrinsic factors. By characterizing the identities of cells at each step on each branch of the lineage tree and collecting samples to produce DNA arrays, the Nishikawa lab is working to build a database of cDNA profiles of the intermediate stages in ES cell differentiation in culture, which can be queried and visualized using software developed in the lab. Analysis of this cDNA chip database promises to lead to a better understanding of the process of endodermal differentiation, and of stem cell specification in general. This powerful new DNA analysis software application, which promises to make a great contribution to the harmonization of multiple DNA expression databases, is being developed in collaboration with the Institute of Biomedical Research and Innovation, located adjacent to the CDB in the Kobe Bio-medical Industry Park.

Differential expression of endoderm-specific genes

In another ongoing project, members of the Nishikawa group are developing software to allow for improved DNA chip analysis and using it to build a database of the progressive intermediate stages that endodermal cells pass through in the journey from ES (embryonic stem cell) to terminally differentiated cells. A number of mesoderm and endoderm cell lineages and their intermediates are being used, as these are available in pure cultures and in vitro differentiation can be steered by the addition of the appropriate factors.

The mesodermal lineage, which has been clearly defined at cell level by the Nishikawa group, includes a number of important cell types, including endodermal cells, the primary constituent cells in many important organs including the lungs, liver and pancreas. Mesodendodermal differentiation is characterized by the activity of specific marker genes whose expression is switched on or off, depending on the pathway a cell has followed and the stage it has reached. Analysis of the stages in this stepwise process has also made it possible to develop a system for guiding differentiation from ES cell to endodermal, mesenchymal or mesodermal (paraxial and lateral) fates in vitro using combinations of extrinsic factors. By characterizing the identities of cells at each step on each branch of the lineage tree and collecting samples to produce DNA arrays, the Nishikawa lab is working to build a database of cDNA profiles of the intermediate stages in ES cell differentiation in culture, which can be queried and visualized using software developed in the lab. Analysis of this cDNA chip database promises to lead to a better understanding of the process of endodermal differentiation, and of stem cell specification in general. This powerful new DNA analysis software application, which promises to make a great contribution to the harmonization of multiple DNA expression databases, is being developed in collaboration with the Institute of Biomedical Research and Innovation, located adjacent to the CDB in the Kobe Bio-medical Industry Park.
Stem cell biology

Stem cell biology

Stem cells of various types, characterized by their abilities to self-renew and to generate more highly specialized cells indefinitely, are the key to repopulating the body’s cells. The study of these cells stands at the heart of some of the central questions in development, but many aspects of stem cell biology, such as the limits (if any) of the ability of the body to reprogram them to produce progeny cells of diverse lineages, and the identities of the intrinsic and extrinsic molecular factors that regulate a cell’s decisions whether to maintain stemness or to commit to differentiation have yet to be resolved.

A stem cells location and immediate environment can be important factors in defining its fate. Recent findings that stem cells may arise from one tissue type and then later be directed to give rise to another strongly substantiates the model in which a stem cell’s behavior and properties are influenced by external signals. By studying the microenvironments, known as ‘niches,’ in which stem cells originate, abide and prolifeate, the Nishikawa research group sees gain insight into the identity and function of molecules involved in stem cell activity, and the potential of stem cell-based therapeutic applications.

Characterizing the stem cell niche

Working to isolate the defining characteristics of specific stem-cell niches, the Nishikawa group has chosen melanocyte stem cells as one of the main model systems for their research, as melanocytes are readily identified by the presence of a melanocyte-specific promoter, Dut, and are amenable to experimental manipulations that allow specific cell types to be isolated from mixed populations. As the existence of niche cells and the identification of their characteristics can only be determined by first isolating the stem cells they support, Nishikawa’s lab has developed an approach in which stem cells are dissected from their surroundings, dissociated and used to construct single cell libraries, a process involving the technically challenging initial step of isolating single intact hair follicles from the skin. Targeted GFP expression is next used to label the follicle, as well as all of the GFP-negative cells from the bulge and the subset of GFP-negative cells found specifically to adhere to bulge melanocytes.

These follicular regions were selected based on the results of a previous study that showed that the bulge and matrix are the two main sites of localization of melanocyte stem cells, making them excellent sites for studies of niche properties. Each of these areas hosts a specific sub-population of stem cells. The matrix niche, the site of active hair growth and pigmentation, provides an environment in which amplifying progeny become committed to a stem cell fate, while the bulge serves as a reservoir for stem cells in their resting, or quiescent, state. Subtractive comparisons of cDNAs from stem cell, differentiated cell and ‘niche-like’ (keratinocyte) libraries revealed approximately 200 non-redundant genes, providing the lab with targets for further experiments in which the individual genes will be conditionally knocked out and the resulting phenotypes examined and analyzed. Other related studies, for which single cell libraries of other resting stem cells are now being constructed, will entail profiling gene expression in melanocytes and resting stem cells, which promises to provide a new resource for scientists investigating stem cells’ genetic signatures.

Profiling gene expression in melanocytes and resting stem cells promises to provide a new resource for scientists investigating stem cells’ genetic signatures

Stem cell types

Stem cells of various types, characterized by their abilities to self-renew and to generate more highly specialized cells indefinitely, are the key to repopulating the body’s cells. The study of these cells stands at the heart of some of the central questions in development, but many aspects of stem cell biology, such as the limits (if any) of the ability of the body to reprogram them to produce progeny cells of diverse lineages, and the identities of the intrinsic and extrinsic molecular factors that regulate a cell’s decisions whether to maintain stemness or to commit to differentiation have yet to be resolved.

A stem cells location and immediate environment can be important factors in defining its fate. Recent findings that stem cells may arise from one tissue type and then later be directed to give rise to another strongly substantiates the model in which a stem cell’s behavior and properties are influenced by external signals. By studying the microenvironments, known as ‘niches,’ in which stem cells originate, abide and prolifeate, the Nishikawa research group sees gain insight into the identity and function of molecules involved in stem cell activity, and the potential of stem cell-based therapeutic applications.

Characterizing the stem cell niche

Working to isolate the defining characteristics of specific stem-cell niches, the Nishikawa group has chosen melanocyte stem cells as one of the main model systems for their research, as melanocytes are readily identified by the presence of a melanocyte-specific promoter, Dut, and are amenable to experimental manipulations that allow specific cell types to be isolated from mixed populations. As the existence of niche cells and the identification of their characteristics can only be determined by first isolating the stem cells they support, Nishikawa’s lab has developed an approach in which stem cells are dissected from their surroundings, dissociated and used to construct single cell libraries, a process involving the technically challenging initial step of isolating single intact hair follicles from the skin. Targeted GFP expression is next used to label the follicle, as well as all of the GFP-negative cells from the bulge and the subset of GFP-negative cells found specifically to adhere to bulge melanocytes.

These follicular regions were selected based on the results of a previous study that showed that the bulge and matrix are the two main sites of localization of melanocyte stem cells, making them excellent sites for studies of niche properties. Each of these areas hosts a specific sub-population of stem cells. The matrix niche, the site of active hair growth and pigmentation, provides an environment in which amplifying progeny become committed to a stem cell fate, while the bulge serves as a reservoir for stem cells in their resting, or quiescent, state. Subtractive comparisons of cDNAs from stem cell, differentiated cell and ‘niche-like’ (keratinocyte) libraries revealed approximately 200 non-redundant genes, providing the lab with targets for further experiments in which the individual genes will be conditionally knocked out and the resulting phenotypes examined and analyzed. Other related studies, for which single cell libraries of other resting stem cells are now being constructed, will entail profiling gene expression in melanocytes and resting stem cells, which promises to provide a new resource for scientists investigating stem cells’ genetic signatures.

Differeation of mesoderm and endoderm

In another ongoing project, members of the Nishikawa group are developing software to allow for improved DNA chip analysis and using it to build a database of the progressive intermediate states that endodermal cells pass through in the journey from ES (embryonic stem) cell to terminally differentiated cells. A number of mesoderm and endoderm cell lineages and their intermediates are being used, as these are available in pure cultures and their in vitro differentiation can be steered by the addition of the appropriate factors.

The mesodermal endodermal lineage, which has been clearly defined at cell level by the Nishikawa group, includes a number of important cell types, including endodermal cells, the primary constituent cells in many important organs including the lungs, liver and pancreas. Mesodendrenal differentiation is characterized by the activity of specific marker genes whose expression is switched on or off, depending on the pathway a cell has followed and the stage it has reached. Analysis of the stages in this stepwise process has also made it possible to develop a system for guiding differentiation from ES cell to endodermal, mesenchymal or mesodermal (paraxial and lateral) fates in vitro using combinations of extrinsic factors. By identifying the characteristic markers of cells at each step on each branch of the lineage tree and collecting samples to produce DNA arrays, the Nishikawa lab is working to build a database of cDNA profiles of the intermediate stages in ES cell differentiation in culture, which can be queried and visualized using software developed in the lab. Analyses of this cDNA chip database promise to lead to a better understanding of the process of endodermal differentiation, and of stem cell specification in general. This powerful new DNA analysis software application, which promises to make a great contribution to the harmonization of multiple DNA databases, is being developed in collaboration with the Institute of Biomedical Research and Innovation, located adjacent to the CDB in the Kobe Bio-Medical Industry Park.
organogenesis and neurogenesis

Induction and patterning of the nervous system

The interactions between cells from different germ layers and their distributions along body axes are established in early embryogenesis and serve to determine the spatial disposition and normal development of tissues and organs. The specification of the dorsal-ventral (back-belly) axis is significant in neural development in that the central nervous system forms on the dorsal side of the body in all vertebrate orders. This process is dictated by the effects of a number of signaling factors that diffuse from organizing centers and direct dorsal ectoderm to maintain a neural fate. These molecules, which include Noggin, Chordin, Follistatin and their homologs, participate in elaborate signaling networks in which factors collaborate and compete to initiate the embryonic nervous system.

Beginning with the identification of the neural inducing factor Chordin in the early 1990s, Yoshiki Sasai’s research has focused on elucidating the molecular signaling mechanisms that enable 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 have been 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, work which led to the recent identification of Tweek, a novel dorsalization factor. The group is now also actively developing effective methods of inducing neuralization in mammals, work which has potential for application in the treatment of neurodegenerative disorders, such as Parkinson’s disease.

Inducing neural differentiation in mammals

The Sasai lab previously reported the development of 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), inducing nerve cells and precursors at high rates of efficiency. In 2003, the lab built on that previous work, setting out to address the question of whether the neural differentiation of ES cells could be guided with even greater precision and diversity by adding patterning factors — soluble molecules that work to determine the identities of cells in a developmental region — to the SDIA-cultured cells.

Although BMP signals have an inhibitory effect on neural development in earlier embryonic stages, they work to promote the neural crest at a later stage in development.

Using BMP4, Sasai’s group succeeded in causing the SDIA-treated ES cells to differentiate into cell types that normally derive from the neural crest, a developmental region that gives rise to nerve cells in the peripheral nervous system, including sensory, autonomic and enteric (gut) neurons. Although BMP signals have an inhibitory effect on neural development in earlier embryonic stages, the same signals work to promote the neural crest at a later stage in development. The group found that they were able to induce peripheral neural types that are naturally derived from the neural crest, including autonomic and sensory neurons and smooth muscle cells, when they treated the SDIA-treated cells with medium containing BMP4. A lower concentration prompts the same cells to differentiate into dorsal central nervous system neurons.

The neural crest arises from the dorsalmost region of the developing nervous system, and BMP4 is regarded as a ‘dorsalizing’ factor. Other factors have an antagonistic effect to BMP4, and cause the cells they act upon to take on a ventral character. Peripheral neurons are generally dorsal in their origins, while much of the central nervous system derives from more ventral developmental regions. Normal development involves the constant, dynamic interplay between patterning factors that steer neural precursors toward either a ventral or dorsal fate.

Sasai’s group also investigated the effects of Shh ( Sonic hedgehog), a factor that suppresses dorsal and promotes ventral development in vivo. As with BMP4, Shh provides concentration-dependent differentiation in SDIA-cultured cells, analogous to its function in the body. A low concentration guides SDIA-derived neural precursors to develop into motor neurons, while higher concentrations bring about differentiation into neural types that in normal development would derive from the floor plate, the ventralmost region of neural development. Tests revealed that these SDIA and Shh-treated neurons display both the molecular markers and physiological attributes (such as electrical activity and guidance characteristics of the same types of neurons in vivo, demonstrating that the cells produced by this method are structurally and functionally similar to natural floor plate cells.

Taken together, these findings show that SDIA-generated neural precursors respond to patterning factors with a versatility similar to that of their in vivo counterparts. Higher concentrations of the dorsalis- ing factor BMP4 induce increasingly dorsal neural types, while greater doses of Shh have the same effect in ventralizing neural precursors. This work represents the first efficient generation of a broad spectrum of neuronal types from ES cells in culture, and the fact that the process has been demonstrated not only in mice, but also in primate cells, marks a significant step toward the ability to induce specific types of neuron from ES cells by cell-derived precursors that is one of the foremost challenges confronting researchers in regenerative medicine.
organogenesis and neurogenesis

Induction and patterning of the nervous system

The interactions between cells from different germ layers and their distributions along body axes are established in early embryogenesis and serve to determine the spatial disposition and normal development of tissues and organs. The specification of the dorsal-ventral (back-belly) axis is significant in neural development in that the central nervous system forms on the dorsal side of the body in all vertebrate orders. This process is dictated by the effects of a number of signaling factors that diffuse from organizing centers and direct dorsal ectoderm to maintain a neural fate. These molecules, which include Noggin, Chordin, Follistatin and their homologs, participate in elaborate signaling networks in which factors collaborate and compete to initiate the embryonic nervous system.

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 enable 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, work which led to the recent identification of Jami, a novel dorsalization factor. The group is now also actively developing effective methods of inducing neuralization in mammals, work which has potential for application in the treatment of neurodegenerative disorders, such as Parkinson’s disease.

Inducing neural differentiation in mammals

The Sasai lab previously reported the development of a technique for inducing the differentiation of neural cells by culturing mouse ES cells on plates of mouse and primate sensory neurons and pigmented epithelial cells, demonstrating that the cells produced by this method are structurally and functionally similar to natural floor plate cells.

Using BMP4, Sasai’s group succeeded in causing the SDIA-treated ES cells to differentiate into cell types that normally derive from the neural crest, a developmental region that gives rise to nerve cells in the peripheral nervous system, including sensory, autonomic and enteric (gut) neurons. Although BMP signals have an inhibitory effect on neural development in earlier embryonic stages, the same signals work to promote the neural crest at a later stage in development. The group found that they were able to induce peripheral neural types that are naturally derived from the neural crest, including autonomic and sensory neurons and smooth muscle cells, when they treated the SDIA-treated cells with modulated concentrations of BMP.

A lower concentration of a technique for inducing the differentiation of neural crest cells by culturing mouse ES cells on plates of mouse and primate sensory neurons was found to promote ventral development in vivo. As with BMP4, Shh provokes concentration-dependent differentiation in SDIA-cultured cells, analogous to its function in the body. A low concentration guides SDIA-derived neural precursors to develop into motor neurons, while higher concentrations bring about differentiation into neural types that, in normal development, would derive from the floor plate, the ventral part of the neural region. The group revealed that these SDIA and Shh-treated neurons display both the molecular markers and physiological characteristics of the same types of neurons in vivo, demonstrating that the cells produced by this method are structurally and functionally similar to natural floor plate cells.

Taken together, these findings show that SDIA-generated neural precursors respond to patterning factors with a versatility similar to that of their in vivo counterparts. Higher concentrations of the dorsalizing factor BMP4 induce increasingly dorsal neural types, whereas greater doses of Shh have the same effect in ventralizing neural precursors. This work presents the first efficient generation of such a broad spectrum of neuronal types from ES cells in culture, and the fact that the process has been demonstrated not only in mice, but also in primate cells, marks a significant step toward the ability to induce specific types of neuron from ES cells by cell-derived precursors that is one of the foremost challenges confronting researchers in regenerative medicine.
The development of multicellular systems and organisms depends on the ability of cells to aggregate and cooperate in diverse ways. Individual cells must be able to identify each other and distinguish between diverse cell types. They need to be able to aggregate in order to form tissues, while cells with different missions remain detached from one another. These functions of cell recognition and bonding are achieved by several types of molecules known as CAMs (cell adhesion molecules), which variously bind with partner molecules present on the surfaces of substrates, matrices, and other 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. Cadherin bonds are dynamically stable—although the intercellular junctions they form persist in the whole cell, the individual bonds are regulatable and can respond to changes in cell state. Masatoshi Takeichi has been engaging in exploring cadherin bonds using various models, such as Xenopus laevis, which allow for the study of cell differentiation in vivo.

Masatoshi Takeichi
Ph. D.
Masatoshi Takeichi is director of the RIKEN CDB Institute and the Center for Developmental Biology as well as the Cell Adhesion and Tissue Patterning research group at the same institute. He completed the B. Sc. and M. S. programs in biology at Nagoya University in 1973, before going on to obtain a degree in biological sciences in 1975. After obtaining his Ph. D., he took a research fellowship at the Carnegie Institution Department of Embryology under Richard Palay. He then returned to Kyoto University, attaining a full professorship in the Department of Biophysics (1986-1993), before becoming the head of the Department of Cell and Developmental Biology at the Graduate School of Science at Kyoto University. He assumed his current position in 2002.

Characterizing Fat1 function
Cadherins do not act alone. Streaming both sides of the cell membrane, cadherin molecules bind with other cadherins of like type in the space between cells, while in the cytoplasm they form anchorage complexes with other proteins, such as various members of the catenin family, which in turn bind with molecules present within the cell and participate in important cell signaling networks. In 2004, the Takeichi lab has found that N-catenin, a form of α-catenin specific to the nervous system, is essential to the stabilization of dendritic spines. These projections are important for establishing and maintaining contacts between axons and dendrites, the two principal types of extensions from the neuronal cell body. Previous work showed that cadherins help to regulate the adhesion of dendritic spines to axons, which is a crucial step in synaptic formation, a finding which was corroborated by this year’s elucidation of α-catenin’s role. The functional loss of this molecule resulted in dramatic increases in spine morphology and shape alterations, while N-catenin overexpression caused reduced dendritic spines, which normally extend and retract intermittently, to turn over at a much lower rate, resulting in abnormal accumulations of mature spines over time. Taken together, these results suggest that N-catenin is a critical agent in maintaining the appropriate balance between stability and turnover in synaptic contacts.

Overexpression of α-catenin in hippocampal neurons causes overproduction of dendritic spines (right). Left, control.
The development of multicellular systems and organisms depends on the ability of cells to aggregate and cooperate in diverse ways. Individual cells must be able to identify each other and distinguish between diverse cell types. They need to be able to form bonds discriminatively, such that cells that must aggregate in order to form a tissue do so, while cells with different missions remain detached from one another. These functions of cell recognition and bonding are achieved by several types of molecules known as CAMs (cell adhesion molecules), which variously bind with partner molecules present on the surfaces of substrates, matrices and other 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 complex assemblies with other intracellular factors to create bonds spanning membranes and intercellular spaces to join cells with other cells expressing similar cadherins. Cadherin bonds are dynamically stable — although the intercellular junctions they form persist in terms of the whole cell, the individual bonds are regulatable and can respond to changes in cell state.

Masatoshi Takeichi has been engaged in exploring the structure, function and biological implications of the cadherin superfamily since his discovery of the first cadherin molecules more than twenty years ago.

**Characterizing Fat1 function**

Cadherins do not act alone. Spanning both sides of the cell membrane, cadherin molecules bind with other cadherins of like type in the space between cells, while in the cytoplasm they form anchorage complexes with other proteins, such as various members of the catenin family, which in turn bind with molecules present within the cell and participate in important cell signaling networks. In 2001, the Takeichi lab investigated the function of a mammalian cadherin known as Fat1, which is found in the peripheries of cells. The group found that Fat1 acts to regulate the function of actin, a primary component of the cytoskeleton that functions in the control of cellular motility and changes in cell morphology. Among members of the cadherin family, Fat1 cadherins are distinguished by their unusually large number of extracellular repeat domains, nearly seven times the number found in classic cadherins — and by the uniqueness of the primary sequences of their cytoplasmic domains. Three Fat cadherins have been identified in mammals — Fat1, 2 and 3. It has been shown that the Drosophila homolog of these molecules plays roles in cell proliferation or in the determination of planar polarity, but the function of the vertebrate Fats has remained unknown.

Using RNA interference (RNAi) to knock down Fat1 function in researchers in the Laboratory for Cell Adhesion and Tissue Patterning discovered that Fat1 is required for the stable maintenance of tight associations between cells and the normal organization of actin. This inhibition of Fat1 also reduced the stability of cadherin-catenin function in interneuronal junctions. Takeichi's lab has found that N-catenin, a form of α-catenin specific to the nervous system, is essential to the stabilization of dendritic spines. These projections are important for establishing and maintaining contacts between axons and dendrites, the two principal types of extensions of the neuronal cell body. Previous work showed that cadherins help to regulate the adhesion of dendritic spines to axons, which is a crucial step in synapse formation, a finding which was corroborated by this year's elucidation of α-catenin's role. The functional loss of this molecule resulted in dramatic increases in spine motility and shape alterations, while N-catenin overexpression caused spines, which normally extend and retract intermittently, to turn over at a much lower rate, resulting in abnormal accumulations of mature spines over time. Taken together, these results suggest that N-catenin is a critical agent in maintaining the appropriate balance between stability and turnover in synaptic contacts.

**Contacts between neurons**

Cadherin complexes are known to play diverse roles in a cell-type-specific manner. In an ongoing study of cadherin-catenin function in interneuronal junctions, Takeichi's lab has found that N-catenin is a critical agent in maintaining the appropriate balance between stability and turnover in synaptic contacts.

**Overexpression of α-catenin in hippocampal neurons causes overproduction of dendritic spines**

**Core Program**

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

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

Blood vessels are formed by two distinct physiologi cal processes in the adult body. In angiogenesis, new blood vessels are generated from pre-existing, differentiated endothelial cells. Vasculogenesis, on the other hand, involves the recruitment and differentiation of previously undifferentiated EPCs at the site of new blood vessel growth. These EPCs are thought to be progeny of adult stem cells in the bone marrow, but the mechanisms underlying vasculogenesis remain unknown. The vascularization of regenerative tissue is a critical component of the natural healing process as well as fundamental to the recovery of blood vessels that have been damaged, blocked, or lost, and the ability to promote and regulate the growth of new blood vessels using EPCs will provide new insights into the vascularization of regenerative tissue. The emerging field of stem cell-based therapy continues to receive attention as one of the most promising frontiers of medical science. Building on previous work in which he identified bone marrow-derived endothelial progenitor cells (EPCs) and demonstrated their role in the generation of new blood vessels, Takayuki Asahara seeks to characterize adult stem and progenitor cells with even greater differentiation potential, and simultaneously to translate that research into clinically relevant advances.


takayuki asahara

M.D., Ph.D.

Takayuki Asahara received his M.D. from Tokyo Medical College in 1984, and his Ph.D. in experimental medicine in cardiology and emergency medicine in 1991. He worked as a research fellow in cardiology at the Tokyo Medical College Hospital from 1989 to 1991, before joining a fellowship in cardiovascular research at St. Elizabeth’s Hospital in Boston (USA). He was appointed assistant professor at Tufts School of Medicine in 1993, and associate professor at the Tufts University School of Medicine in 1999. 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 Kobe University School of Medicine.


The vascularity of regenerative tissue is a critical component of the natural healing process. This belief was borne out by the EPC transplantation study, in which the researchers first isolated large numbers of human EPCs for transplantation into mouse models of limb ischemia — localized oxygen starvation of tissue resulting in damage to blood vessels. The transplanted human EPCs were found to improve neovascularization in the ischemic hindlimbs of mice, as well as to reduce the secondary effects of prolonged ischemia, which include necrosis and autolysis. The incorporation of the transplanted EPCs at ischemic sites was determined by measuring the uptake of labeled human progenitor cells in tissue sections from the SDF-1 and unrelated groups, which showed that the EPCs accumulated at nearly two times the rate in the limbs which had been injected with SDF-1 compared to that in the control group. This represents an important first proof-of-principle demonstration that the homing of EPCs to a damaged area can be augmented by an extrinsic factor.

Targeted and autologous transplantation

EPCs represent an extremely promising new mode of promoting the therapeutic growth of blood vessels, but significant obstacles must be overcome before that promise can be realized. Previous studies in rats have used quantities of EPCs that would require impractically large supplies of peripheral blood to derive equivalent amounts of EPCs for use in humans, and the possibility of rejection of blood from non-self sources by the immune system remains another important potential barrier. Members of the Asahara team sought to address these questions by developing a system for the local, rather than systemic, transplantation of EPCs freely isolated from the host. These studies were conducted using a swine model of myocardial (heart muscle) ischemia, from which small quantities of blood were drawn to allow the isolation and expansion of EPCs, identified by the presence of the marker molecule CD31. Catecholamines were used to inject similar quantities of either CD31+ or CD31- cells, or of a control solution containing no cells, directly into ischemic sites in the hearts of pigs, to test the effects of the concentrated delivery of autologous EPCs.

The results showed that the targeted transplantation of CD31+ hematopoietic cells reduced ischemic damage and promoted neovascularization, resulting in improved cardiac function, while pigs receiving CD31+ or control solutions experienced no such benefits. A complementary study involving the targeted transplantation of human CD34+ cells into ischemic rat hearts showed that non-autologous cells can also incorporate into damage sites and differentiate into functioning endothelial cells, even when the transplanted quantities are one-twentieth the amounts used in similar previous experiments.

Clinical work

In addition to his role as team leader of the CDB Laboratory for Stem Cell Translational Research, Asahara also serves as director of the regenerative medicine program at the neighboring Institute for Biomedical Research and Innovation. In its role as a translational research lab, Asahara’s team is beginning clinical tests of the therapeutic uses of EPCs in the treatment of Bluerger disease (thrombomangitis obliterans), an inflammatory condition involving the obstruction of small and medium-sized blood vessels in the limbs, and linked with heavy tobacco use. Plans are in place to expand the patient population of this preliminary study into the therapeutic transplantation of EPCs, which may offer a new mode of treatment for this disease which at present frequently necessitates the surgical amputation of ischemic digits or limbs.

Publications

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

Stimulating EPC accumulation
Researchers in the Asahara lab found that EPCs taken from human subjects and expanded ex vivo contribute to the vasculization of the damaged limbs of mice at higher efficiencies if the limbs were first injected with a factor known as SDF-1 (stromal cell-derived factor 1), a chemokine originally identified in mouse bone marrow and whose only known receptor is expressed in hematopoietic stem cells. The CXCR4 transmembrane receptor, present in CD34+ hematopoietic cells, shows a strong attraction to SDF-1 and is believed to help recruit stem cells to sites in need of vascular replacement or repair.

This belief was borne out by the EPC transplantation study, in which the researchers first isolated large numbers of human EPCs for transplantation into mouse models of ischemia — localized oxygen starvation of tissue resulting in damage to blood vessels. The transplanted human EPCs were found to improve neovascularization in the ischemic murine hindlimbs, as well as to reduce the secondary effects of prolonged ischemia, which include necrosis and autophagocytosis. The incorporation of the transplanted EPCs at ischemic sites was determined by measuring the uptake of labeled human progenitor cells in tissue sections from the SDF-1 and untransplanted groups, which showed that the EPCs accumulated at nearly two times the rate in the limbs which had been injected with SDF-1 compared to that of the control group. This represents an important first proof-of-principle demonstration that the homing of EPCs to a damaged area can be augmented by an extrinsic factor.

Targeted and autologous transplantation
EPCs represent an extremely promising new mode of promoting the therapeutic growth of blood vessels, but significant obstacles must be overcome before that promise can be realized. Previous studies in rats have used quantities of EPCs that would require impractically large supplies of peripheral blood to derive equivalent amounts of EPCs for use in humans, and the possibility of rejection of blood from non-self sources by the immune system remains another important potential barrier. Members of the Asahara team sought to address these questions by developing a system for the local, rather than systemic, transplantation of EPCs freshly isolated from the host. These studies were conducted using a swine model of myocardial (heart muscle) ischemia, from which small quantities of blood were drawn to allow the isolation and expansion of EPCs, identified by the presence of the marker molecule CD31. Catheters were used to inject similar quantities of either CD31+ or CD31- cells, or of a control solution containing no cells, directly into ischemic sites in the hearts of pigs, to test the effects of the concentrated delivery of autologous EPCs.

The results showed that the targeted transplantation of CD31+ hematopoietic cells reduced ischemic damage and promoted neovascularization, resulting in improved cardiac function, while pigs receiving CD31- or control solutions experienced no such benefits. A complementary study involving the targeted transplantation of human CD34+ cells into ischemic rat hearts showed that non-autologous cells can also incorporate into damaged sites and differentiate into functioning endothelial cells, even when the transplanted quantities are one-twentieth the amounts used in similar previous experiments.

Clinical work
In addition to his role as team leader of the CDB Laboratory for Stem Cell Translational Research, Asahara also serves as director of the regenerative medicine program at the Tokyo Medical and Dental University Institute for Biomedical Research and Innovation. In its role as a translational research lab, Asahara’s team is beginning clinical tests of the therapeutic uses of EPCs in the treatment of Buerger Disease (thromboangiitis obliterans), an inflammatory condition involving the obstruction of small and medium-sized blood vessels, and linked with heavy tobacco use. Plots are in place to expand the patient population of this preliminary study into the therapeutic transplantation of EPCs, which may offer a new mode of treatment for this disease which at present frequently necessitates the surgical amputation of ischemic digits or limbs.

Takayuki Asahara
M.D., Ph.D.

Takayuki Asahara received his M.D. from Tokyo Medical College in 1984, and his M.S. and Ph.D. in cardiology and emergency medicine from St. Elizabeth’s Hospital in 1990. He worked as a research fellow in cardiology at the Tokyo Medical College Hospital from 1989 to 1990, 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 Tufts University School of Medical Sciences in 2000. In addition to his current position as C DB team leader, Dr. Asahara serves as director of Regenerative Medicine and Research at the RIKEN Institute of Biomedical Research and Innovation, and Professor of Physiology at the Tokyo University School of Medicine.

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. Vasculogenesis, on the other hand, involves the recruitment and differentiation of previously undifferentiated EPCs at the site of new blood vessel growth. These EPCs are thought to be progeny of adult stem cells and progenitors in the hematopoietic system, which can be induced to demonstrate true pluripotency under the right culture conditions. The vasculization of regenerating tissue is a critical component of the natural healing process, as well as to the recovery of blood vessels that have been damaged, blocked or lost, and the ability to promote and regulate the growth of new blood vessels using EPCs will provide new and open areas to explore for researchers and clinicians working to develop treatments for disorders of the cardiovascular and other systems.

A colony of endothelial progenitor cells on the surface of an injured blood vessel

Ongoing studies in the Asahara lab have also involved the study of therapeutic angiogenesis in patients suffering from diabetic peripheral arteriopathy, a condition that affects up to 50% of all diabetic individuals and is associated with amputation of the lower extremities. Asahara’s team has previously performed the world’s first clinical study involving the transplantation of human CD34+ cells into ischemic limbs which had been injected with SDF-1, which showed that the EPCs accumulated at nearly two times the rate in the limbs which had been injected with SDF-1 compared to that of the control group. This represents an important first proof-of-principle demonstration that the homing of EPCs to a damaged area can be augmented by an extrinsic factor.

Clinical study
In addition to his role as team leader of the CDB Laboratory for Stem Cell Translational Research, Asahara also serves as director of the regenerative medicine program at the Tokyo Medical and Dental University Institute for Biomedical Research and Innovation. In its role as a translational research lab, Asahara’s team is beginning clinical tests of the therapeutic uses of EPCs in the treatment of Buerger Disease (thromboangiitis obliterans), an inflammatory condition involving the obstruction of small and medium-sized blood vessels, and linked with heavy tobacco use. Plots are in place to expand the patient population of this preliminary study into the therapeutic transplantation of EPCs, which may offer a new mode of treatment for this disease which at present frequently necessitates the surgical amputation of ischemic digits or limbs.
Neurotrophic factors

The early stages of neural network development see the growth of a number of neurons in excess of the number that will ultimately populate any given region. Neurons that have formed synaptic connections establish what is known as a trophic interaction in which the target tissue 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 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 signals provides the earliest evidence for the survival of nerve growth factor (NGF). When injecting the substance into mice, the researchers observed a dramatic increase in the number of neurons within the target tissue. This effect was not seen in mice that had been treated with a control substance, indicating that NGF had a specific role in promoting neuron survival.

Hideaki Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1, GDNF, GFRα2, ARTN, and GFRα3, ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.

Kapilno Inoue
Kazumi Nogicchi
Yoshio Nakanishi
Yasufumi Iwatsuki
Yusuke Kubo
Masaaki Takahashi
Satoshi Kudo
Kazuo Hattori

GDNF ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1 and GDNF, GFRα2 and ARTN, and GFRα3 and ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.

GFP staining of GDNF-expressing motor and sensory neurons in the binucleated RET-GFP mice

GDNF ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1 and GDNF, GFRα2 and ARTN, and GFRα3 and ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.

X-gal staining of a mouse embryo expressing tau-lacZ under the GFRα1 promoter

GFP staining of GDNF-expressing motor and sensory neurons in the binucleated RET-GFP mice

GDNF ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1 and GDNF, GFRα2 and ARTN, and GFRα3 and ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.

GFP staining of GDNF-expressing motor and sensory neurons in the binucleated RET-GFP mice

GDNF ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1 and GDNF, GFRα2 and ARTN, and GFRα3 and ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.

GFP staining of GDNF-expressing motor and sensory neurons in the binucleated RET-GFP mice

GDNF ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1 and GDNF, GFRα2 and ARTN, and GFRα3 and ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.

GFP staining of GDNF-expressing motor and sensory neurons in the binucleated RET-GFP mice

GDNF ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL members were disrupted and found that, in receptor-ligand interactions in vivo, there are affinitive pairings between GFRα1 and GDNF, GFRα2 and ARTN, and GFRα3 and ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory, and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. GDNF binds to GFRα1 and GFRα2 receptor, and formation of receptor-ligand complexes is essential for migration of enteric neurons and differentiation of autonomic, sensory, and motor neurons.
Neurotrophic factors

The early stages of neural network development see a growth of a number of neurons in excess of the number that will ultimately populate any given region. Neurons that have formed synaptic connections establish what is known as a trophic interaction in which the target tissue 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 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 Rex-Montagui 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), neuropeptides, neurotactin factors, and the transforming growth factor family. Several neurotrophins (of which NGF is a member) are particularly interested in this last group, the GDNF Family Ligands (the GFLs) in particular. This family of neurotrophic factors includes four known members — GDNF (for Glial cell line Derived Neurotrophic Factor), Neurturin, Artemin and Pareaun. The GFLs signal via receptor complexes formed by the RET receptor tyrosine kinase and one of four co-receptors, GFRα1-4. In vitro, these four receptors show differential affinities for specific GFLs, with GFRα1 showing the greatest ability to interact with the range of GFL family members.

GDFN ligand-receptor pairings

Enomoto studied phenotypes of mice in which individual GFL, GFRαs or Ret genes are disrupted and found that, in receptor-ligand interactions in vivo, there are allfinite pairings between GFRα1-GDNF, GFRα2-NRTN and GFRα3-ARTN. The loss of GDNF-GFRα1 system had the strongest developmental effects, causing defects in enteric, autonomic, sensory and motor neurons as well as failures in the development of the kidney, and resulting in lethality by the P0 stage of development. Birth. Similar neuronal populations are affected by Neurturin or GFRα2 deficiency, but the phenotypes are less severe, with kidneys forming normally and infertile effects on sympathetic and motor neural development. Analysis of the consequences of these mutations in developing enteric and parasympathetic neurons suggests that GDNF-GFRα1 functions in neural precursors to regulate fundamental neurodevelopmental processes such as migration and proliferation, while the later expression of Neurturin-GFRα2 in target tissues serves to maintain previously established neurons.

Artemin-GFRα1 signaling seems to operate in a more specific subset of cells, sympathetic neurons, with no apparent function in enteric or central neural development. The sympathetic nervous system arises from the neural crest and follows a stepwise developmental pathway that directs its neurons to establish their appropriate functional roles and positions throughout the body. In a series of signal-directed decisions, sympathetic precursor cells migrate and differentiate into neurons soon after they undergo lineage commitment and restriction to a catecholaminergic (sympathetic neurotransmitter-producing) fate. Enomoto found that Artemin is expressed in blood vessels and attracts growing axons emanating from sympathetic ganglion that expresses RET. This interaction is crucial for sympathetic axons to follow vascular pathways and to reach the final target tissues. The fourth GFRα-receptor pairing of Persephin and GFRα4 is known to be expressed ubiquitously at low levels, but its developmental activity remains obscure.

In related work, members of the Enomoto lab studied the roles of RET and TRKA (neurotrophin receptor) in sympathetic neurogenesis and maintenance. Both RET and TRKA are expressed in developing sympathetic neurons, but the balance between these two receptor types shifts over time, with RET predominating during earlier phases of axon growth and migration, while TRK assumes a more important role in ensuring the perinatal survival and maintenance of these cells.

Studies of RET signaling are of particular interest for their value in biomedical research. Hirschspring’s disease, a congenital disorder affecting about 1 in 5,000 newborn children in which enteric neuron precursors fail to colonize the distal part of the gut resulting in a loss of bowel motility, chronic constipation and bowel obstruction, is caused by a loss-of-function mutation in the Ret gene. Hyperactivation of RET results in abnormal development of the neural crest, a developmentally transient structure that normally gives rise to neurons, glia, endocrine cells and mesenchymal cells, and has been linked to the occurrence of endocrine tumors, such as thyroid carcinoma and pheochromocytoma.

In the future, the Enomoto research team plans to conduct systematic analyses of RET-bearing neurons to achieve a better understanding of the molecular mechanisms involved in RET and GFRα receptor signaling. One immediate focus will be on investigating roles for RET, GDNF and GFRα1 in the postnatal maintenance of motor, sensory and dopaminergic neurons, using a Cre/lox c o d i o n a l a n d knockstrat e. By providing insights into the functions of GFL-specific neurotrophic factor signaling pathways, Eno moto hopes to contribute to the development of stem cell-based therapies for nervous system disorders. 

X-gal staining of a mouse embryo expressing tau-lacZ under the GFRα1 promoter

GFP staining of RET-expressing motor and sensory neurons in the brainstem of RET-GFP mice

GFP staining of RET-expressing motor and sensory neurons in the brainstem of RET-GFP mice

GFP staining of RET-expressing motor and sensory neurons in the brainstem of RET-GFP mice
Chihiro Hama is working to address these questions through investigations into the neural network of the fruit fly Drosophila, a system which, in spite of its comparatively simple structure, demonstrates the capacity for instinctive behaviors, learning, and memory. While there are profound differences in degrees of network sophistication and cell type diversity between Drosophila and more highly evolved organisms such as mammals, 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.

**Neural networks**

Neural network development involves the orchestration of complex processes from neuronal differentiation to axon growth to the formation of synapses. 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 bewildering intricacy and depth.

Chihiro Hama, Ph.D.

Chihiro Hama received his B. Sc. and M. Sc. from the University of Tokyo Department of Biochemistry and Chemistry and was awarded a Ph. D. from the same institution in 1985 for his work on epidermal growth factor. He spent the period from 1985 to 1988 as a postdoc in the laboratory of Thomas H. Nagai 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.

Neural networks in the Mushroom body of the Drosophila brain.

Mushroom body in the Drosophila brain.

**Slender lobes**

The mushroom body, containing on the order of 2,000 neurons, is the functional center for olfactory learning and memory in Drosophila. Michiko Othira in the Hama lab has identified the gene slender lobes (sle), whose homozygous mutation results in sterile flies with thinner axonal bundles and reduced neuron counts in the mushroom body, a phenotype attributable to the failure of flies lacking the sle gene to sustain the normally high rate of cellular proliferation by mushroom body neuroblasts (neural progenitor cells).

The protein encoded by the sle gene is found in the nuclei of a variety of cells, including neuroblasts, and is abundant in the perinuclear region during interphase. Genetic analyses have revealed that a majority of interphase nucleoli in the mutant are shifted to a particular state of assembly that is transiently or rarely occurs in the wild type. Electron microscopy has showed that the nucleoli in mutant neuroblasts are unusually densely packed, and are separated from the perinuclear heterochromatin that surrounds or tightly associates with nucleoli in wild type. Similar phenotypes were also observed in neurons that are qui
dent during mitosis and DNA synthesis. The Hama lab’s studies indicate that Sle, a novel perinucleolar protein, may modulate nucleolar assembly and consequently promotes the proliferation of neuroblasts.

**Olfactory axon guidance**

The Drosophila olfactory sensory system comprises around 1,300 neurons that are positioned in the head appendages and project into the antennal lobe of the brain, which contains 43 glomerular synapses clusters of sensory neurons and interneurons. Each individual sensory neuron expresses only one or two types of the approximately 60 known types of odorant receptors, and all neurons expressing the same odorant receptor project to the same glomerulus.

The Hama lab is interested in resolving the mechanisms by which these specialized neurons are able to identify and home to specific targets over considerable distances with such great accuracy during the organization of the olfactory network. This research seeks both to clarify the roles of known genetic elements and to uncover new pieces needed to solve this intricate puzzle through screening for new mutations that affect the projection of olfactory neurons.

Keita Endo in the Hama lab has developed a system to generate mutant flies and screen them for olfactory neuron projection phenotypes by visualizing specific projection patterns. The first step of this approach takes advantage of an enhancer trap line that enables the forced expression of marker genes in specific subsets of olfactory sensory neurons, making it possible to visualize the extension of their axons toward the glomeruli. The second phase entails 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 both normal neural development, members of the Hama lab are now analyzing 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.

**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 neural activities.

Targeting of olfactory sensory neurons into specific glomeruli in the antennal lobes.

The Hama lab is interested in resolving the mechanisms by which olfactory neurons identify and home to specific targets.

**Publications**

- Hama C, Nabeshima Y and Hama C. The Hama lab is interested in resolving the mechanisms by which olfactory neurons identify and home to specific targets. 

**Mushroom body in the Drosophila brain.**

 stattibs.png
neural network development

Neural networks

Neural network development involves the orchestration of complex processes from neuronal differentiation to axon growth to the formation of synapses. 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 bewildering intricacy and depth.

Chihiro Hama is working to address these questions through investigations into the neural network of the fruit fly Drosophila, a system which, in spite of its comparatively simple structure, demonstrates the capacity for instinctive behaviors, learning and memory. While there are profound differences in degrees of network sophistication and cell type diversity between Drosophila and more highly evolved organisms such as mammals, 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.

Slender lobes

The mushroom body, containing on the order of 2000 neurons, is the functional center for olfactory learning and memory in Drosophila. Miwako Ohtsuki in the Hama lab has identified the gene slender lobes (sle), whose homozygous mutation results in sterile flies with thinner axonal bundles and reduced neuron counts in the mushroom body, a phenotype attributable to the failure of flies lacking the sle gene to sustain the normally high rate of cellular proliferation by mushroom body neuroblasts (neural progenitor cells). The protein encoded by the sle gene is found in the nuclei of a variety of cells, including neuroblasts, and is abundant in the perinucleolar region during interphase. Genetic analyses have revealed that a majority of interphase nucleoli in the mutant are shifted to a particular state of assembly that transiently or rarely occurs in the wild type. Electron microscopy has shown that the nucleoli in mutant neuroblasts are unusually densely packed, and are separated from the perinucleolar heterochromatin that surrounds or tightly associates with nucleoli in wild type. Similar phenotypes were also observed in neurons that are quiescent during mitosis and DNA synthesis. The Hama lab's studies indicate that Sle, a novel perinuclear protein, may modulate nuclear assembly and consequently promotes the proliferation of neuroblasts.

Olfactory axon guidance

The Drosophila olfactory sensory system comprises around 1,300 neurons that are positioned in the head appendages and project into the antennal lobe of the brain, which contains 43 glomerular synaptic clusters of sensory neurons and interneurons. Each individual sensory neuron expresses only one or two types of the approximately 60 known types of odorant receptors, and all neurons expressing the same odorant receptor project to the same glomerulus. The Hama lab is interested in resolving the mechanisms by which these specialized neurons are able to identify and home to specific targets over considerable distances with such great accuracy during the organization of the olfactory network. This research seeks both to clarify the roles of known genetic elements and to uncover new pieces needed to solve this intricate puzzle through screening for new mutations that affect the projection of olfactory neurons.

Keita Endo in the Hama lab has developed a system to generate mutant flies and screen them for olfactory neuron projection phenotypes by visualizing specific projection patterns. The first step of this approach takes advantage of an enhancer trap line that enables the forced expression of marker genes in specific subsets of olfactory sensory neurons, making it possible to visualize the extension of their axons toward the glomeruli. The second phase entails 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 into microtubules in a specific orientation. To clarify how vesicle transport is involved in neuronal development, members of the Hama lab are now analyzing 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.

Targeting of olfactory sensory neurons into specific glomeruli in the antennal lobe

The Hama lab is interested in resolving the mechanisms by which olfactory neurons identify and home to specific targets.

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 into microtubules in a specific orientation. To clarify how vesicle transport is involved in neuronal development, members of the Hama lab are now analyzing 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.

The Hama lab is interested in resolving the mechanisms by which olfactory neurons identify and home to specific targets.

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 into microtubules in a specific orientation. To clarify how vesicle transport is involved in neuronal development, members of the Hama lab are now analyzing 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.

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 into microtubules in a specific orientation. To clarify how vesicle transport is involved in neuronal development, members of the Hama lab are now analyzing 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.
Vertebrate embryogenesis begins with a seemingly formless egg that, through processes of division, growth, differentiation, migration and rearrangement, rapidly gives rise to a highly organized structure characterized by a number of definitive axes. This transformation is particularly striking and rapid in the zebrafish (Danio rerio), which in the space of a single day develops from an ovum to a recognizable vertebrate body laid down in respect to multiple polar vectors. The speed of its growth and reorganization, coupled with its amenability to genetic studies and the revealing peculiarity of its embryo, make this organism an ideal system for the study of axis formation in early vertebrate development.

Regulating the dorsal-ventral axis

Masahiko Hibi’s laboratory focuses on the molecular genetic cascade of events stemming from organizing centers in the zebrafish embryo. These centers, which emerge soon after fertilization, play pivotal roles in setting up positional axes. Hibi’s lab is particularly interested in the formation of the dorsal-ventral (back-belly) axis, an essential step in the establishment of the body plan. An embryonic region known as zebrafish as the dorsal organizer chordina. Induced neuroectoderm is generated in a stepwise fashion.

Vertebrate neuronal tissues are generated in a stepwise fashion. These steps include neural induction, anterior-posterior patterning, and neurogenesis. In amphibians and teleosts (bony fish) embryos, neuroectoderm is induced by BMP inhibitors derived from organizing centers. Induced neuroectoderm is by default anterior in character, but a subset in some tissues is subsequently subject to posteriorizing transformations. The hindbrain and spinal cord, which are posteriorized regions, are specified by a signal from a region known as non-axial mesendoderm, while anterior neuroectoderm that does not receive this posteriorizing signal develops into fore- and midbrain. After these initial processes of neural induction and patterning, neurogenic regions, the domains in which neurogenesis takes place, are established.

The Hibi lab has been working to identify genes involved in the control of this patterning and neurogenesis, focusing on two genes in particular: the zinc finger gene fox-like expressed in the fore-brain, and the posterior neuron-specific homeobox gene pnx. In collaborative work, they showed that fox-like is required for the formation of hypothalamic monoaminergic neurons in zebrafish. These neurons produce neurotransmitters such as dopamine and serotonin, which are centrally important to the regulation of mood, behavior, endocrine and cognitive functions in humans.

In work published in 2003, the Hibi lab analyzed and isolated the responsible gene for a mutant named ogon, which displays ventralized phenotypes, and found that the ogon locus encodes a zebrafish homologue of the protein Secreted Frizzled (Siz- zled), which functions as negative feedback regulator of BMP signaling. While numerous mutations are known to result in dorsalized phenotypes, ogon is only the second zebrafish mutant reported to show clearly ventralized phenotypes, such as expanded ventral tail fins, blood, pronephros, and other posterior or structures. These patterns are similar to the phenotype observed in mutants of the gene for the BMP antagonist, Chordin, which suggested that Ogon might also be involved in BMP inhibition.

Hibi and colleagues set out to clone the ogon gene (which is also known as mercedes and short tail), in order to determine its precise relationship with Chordin, and the molecular bases for its action in regulating the establishment of the dorsal-ventral axis. The results of that positional cloning showed that ogon encodes a homolog of the Secreted Frizzled (Sizzled) protein, which has similarities to the Wnt recep- tor, Frizzled. The canonical Wnt pathway is known to play a role in axis formation, but it has been suggested that ogon/sizzled influences the dorsal-ventral axis independently of its effect on Wnt. To test this idea, members of the Hibi lab compared the activity of Ogon/Sizzled with that of Wnt inhibitors in ectopic expression studies, and found that the effects of Ogon/Sizzled misexpression resembled those of BMP, but not Wnt, inhibitors, suggesting an exceptional mode of action.

They next looked at the possible involvement of Chordin (which is known to be essential in the process of dorsalization) in Ogon/Sizzled effects by injecting ogon/sizzled RNA into mutants (chordina) lacking a functional chordina gene. This failed to rescue the ventralized phenotype of homozygous chordina mutants, indicating that Ogon/Sizzled activity depends on Chordin. The detailed functional relationship between these two dorsalizing factors remains to be worked out, but these initial findings suggest that Ogon/Sizzled augments Chordin activity either by inhibiting a Chordin inhibitor, by upregulating Chordin activity, or by making BMP more sensitive to Chordin’s inhibitory effects.

Publications


The material effect mutant ogon knockdown displays a completely ventralized phenotype.
Vertebrate embryogenesis begins with a seemingly formless egg that, through processes of division, growth, differentiation, migration and rearrangement, rapidly gives rise to a highly organized structure characterized by a number of definitive axes. The transformation is particularly striking and rapid in the zebrafish (Danio rerio), which in the space of a single day develops from an ovum to a recognizable vertebrate body laid down in respect to multiple polar vectors. The speed of its growth and reproduction, coupled with its amenability to genetic studies and the revealing pellucidity of its embryo, make this creature characterized by a number of definitive axes. Neurogenesis, focusing on two genes in particular: the zinc finger gene fez-like expressed in the forebrain, and the posterior neuron-specific homeobox gene pnx. In collaborative work, they showed that fez-like is required for the formation of hypothalamic monoaminergic neurons in zebrafish. These neurons produce neurotransmitters such as dopamine and serotonin, which are centrally involved in the control of mood, behavior, endocrine and cognitive functions in humans. A separate study identified a homeobox gene, pnx, involved in the development of posterior neurons. This gene, which is regulated by a signal from the posteriorizing non-axial mesendoderm and Notch signaling, acts as a transcriptional repressor. Misexpression of pnx results in an increase in neural precursor cells and neurons, while its repression caused reductions in certain populations of posterior neurons, indicating that the gene participates in posterior neurogenesis.

**Organizing centers, which emerge soon after fertilization, play pivotal roles in setting up positional axes in the zebrafish embryo**

Ogon/Sizzled misexpression results in a mutant-like phenotype with that of Wnt inhibitors in ectopic expression studies, and found that the effects of Ogon/Sizzled misexpression resemble those of BMP, but not Wnt, inhibitors, suggesting an exceptional mode of action.

They next looked at the possible involvement of Chordin (which is known to be essential in the process of dorsalization) in Ogon/Sizzled effects by injecting Ogon/sizzled RNA into mutants (chordin-/-) lacking a functional chordin gene. This failed to rescue the ventralized phenotype of homozygous chordin mutants, indicating that Ogon/Sizzled activity depends on Chordin. The detailed functional relationship between these two dorsalizing factors remains to be worked out, but these initial findings suggest that Ogon/Sizzled augments Chordin activity either by inhibiting a Chordin inhibitor, by upregulating Chordin activity, or by making BMP more sensitive to Chordin’s inhibitory effects.

In 2003, the Hibi lab isolated the responsible gene for a mutant named ogon, which displays ventralized phenotypes, and found that the ogon locus encodes a zebrafish homologue of the protein Secreted Frizzled (Sizzled), which functions as a negative feedback regulator of BMP signaling. While numerous mutations are known to result in dorsalized phenotypes, ogon is the second zebrafish mutant reported to show clearly ventralized phenotypes, such as expanded ventral tail fins, blood, pronephros, and other posterior structures. These patterns are similar to the phenotype observed in mutations of the gene for the BMP antagonist, Chordin, which suggested that ogon might also be involved in BMP inhibition.

Hibi and colleagues set out to clone the ogon gene (which is also known as mercedes and short tail), in order to determine its precise relationship with Chordin, and the molecular bases for its action in regulating the establishment of the dorsal-ventral axis. The results of that positional cloning showed that ogon encodes a homolog of the Secreted Frizzled (Sizzled) protein, which has similarities to the Wnt recep-}

**Vertebrate neuronal tissues are generated in a stepwise fashion.** These steps include neural induction, antero-posterior patterning, and neurogenesis. In amphibian and teleost (bony fish) embryos, neuroectoderm is induced by BMP inhibitors derived from organizing centers. Induced neuroectoderm is by default anterior in character, but a subset in some tissues is subsequently subject to posteriorizing transformations. The hindbrain and spinal cord, which are posteriorized regions, are specified by a signal from a region known as non-axial mesendoderm, while anterior neuroectoderm does not receive this posteriorizing signal develops into fore- and midbrain. After these initial processes of neural induction and patterning, neurogenic regions, the domains in which neurogenesis takes place, are established.

The Hibi lab has been working to identify genes involved in the control of this patterning and neurogenesis, focusing on two genes in particular: the zinc finger gene fez-like expressed in the forebrain, and the posterior neuron-specific homeobox gene pnx. In collaborative work, they showed that fez-like is required for the formation of hypothalamic monoaminergic neurons in zebrafish. These neurons produce neurotransmitters such as dopamine and serotonin, which are centrally important to the regulation of mood, behavior, endocrine and cognitive functions in humans. A separate study identified a homeobox gene, pnx, involved in the development of posterior neurons. This gene, which is regulated by a signal from the posteriorizing non-axial mesendoderm and Notch signaling, acts as a transcriptional repressor. Misexpression of pnx results in an increase in neural precursor cells and neurons, while its repression caused reductions in certain populations of posterior neurons, indicating that the gene participates in posterior neurogenesis.

In work published in 2003, the Hibi lab analyzed and isolated the responsible gene for a mutant named ogon, which displays ventralized phenotypes, and found that the ogon locus encodes a zebrafish homologue of the protein Secreted Frizzled (Sizzled), which functions as a negative feedback regulator of BMP signaling. While numerous mutations are known to result in dorsalized phenotypes, ogon is the second zebrafish mutant reported to show clearly ventralized phenotypes, such as expanded ventral tail fins, blood, pronephros, and other posterior structures. These patterns are similar to the phenotype observed in mutations of the gene for the BMP antagonist, Chordin, which suggested that ogon might also be involved in BMP inhibition.
The patterning of the natural world

The question of how complex patterns arise from seemingly disorganized or formless initial structures represents an intriguing challenge to mathematicians, physicists, chemists and biologists alike. Theoretical work indicates that the mechanisms underlying pattern formation are similar in both biological and non-biological systems, and a number of mathematical models capable of describing pattern formation in chemical media have been proposed. But the greater complexity of living systems has made it much more difficult to demonstrate a mathematical basis for biological patterns. In 1952, the British mathematician Alan Turing proposed a simple mathematical equation capable of generating periodic structures such as stripes, spots and reticulations. This model, known as the reaction-diffusion model, demonstrates that the interaction between a local activator and a long-range inhibitor can give rise to mathematical similarity between a well-characterized reaction that produces wave-like patterns in chemical media and a pattern-forming phenomenon in the skin of a mutant mouse. These mice, which have defects in a gene responsible for hair follicle development, develop bands of darkened skin that traverse the body surface in waves. Kondo, working in collaboration with researchers from the Medical School of Mie University, showed that these traveling waves of skin coloration are strikingly similar to nonlinear waves produced by the Belousov-Zhabotinskii (BZ) reaction in chemical systems, suggesting a shared underlying principle. The mathematical analysis suggests a candidate molecule, Shh, which satisfies the necessary criteria for an activator and a long-range inhibitor can give rise to periodic structures in response to differences in the individual diffusion rates.

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.

Traveling wave mice

In research published this year, Kondo identified a mathematical similarity between a well-characterized reaction that produces wave-like patterns in chemical media and a pattern-forming phenomenon in the skin of a mutant mouse. These mice, which have defects in a gene responsible for hair follicle development, develop bands of darkened skin that traverse the body surface in waves. Kondo, working in collaboration with researchers from the Medical School of Mie University, showed that these traveling waves of skin coloration are strikingly similar to nonlinear waves produced by the Belousov-Zhabotinskii (BZ) reaction in chemical systems, suggesting a shared underlying principle. The mathematical analysis suggests a candidate molecule, Shh, which satisfies the necessary criteria for an activator and a long-range inhibitor can give rise to periodic structures in response to differences in the individual diffusion rates.

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.
The patterning of the natural world

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

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.

Traveling wave mice

In research published this year, Kondo identified a mathematical similarity between a well-characterized reaction that produces wave-like patterns in chemical media and a pattern-forming phenomenon in the skin of a mutant mouse. These mice, which have defects in a gene responsible for hair follicle development, develop bands of darkened skin that traverse the body surface in waves. Kondo, working in collaboration with researchers from the Medical School of Mie University, showed that these traveling waves of skin coloration are strikingly similar to nonlinear waves produced by the Belousov-Zhabotinsky (BZ) reaction in chemical systems, suggesting a shared underlying principle. The research began when Kondo learned of a mutant strain of mouse with an unusual striped phenotype. The mutation, a splicing defect in the Foxn1 gene, causes hair follicle development to terminate just after skin pigments begin to accumulate. The immature follicles die off and are quickly replaced by a new hair cycle. The cyclical nature of this follicular attrition and the subsequent re-activation by neighboring follicles produces a phenotype in which the mouse’s skin color at first uniformly oscillates between dark and light coloration, then begins to take on a remarkable striped appearance, with bands of pigmentation that originate from the region under the feathers and travel across the body surface in all directions. These waves first appear at about three months after birth, and continue to arise and propagate throughout the life of the animal.

Kondo identified similarities between wave-like patterns observed in chemical media and a pattern-forming phenomenon in the skin of a mutant mouse

By tracking the movements of traveling waves in the Foxn1 mouse skin pattern, Kondo ascertained that their formation was fundamentally similar to that of waves generated by the BZ reaction. Although this finding does not directly identify the specific molecular mechanism at work in the Foxn1 phenotype, mathematical analysis suggests a candidate molecule, Shh, which satisfies the necessary criteria for a molecule key to traveling wave pattern formation. And, as many other species demonstrate similar stripe mechanisms, this report may help to provide biologists with a mathematical model to explain this skin patterning phenomenon.
Darwin conceived evolution as a process driven by the interplay between random mutation and natural selection, but the advent of molecular developmental biology in the 1980s led to the recognition that inherent genetic constraints also have a role in defining the trajectories that evolutionary processes are most likely to follow. The homeobox (Hox) genes, a developmentally important set of genes provide one instantiation of this concept; they play central roles in regulating the morphological development of organisms ranging from yeast and plants to animals from every branch of the phylogenetic tree and share a highly conserved domain (the 180-base-pair homeodomain) that indicates a non-random mode of evolutionary selection.

Evodevo

Combining experimental and analytic techniques from molecular biology, phylogeny, 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 divergence of species. His approach involves examining related genes in phylogenetically distinct animals to uncover the ways in which context affects gene expression, and thereby influences body development. By comparing the molecular bases of the emergence of discrete structures, Kuratani hopes to illustrate the means by which developmental mechanisms mediate the translation of changes in the genome to changes in morphology.

The turtle's shell

The abrupt advent of the turtle's shell, or 'carapace,' which appeared with few recognizable precursors, provides a remarkable exception to the general rule of gradual evolution in incremental steps. Such a phenomenon is 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. Evodevo (evolutionary developmental biology) theory predicts instead that the genes responsible for carapace development actually belong to a set of genes shared by groups related to the turtle, 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.

In other closely related species, ribs are entirely internal and reside interior to the scapulae (corresponding to the shoulder blades in humans). In the turtle, however, the rib bones grow exterior to the carapace to form the external carapace. Using cross-species expression, cDNA library and cell sorting studies, researchers in the Kuratani lab have identified a number of developmental switching points that enable this exteriorization of this portion of the turtle endoskeleton. Their findings indicate that alterations in a number of essentially conserved genes, rather than genetic novelty, have resulted in the turtle's unique anatomical phenotype.

Hindbrain evolution

In vertebrates, the hindbrain is a segmented structure, subdivided into clearly demarcated units called rhombomeres, which generate specific sets of neurons. The larval Amphioxus, a more primitive chordate, however, lacks this hindbrain segmentation. The lamprey, a jawless fish that arose in the interval originally independent of the lamprey, hindbrain segmentation. In work published in 2004, investigators in the Kuratani lab labeled reticulospinal and branchial motor neurons (which derive from rhombomeres) to reveal the neuronal organization of the hindbrain of the Japanese lamprey, Lethenteron japonicum, and studies the expression patterns of rhombomere-specific genes. They found that lamprey reticular neurons develop in conserved rhombomere-specific positions, similar to those observed in the gnathostome zebrafish. Interestingly, in lamprey the positions of other sets of hindbrain neurons — the trigeminal and facial motor nuclei — do not map neatly to rhombicomm field processes, as they do in gnathostomes. Rather, the trigeminal and facial motor nuclei originate, in the middle of rhombomere 4, in the region of expression of the lamprey Hox gene HoxV3. When retinoic acid (which is known to alter Hox gene expression and associated developmental programs) was introduced to the developing hindbrain region, it caused positional shifts of both HoxV3 expression and branchiomotor nuclei, but no apparent changes in segmentation or the positions of reticol neurons.

These findings indicate that, in the lamprey, hindbrain neural identity and hindbrain segmentation are governed by independent mechanisms, providing strong counter-evidence to one prevailing model that suggests that the establishment of neurons identity and specific subsets of hindbrain neurons is the result of a convergent process in which originally independent mechanisms became linked over evolutionary time.

In vertebrates, the hindbrain is a segmented structure, subdivided into clearly demarcated units called rhombomeres, which generate specific sets of neurons. The larval Amphioxus, a more primitive chordate, however, lacks this hindbrain segmentation. The lamprey, a jawless fish that arose in the interval originally independent of the lamprey, hindbrain segmentation. In work published in 2004, investigators in the Kuratani lab labeled reticulospinal and branchial motor neurons (which derive from rhombomeres) to reveal the neuronal organization of the hindbrain of the Japanese lamprey, Lethenteron japonicum, and studies the expression patterns of rhombomere-specific genes. They found that lamprey reticular neurons develop in conserved rhombomere-specific positions, similar to those observed in the gnathostome zebrafish. Interestingly, in lamprey the positions of other sets of hindbrain neurons — the trigeminal and facial motor nuclei — do not map neatly to rhombicomm field processes, as they do in gnathostomes. Rather, the trigeminal and facial motor nuclei originate, in the middle of rhombomere 4, in the region of expression of the lamprey Hox gene HoxV3. When retinoic acid (which is known to alter Hox gene expression and associated developmental programs) was introduced to the developing hindbrain region, it caused positional shifts of both HoxV3 expression and branchiomotor nuclei, but no apparent changes in segmentation or the positions of reticol neurons.

These findings indicate that, in the lamprey, hindbrain neural identity and hindbrain segmentation are governed by independent mechanisms, providing strong counter-evidence to one prevailing model that suggests that the establishment of neurons identity and specific subsets of hindbrain neurons is the result of a convergent process in which originally independent mechanisms became linked over evolutionary time.
Evo-devo

Dawson conceived evolution as a process driven by the interplay between random mutational and natural selection, but the advent of molecular developmental biology in the 1980s led to the recognition that inherent genetic constraints also have a role in defining the trajectories that evolutionary processes are most likely to follow. The homeobox (Hox) genes, a developmentally important set of genes provide one instantiation of this concept; they play central roles in regulating the morphological development of organisms ranging from yeast and plants to animals from every branch of the phylogenetic tree and share a highly conserved domain (the 180-base pair homeodomain) that indicates a non-random mode of evolutionary selection.

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

The turtle’s shell

The abrupt advent of the turtle’s shell, or ‘carapace,’ which appeared with few recognizable precursors, provides a remarkable exception to the general rule of gradual evolution in incremental steps. Such a phenomenon is difficult to explain by mutational 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 (evolutionary developmental biology) theory predicts instead that the genes responsible for carapace development actually belong to a set of genes shared by groups related to the turtle, 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.

In other closely related species, ribs are entirely internal and reside interior to the scapulae (corresponding to the shoulder blades in humans). In the turtle, however, the rib bones grow exterior to the scapulae to form the external carapace. Using cross-species expression, cDNA library and cell sorting studies, researchers in the Kuratani lab have identified a number of developmental switching points that enable this exteriorization of this portion of the turtle endoskeleton. Their findings indicate that alterations in a number of essentially conserved genes, rather than genetic novelty, have resulted in the turtle’s unique anatomical phenotype.

Hindbrain evolution

In vertebrates, the hindbrain is a segmented structure, subdivided into clearly demarcated units called rhombomeres, which generate specific sets of neurons. The larval amphibian, a more primitive chordate, however, lacks this hindbrain segmentation. The lamprey, a jawless fish that arose in the interval between non-vertebrate chordates (such as amphioxus and hagfish) and the jawed vertebrates, provides a readily available model for studying the emergent nature of the hindbrain developmental plan.

In work published in 2004, investigators in the Kuratani lab labeled reticulospinal and branchiomotor neurons (which derive from rhombomeres) to reveal the neuronal organization of the hindbrain of the Japanese lamprey, Lethenteron japonicum, and studied the expression patterns of rhombomere-specific genes. They found that lamprey reticulospinal neurons develop in conserved rhombomere-specific positions, similar to those observed in the gnathostome zebrafish. Interestingly, in lamprey the positions of other sets of hindbrain neurons—the trigeminal and facial motor nuclei—do not map neatly to rhombomeric borders, as they do in gnathostomes. Rather, the trigeminal facial nerve originates in the middle of rhombomere 4, in the region of expression of the lamprey Hox gene, Hlx2. When retinoic acid (which is known to alter Hox gene expression and associated developmental programs) was introduced to the developing hindbrain region, it caused positional shifts of both Hlx2 expression and branchiomotor nuclei, but no apparent changes in segmentation or the positions of reticulospinal neurons.

Removal of carapacial ridge results in a characteristic fan-shape splaying of turtle ribs.
Sensory cellular differentiation

The conversion of uncommitted precursor cells in the otic placode to differentiated, functional otic subtypes is very precisely timed. If it occurs prematurely, the resulting organ is underdeveloped, with too few cells forming the adult structure. If it happens too late, there is a danger that these cells will still be immature and unable to fulfill their needed roles in subsequent developmental processes. An example of this temporal regulation is seen in the wiring of the inner ear to the brain, which requires the formation of neurons from the otic placode. If these cells are not formed when this process occurs, the function of the auditory system is compromised. But what is the nature of this control? By using embryological studies, combined with gene manipulation and ex-ovo culture, Raj’s lab is addressing this question, finding the basis for this precise regulation in the functional characteristics of specific signaling molecules. Their control and effects on the development of the inner ear represents one of the most exciting aspects of research in Raj’s lab, with implications in regenerative technologies and tissue engineering based therapeutics.

Morphogenesis of the otic placode

Although the adult inner ear is located in the interior of the animal it is nonetheless an ectodermal structure, originating from a placode embedded within the outermost layer of the embryo. Through a process known as invagination, the otic placode pinches off from the ectoderm. This represents one of the earliest stages of organogenesis. Signals in 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 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. Signaling interactions, acting upon the cell surface, trigger a series of transcription events that are interpreted in the nucleus of the responding cell as transcription factors. Raj’s lab is characterizing the transcriptional response to the different signals that act to collectively specify the inner ear. By understanding their function, Raj hopes to be able to determine a mechanism of molecular synergy.

First cellular consequences of inner ear induction, and Raj has focused on this process to understand the functional significance of signaling factors and their transcriptional responses. Working from the hypothesis that extracellular cues choreograph this complex dance of cells, modifying their shape, motility, growth and the location of subcellular components, Raj hopes to be able to reconcile the events that occur outside the cell with the biological changes occurring within.

Development of other sensory organs

As well as the inner ear, Raj’s lab is also investigating the development of other sensory organs, in particular the nose and the eye. Using embryological manipulations, the Lab for Sensory Development is mapping the tissues that are responsible for the formation and differentiation of these structures. Though still in their infancy, these research projects do indicate that tissue interactions distinct from each other and from those operating to induce the inner ear are responsible for the control of their development.

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 of Fgf-19 is in purple. Shown is a section through a stage 20 embryo. Fgf-19 is expressed in the condensing otic placode at these stages, suggesting a role in the later development of the inner ear as well as its induction.

Fgf-19 is expressed in the mesoderm of the chicken embryo from early stages.

Publication


Sensory cellular differentiation

The conversion of uncommitted precursor cells in the otic placode to differentiated, functional otic subtypes is very precisely timed. If it occurs prematurely, the resulting organ is underdeveloped, with too few cells forming the adult structure. If it happens too late, there is a danger that these cells will still be immature and unable to fulfill their needed roles in subsequent developmental processes. An example of this temporal regulation is seen in the wiring of the inner ear to the brain, which requires the formation of neurons from the otic placode. If these cells are not formed when this process occurs, the function of the auditory system is compromised. But what is the nature of this control? By using embryological studies, combined with gene manipulation and ex-ovo culture, Raj’s lab is addressing this question, finding the basis for this precise regulation in the functional characteristics of specific signaling molecules. Their control and effects on the development of the inner ear represents one of the most exciting aspects of research in Raj’s lab, with implications in regenerative technologies and tissue engineering based therapeutics.

Development of the sensory organs

As well as the inner ear, Raj’s lab is also investigating other sensory organs. As the inner ear, Raj’s lab is also investigating the development of other sensory organs, in particular the nose and the eye. Using embryological manipulations, the Lab for Sensory Development is mapping the tissues that are responsible for the formation and differentiation of these structures. Though still in their infancy, these research projects do indicate that tissue interactions distinct from each other and from those operating to induce the inner ear are responsible for the control of their development.

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 of Fgf-19 in purple. Shown is a section through a stage 17 chick embryo. Fgf-19 is expressed in the condensing acoustic ganglia at these stages, suggesting a role in the later development of the inner ear as well as in its induction.

Figure 19 is expressed in the mesoderm of the chicken embryo from early stages.
Germ cells are the only cell types capable of transmitting genetic information across generations, and this function is characterized by unique developmental processes as well. In many types of animals, including the Drosophila fruit fly, the formation and differentiation of germ cells is controlled by mRNAs and proteins localized in a specific cytoplasmic region within eggs, called germ plasm. Germ plasm mRNAs are translated in a spatiotemporally regulated manner, but the means by which the germ plasm is formed and achieves this controlled translation remain largely unknown. 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 regulation. As an ovarian protein, Cup binds to the 3’ UTR of oskar mRNA, but the underlying function in vivo, that Cup interacts with Bruno, which serves as a form of supportive scaffold for the translational repression of the oskar mRNA. A similar model of protein interactions is observed in the ascidian Pgc RNA in oogenesis. The mechanism by which the Cup-Bruno complex is coupled with RNA localization remains to be solved.

Polar granule maintenance

Polar granules are large complexes of ribonucleoproteins that store RNAs and proteins required for the formation of germ cells in the fruit fly. A non-coding Polar granule component (Pgc) RNA has been shown to be important in the maintenance of these germ plasm organelles; in Pgc antisense knockdown embryos, germ cells are formed but subsequently degenerate, which has led to the hypothesis that Pgc serves as a form of supportive scaffolding maintaining the structural integrity of polar granules. The Nakamura lab is now exploring Pgc function in more detail by isolating a complete loss-of-function mutant, allowing for more specific analyses of the gene’s role. They also plan to look for the gene’s functional domains, using knockin embryos possessing a notochord, an evolutionary forebear of the spinal cord, and a rudimentary nervous system, both of which are lost when it enters its immmobile adult stage.

Nakamura is now investigating the roles of two genes in the maintenance and migration of germ cells.

In recent work, the Nakamura lab demonstrated that an ovarian protein, Cup, is another protein required for the formation of germ cells in the fruit fly. A non-coding Polar granule component (Pgc) RNA, which germ cells fail to migrate to the zona pellucida, germ cells are formed but subsequently degenerate, which has led to the hypothesis that Pgc serves as a form of supportive scaffolding maintaining the structural integrity of polar granules.

In other ongoing research, members of the Nakamura lab demonstrated that Pgc is required for the formation of germ cells in the fruit fly. A non-coding Polar granule component (Pgc) RNA, which germ cells fail to migrate to the zona pellucida, germ cells are formed but subsequently degenerate, which has led to the hypothesis that Pgc serves as a form of supportive scaffolding maintaining the structural integrity of polar granules.


Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the Drosophila fruit fly, the formation and differentiation of germ cells is controlled by mRNAs and proteins localized in a specific cytoplasmic region within eggs, called germ plasm. Germ plasm mRNAs are translated in a spatiotemporally regulated manner, whereas the means by which the germ plasm is formed and achieves this controlled translation remain largely unknown. 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 spatiotemporal regulation, which are important to a number of other developmental processes including asymmetric cell division, cell migration and axis formation and likely synaptic plasticity.

Translational repression

RNA activity during Drosophila oogenesis involves a number of sequential processes. The Drosophila oocyte shares cytoplasm with neighboring nurse cells via an incomplete cell membrane, allowing mRNAs and proteins from the nurse cells to be transported to the oocyte in the form of ribonucleoproteins. Following their export from the nurse cell nuclei, mRNAs are translationally repressed, or ‘masked,’ and transported to specified regions of the oocyte, where they establish fixed and precise localizations and retain their ability to undergo translation. In one example of this critically important regulation, the translation of the RNA for the maternal gene oskar, which has critical functions in embryonic patterning and the formation of germ cells, is repressed during its transport to the posterior pole of the oocyte. This transport-specific repression is known to be mediated by the protein Bruno, which binds to the 3' UTR of oskar mRNA, but the underlying mechanisms have remained obscure.

Following their export from the nurse cell nuclei, mRNAs are translationally repressed, or ‘masked,’ and transported to specified regions of the oocyte.

In recent work, the Nakamura lab demonstrated that an ovarian protein, Cup, is another protein required to inhibit the premature translation of oskar mRNA, and that Cup achieves this by binding to a second protein, elf4e, a 5' cap-binding general translation initiation factor. The binding with Cup prevents elf4e from binding with a different partnering molecule, elf4g, and thereby initiates the inhibition of translation. Findings that a mutant form of Cup lacking the sequence with which it binds elf4e failed to repress oskar translation in vivo, that Cup interacts with Bruno in a yeast two-hybrid assay, and that the Cup-elf4e complex associates with Bruno in vivo suggest that these three proteins form a complex that achieves translational repression by interactions with the 5' and 3' ends of the oskar RNA. A similar model of protein interactions is observed in the translational repression of the Drosophila btl RNA in the Xenopus African clawed frog, indicating that this paradigm of translational repression through the 5'/3' interactions is conserved across species.

Nakamura next intends to look into the means by which the repressor effects of the elf4e-Cup-Bruno complex are alleviated at the appropriate developmental stage, after the oskar ribonucleoprotein complex has reached and anchored to its appropriate destination at the pole of the oocyte.

After fertilization, the ascidian egg develops in a small, free-swimming tadpole possessing a notochord, an evolutionary forebear of the spinal cord, and a rudimentary nervous system, both of which are lost when it enters its immobile adult stage.

Nakamura seeks to analyze the regulatory mechanisms of germ cell development in Ciona, which are interesting in that while several lines of evidence indicate that germ cells are formed from maternally derived germ plasm, it also appears that germ cells can be regenerated after metamorphosis, suggesting that two independent mechanisms may be at work. In research conducted in collaboration with Suwa Akiyama, a local municipal aquarium, Nakamura's team will explore the genetic regulation of ascidian germ cell development by selecting candidate genes from EST and genome databases (a draft sequence of the Ciona genome is available) and confirming the spatial and temporal patterns of their expression and characterizing promoter regions and trans-acting factors.
Heterochromatin also functions in telomeres, which play key roles in replicative senescence and cancer, and centromeres, the linchpins of mitosis.

In previous studies, Nakayama demonstrated that heterochromatin protein binding states play a role in the regulation of gene silencing. Nakayama performed detailed analyses of the binding states of Swi6, a homolog of the mammalian HP-1 heterochromatin protein at the 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 at the mat locus is required for genomic integrity.

Nakayama has now linked Swi6 function to a number of other chromodomain proteins in fission yeast. Chemical modifications to histones at specific sites on the genome are known to regulate gene expression. Through his work on heterochromatin histone modifications, Nakayama has uncovered potentially important new roles for proteins in the establishment, maintenance and transmission of epigenetic information.

Through his work on heterochromatin histone modifications, Nakayama has uncovered potentially important new roles for proteins in the establishment, maintenance and transmission of epigenetic information. These findings show that the definition 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.
Modifications to the nuclear DNA-protein chromatin complex are central to the epigenetic regulation of gene transcription, an activity that must be maintained and propagated across mitotic cycles and throughout the development of the organism proper in order for cells to establish and maintain their identities. Chromatin occurs in highly-condensed and less spatially concentrated states, known as heterochromatin and euchromatin, respectively. Heterochromatin regions have fewer genes overall than euchromatic stretches of the genome, and many of the genes that are found there remain unexpressed. However, heterochromatin functions as more than a locked closed to store stretches of unused DNA; in fact, there appear to be a number of functionally distinct types of heterochromatin serving in a spectrum of developmentally important capacities, from the transcriptional regulation of cell-type specific genes to genomic self-defense by compartmentalizing and neutralizing foreign mobile genetic elements that might otherwise interfere with proper gene function. Heterochromatin also functions in two genetically silent chromosomal regions: telomeres, which play key roles in replicative senescence and cancer, and centromeres, the linchpins of mitosis.

Heterochromatin also functions in telomeres, which play key roles in replicative senescence and cancer, and centromeres, the linchpins of mitosis.

In previous studies, Nakayama demonstrated that heterochromatin protein binding states play a role in the regulation of gene silencing. Nakayama performed detailed analyses of the binding states of Swi6, a homolog of the mammalian HP-1 heterochromatin protein, is a component of fission yeast Clr6 histone deacetylase complex required for genomic integrity. Through his work on heterochromatin histone modifications, Nakayama has uncovered potentially important new roles for proteins in the establishment, maintenance and transmission of epigenetic information. These findings show that the definition 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.
Cell migration and organogenesis

Cell division, cell migration and changes in cell shape are coordinated to generate complex tissue and organ systems during animal development and organogenesis. One important aspect of this minutely regulated process is the movement of cells in sheets, an activity orchestrated by mechanisms that remain poorly understood. During organogenesis, sheets of cells migrate and spread over the underlying tissues while maintaining selective adhesive bonds with cells of similar type, and make their way to destinations in the body, following molecular guidance cues generated by mechanisms that remain enigmatic. Kiyoji Nishiwaki studies gonadogenesis in the nematode C. elegans as a model system to improve the understanding of the molecular bases of cell migration during organ development. By developing a clearer picture of evolutionarily conserved mechanisms, this research promises to provide insights into cell migration and organ development in humans, and contribute to the understanding of human diseases involving disturbances in coordinated cellular movement.

Guidance of the gonad

Interactions between the basement membrane of the migrating gonad and that of the body wall are keystone processes in gonadal development. The C. elegans gonad is U-shaped, the result of the directed migration of cells known as distal tip cells present at the leading ends of the developing gonad in larvae. The gene mgl-17, which Nishiwaki previously showed to be essential to this guided movement, is expressed in muscle cells and secreted into the body cavity where it binds to the migrating gonadal distal tip cells. Genetic analyses demonstrated that MIG-17 molecules in MIG-23 mutants had lower than normal molecular weights consistent with the loss of glycosylation, while immunoblotting studies confirmed that in these mutants MIG-17 failed to bind sugars at the normal levels. MIG-17 molecules designed with deficient glycosylation sites also failed to localize at the gonad and showed no ability to direct its migration. Genetic analyses revealed that MIG-23 is expressed in muscle cells, which receive signals with MIG-17’s muscle cell origin. Taken together, these results indicate that MIG-23 molecules are secreted from muscle cells after glycosylation by MIG-23, a modification which enables their recruitment to the migrating gonad.

Nishiwaki’s team has analyzed a number of mutations that result in aberrant gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protein was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Goji apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose roles are incompletely understood, but which is thought to control cell migration in Caenorhabditis elegans. Because of the complexity of the CrkII/Dock180/Rac pathway, is required for gonadogenesis and cell migration. Cell 107:27-41 (2000).

Kiyotaka Ohkura
Shinji Ihara
Yukihiko Kubota
Kiyoji Nishiwaki
Research Scientist
Staff

This research promises to provide insights into cell migration and organ development in humans.

Questions and implications

Although Goji body NDPases such as MIG-23 are capable of modifying a number of other secreted proteins, the Nishiwaki study indicates that the effects of the NDPase dysfunction are especially prominent in the abnormal glycosylation and function of an ADAM family protease, suggesting that glycosylation may play a more important role in the function of proteins in this family than it does in that of other secreted proteins. ADAM family proteases are known to be involved in a number of human diseases and the question of whether glycosylation defects play a role in their pathogenesis merits further investigation. Research such as this provides insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions by restoring normal ADAM protease glycosylation in cases in which it is aberrant.

MIG-17 is glycosylated by MIG-23 and secreted from the muscle cells to localize on the gonad surface where it is required for the migration of distal tip cells.

This is a highly schematic representation of the cell's migration and organogenesis processes, highlighting the role of MIG-17 and MIG-23 in this coordination. Figure 1 here is not necessary as the text contains the information provided.
Cell migration and organogenesis

Cell migration and changes in cell shape are coordinated to generate complex tissue and organ systems during animal development and regeneration. One important aspect of this minutely regulated process is the movement of cells in sheets, an activity orchestrated by mechanisms that remain poorly understood. During organogenesis, sheets of cells migrate and spread over the underlying tissues while maintaining selective adhesive bonds with cells of similar type, and make their way to destinations in the body, following molecular guidance cues generated by mechanisms that remain enigmatic. Kiyoji Nishiwaki studies gonadogenesis in the nematode C. elegans as a model system to improve the understanding of the molecular bases of cell migration during organ development. By developing a clearer picture of evolutionarily conserved mechanisms, this research promises to provide insights into cell migration and organ development in humans, and contribute to the understanding of human diseases involving disturbances in coordinated cellular movement.

Guidance of the gonad

Interactions between the basement membrane of the migrating gonad and that of the body wall are keystone processes in gonadal development. The C. elegans gonad is U-shaped, the result of the directed migration of cells known as distal tip cells present at the leading ends of the developing gonad in larvae. The gene mig-17, which Nishiwaki previously showed to be essential to this guided movement, is expressed in muscle cells and secreted into the body cavity where it binds to the migrating gonadal tip cells. Distal tip cells are worms engineered to lack the MIG-17 protein fail to steer cell migration properly, resulting in abnormal gonadal development and a ‘meandering’ gonad phenotype. However, the means by which MIG-17 coordinates the timing and direction of the developing gonad’s progress through the larval body has remained unknown.

MIG-17 is an ADAM (a disintegrin and metalloprotease) protease, a family of secreted or membrane proteins first identified as components of various snake venoms, where they have hemorrhagic and anti-coagulatory effects. ADAM family proteases have been implicated in a number of human physiological and pathological processes, including blood coagulation disorders, rheumatoid arthritis and asthma. These proteases act to proteolytically release membrane-bound growth factors or to disrupt components of extracellular matrices such as collagen and proteoglycans (which help to build skin and soft tissue).

Nishiwaki’s team has analyzed a number of mutations that result in abnormal gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protease was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Golgi apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose role is incompletely understood, but which is thought to serve a regulatory function in some signaling molecules. On further investigation, MIG-23 mutants showed reductions in NDPase activity. The wild-type MIG-23 protein successfully rescued yeast mutants for NDPase, lending solid support to the case for the effects of glycosylation on organogenesis.

This research promises to provide insights into cell migration and organ development in humans.

Questions and implications

Although Golgi body NDPases such as MIG-23 are capable of modifying a number of other secreted proteins, the Nishiwaki study indicates that the effects of the NDPase dysfunction are especially prominent in the abnormal glycosylation and function of an ADAM family protease, suggesting that glycosylation may play a more important role in the function of proteins in this family than it does in that of other secreted proteins. ADAM family proteases are known to be involved in a number of human diseases, and the question of whether glycosylation defects play a role in their pathogenesis merits further investigation. Research such as this provides insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions by restoring normal ADAM protease glycosylation in cases in which it is deficient.

Nishiwaki’s team has analyzed a number of mutations that result in aberrant gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protease was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Golgi apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose role is incompletely understood, but which is thought to serve a regulatory function in some signaling molecules. On further investigation, MIG-23 mutants showed reductions in NDPase activity. The wild-type MIG-23 protein successfully rescued yeast mutants for NDPase, lending solid support to the case for the effects of glycosylation on organogenesis.

This research promises to provide insights into cell migration and organ development in humans.

Questions and implications

Although Golgi body NDPases such as MIG-23 are capable of modifying a number of other secreted proteins, the Nishiwaki study indicates that the effects of the NDPase dysfunction are especially prominent in the abnormal glycosylation and function of an ADAM family protease, suggesting that glycosylation may play a more important role in the function of proteins in this family than it does in that of other secreted proteins. ADAM family proteases are known to be involved in a number of human diseases, and the question of whether glycosylation defects play a role in their pathogenesis merits further investigation. Research such as this provides insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions by restoring normal ADAM protease glycosylation in cases in which it is deficient.

Nishiwaki’s team has analyzed a number of mutations that result in aberrant gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protease was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Golgi apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose role is incompletely understood, but which is thought to serve a regulatory function in some signaling molecules. On further investigation, MIG-23 mutants showed reductions in NDPase activity. The wild-type MIG-23 protein successfully rescued yeast mutants for NDPase, lending solid support to the case for the effects of glycosylation on organogenesis.

This research promises to provide insights into cell migration and organ development in humans.

Questions and implications

Although Golgi body NDPases such as MIG-23 are capable of modifying a number of other secreted proteins, the Nishiwaki study indicates that the effects of the NDPase dysfunction are especially prominent in the abnormal glycosylation and function of an ADAM family protease, suggesting that glycosylation may play a more important role in the function of proteins in this family than it does in that of other secreted proteins. ADAM family proteases are known to be involved in a number of human diseases, and the question of whether glycosylation defects play a role in their pathogenesis merits further investigation. Research such as this provides insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions by restoring normal ADAM protease glycosylation in cases in which it is deficient.

Nishiwaki’s team has analyzed a number of mutations that result in aberrant gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protease was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Golgi apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose role is incompletely understood, but which is thought to serve a regulatory function in some signaling molecules. On further investigation, MIG-23 mutants showed reductions in NDPase activity. The wild-type MIG-23 protein successfully rescued yeast mutants for NDPase, lending solid support to the case for the effects of glycosylation on organogenesis.

This research promises to provide insights into cell migration and organ development in humans.

Questions and implications

Although Golgi body NDPases such as MIG-23 are capable of modifying a number of other secreted proteins, the Nishiwaki study indicates that the effects of the NDPase dysfunction are especially prominent in the abnormal glycosylation and function of an ADAM family protease, suggesting that glycosylation may play a more important role in the function of proteins in this family than it does in that of other secreted proteins. ADAM family proteases are known to be involved in a number of human diseases, and the question of whether glycosylation defects play a role in their pathogenesis merits further investigation. Research such as this provides insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions by restoring normal ADAM protease glycosylation in cases in which it is deficient.

Nishiwaki’s team has analyzed a number of mutations that result in aberrant gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protease was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Golgi apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose role is incompletely understood, but which is thought to serve a regulatory function in some signaling molecules. On further investigation, MIG-23 mutants showed reductions in NDPase activity. The wild-type MIG-23 protein successfully rescued yeast mutants for NDPase, lending solid support to the case for the effects of glycosylation on organogenesis.

This research promises to provide insights into cell migration and organ development in humans.

Questions and implications

Although Golgi body NDPases such as MIG-23 are capable of modifying a number of other secreted proteins, the Nishiwaki study indicates that the effects of the NDPase dysfunction are especially prominent in the abnormal glycosylation and function of an ADAM family protease, suggesting that glycosylation may play a more important role in the function of proteins in this family than it does in that of other secreted proteins. ADAM family proteases are known to be involved in a number of human diseases, and the question of whether glycosylation defects play a role in their pathogenesis merits further investigation. Research such as this provides insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions by restoring normal ADAM protease glycosylation in cases in which it is deficient.

Nishiwaki’s team has analyzed a number of mutations that result in aberrant gonadal phenotypes similar to those seen in mig-17 mutants, including the mutation of the gene that encodes MIG-23. This protease was found to be structurally related to nucleoside diphosphatases (NDPases), glycosylating proteins found in the Golgi apparatus, the intracellular processing center where proteins are modified, sorted and released from the cell. Glycosylation involves the addition of a sugar to a site on a protein chain, a post-translational modification whose role is incompletely understood, but which is thought to serve a regulatory function in some signaling molecules. On further investigation, MIG-23 mutants showed reductions in NDPase activity. The wild-type MIG-23 protein successfully rescued yeast mutants for NDPase, lending solid support to the case for the effects of glycosylation on organogenesis.

This research promises to provide insights into cell migration and organ development in humans.
pluripotent cell studies

Self-renewal and pluripotency

Their ability to self-renew indefinitely and to differentiate into cells of all three germ layer types (a differentiate capacity termed ‘pluripotency’), makes embryonic stem (ES) cells one of the most versatile tools in the toolbox of molecular biologists. ES cells are capable of differentiating into any cell type within the body, allowing researchers to study the development of cells and tissues and to potentially engineer replacement tissues and organs for therapeutic use.

Inducing differentiation

The development of methods by which undifferentiated ES cells can be induced to commit to a specific cell lineage is of central importance to the stem cell research community. Studying factors identified in work on knockout mice, the Niwa research team has been engaged in the analysis of transcription factors with potential roles in the development of extraembryonic lineages. Extraembryonic endoderm derivatives include the parietal and visceral endoderm, which give rise to the yolk sac covering embryos in utero during development, and trophectoderm derivatives, the source of the placenta that sustains the developing mammalian embryo.

In previous research, the Niwa lab showed that the overexpression of a transcription factor such as Gata-4 or Gata-6 resulted in the specific conversion of undifferentiated ES cells to extraembryonic endodermal cells, and that the exogenous expression of either of these factors simultaneously induced the expression of the other from the endogenous gene. New work suggests that the homeobox gene Cd2-2 can serve as a similar trigger for the induction of trophectoderm from ES cells expressing low levels of the pluripotency maintaining gene, Oct 3/4. Using regulatable activation of Cd2-2 in vitro, Niwa successfully induced ES cells to differentiate into trophectoderm stem cells. These results, in combination with the GATA study, have led to the development of a model in which Oct 3/4, when maintained at an appropriate mid-range level, inhibits the differentiation of ES cells into either the trophectodermal or primitive endodermal lineages by suppressing Cd2-2 and Gata-6, respectively.

Maintaining pluripotency

The POU-family transcriptional regulator Oct 3/4 was the first factor found to elicit multiple differentiation outcomes dependent on its expression level. ES cells expressing median levels of Oct 3/4 maintain their pluripotency, while their overexpression results in differentiation into primitive endoderm and mesoderm and its inhibition causes the cells to take up a trophectodermal fate. These findings established Oct 3/4 as a primary regulator of pluripotency in ES cells, but the function of this regulator in the commitment of more specific lineages remains an open issue.

In addition to its function as a maintainer of pluripotency, Oct 3/4 may also play a secondary role in regulating neuronal differentiation.

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 ES 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 putative ‘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.

Expressions from either Oct 3/4 or SAF can lead to self-induction of ES cell colonies, as shown in the figure above. This phenomenon has been termed ‘self-renewal and pluripotency’ and is a hallmark of ES cells. ES cells maintain their self-renewal capacity through the expression of a family of transcription factors known as the POU family, which includes Oct 3/4. These factors control the expression of genes involved in cell proliferation and differentiation, allowing ES cells to maintain their pluripotency indefinitely.

In 2003, the Niwa lab contributed to a study that demonstrated a role for Oct 3/4 in neurogenesis promoted by stromal cell derived inducing activity (SDIA, see p. 18-19 for a description of this phenomenon). The researchers found that SDIA acts to maintain Oct 3/4 expression in ES cells, which appears to be important as a promoter of the differentiation of ES cells into neural lineages. ES cells from which Oct 3/4 had been deleted lost their ability to differentiate into neurons, while heightened levels of Oct 3/4 intensified the neurogenic effects of SDIA. This finding raises the possibility that, in addition to its function as a maintainer of pluripotency, Oct 3/4 also plays a secondary role in regulating neuronal differentiation.

Contribution of SDIA to neurogenesis.

Trophectoderm stem (TS) cells, derived from ES cells, grown on feeder cells in the presence of recombinant FGF4
**Self-renewal and pluripotency**

Their ability to self-renew indefinitely and to differentiate into cells of all three germ layer types (a differentiative capacity termed ‘pluripotency’), makes embryonic stem (ES) cells one of the most promising subjects of study in regenerative medicine, as well as an attractive model system for research into a spectrum of developmental processes. However, the mechanisms by which ES cells are able to maintain these capabilities are incompletely understood, and a better understanding of the stemness of these cells will be necessary in order to be able to take best advantage of their remarkable properties.

Two of the biggest challenges that now face stem cell research are the determination of the factors that allow ES cells to generate limitless self-renewable progeny, and the identification of molecules that direct the dividing ES cell to produce daughter cells of specific types. Hitoshi Niwa’s research addresses both of these challenges, with the aims of developing solid scientific foundations and reliable technologies to support this exciting field of biomedicine.

### Inducing differentiation

The development of methods by which undifferentiated ES cells can be prompted to commit to a specific cell lineage is a central topic in the stem cell research community. Studying factors identified in knockout mice, the Niwa research team has been engaged in the analysis of transcription factors with potential roles in the development of extraembryonic endoderm and trophectoderm. Extraembryonic endoderm derivatives include the parietal and visceral endoderm, which give rise to the yolk sac covering embryos in utero during development, and trophectoderm derivatives, the source of the placenta that sustains the developing mammalian embryo.

In previous research, the Niwa lab showed that the overexpression of GATA transcription factors such as Gata-3 and Gata-6 resulted in the specific conversion of undifferentiated ES cells into extraembryonic endodermal cells, and that the exogenous expression of either of these factors simultaneously induced the expression of the other from the endogenous genes. More work suggests that the homeobox gene Cdx-2 can serve as a similar trigger for the induction of trophectoderm from ES cells expressing low levels of the pluripotency maintaining gene, Oct 3/4. Using regulatable activation of Cdx-2 in vitro, Niwa successfully induced ES cells to differentiate into trophectoderm stem cells. These results, in combination with the GATA study, have led to the development of a model in which Oct 3/4, when maintained at an appropriate mid-range level, inhibits the differentiation of ES cells into either the trophectodermal or primitive endodermal lineages by suppressing Cdx-2 and Gata-6, respectively.

### Maintaining 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 median levels of Oct 3/4 maintain their pluripotency, while their overexpression results in differentiation into primitive endoderm and mesendoderm and its inhibition causes the cells to take up a trophectodermal fate. These findings established Oct 3/4 as a primary regulator of placentaly in ES cells, but the function of this regulator in the commitment of more specific lineages remains an open issue.

In addition to its function as a maintainer of pluripotency, Oct 3/4 may also play a second key role in regulating neuronal differentiation.

### 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 putative ‘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 revolutionary important cells.

**Terminally differentiated trophectoderm giant cells derived from ES cells, showing large, flat morphologies and polyploidy.**

Terminally differentiated trophectoderm giant cells derived from ES cells, showing large, flat morphologies and polyploidy.
The methylation of mammalian DNA plays an important role in determining gene expression. The number and locations of methyl tags provide molecular icons marking genes as inactive, or in some cases, active. Certain types of protein recognize and bind to the tagged DNA, affecting the expression patterns of the genes it encodes. During embryonic development, DNA methylation patterns are established in a sequence of steps involving both the removal of methyl tags at the preimplantation stage and the establishment of new sets of tags, a process known as de novo methylation, which occurs after implantation and again during gametogenesis. These methylation patterns can subsequently be maintained and transmitted across generations by maintenance methylation.

Masaki Okano’s research concentrates on the mechanisms by which DNA methylation is established and maintained throughout development, focusing on the properties and activity of the Dmnt family of methyltransferases, which includes Dmnt1, Dmnt3a and Dmnt3b. He particularly focuses on working out and clarifying the roles of de novo methyltransferases in embryogenesis and cell differentiation, seeing these enzymes as necessary agents in maintaining the proper balance between stability and plasticity in the expression of specific genes.

Dmnt3a and Dmnt3b

Methylation lays down developmentally significant gene expression patterns that tend not to alter once established. In previous work, Okano showed that de novo methylation in mice required the expression of a pair of methyltransferase genes, Dmnt3a and Dmnt3b, at a very early stage of embryonic development, as well as during gametogenesis. Prior to this finding, it was believed that a related gene, Dmnt1, was solely responsible for the methylation of DNA. Using lacZ reporter gene studies, Okano showed that these genes are expressed in early embryonic and ES cells, suggesting that they function in the epigenetic reprogramming of DNA methylation states. Based on these findings, it is now known that Dmnt functions primarily in methylation maintenance, which is necessary for the stable inheritance of tissue-specific methylation patterns, but that Dmnt3a and Dmnt3b are the principal determinants in initiating DNA methylation. Recent work has also shown that isoforms of Dmnt3a and Dmnt3b function as maintenance methyltransferases in mouse embryonic stem cells, indicating a broader role for these proteins.

As de novo methylation activity takes place mainly in embryonic stem (ES) cells, early postimplantation embryos and gametogenesis, ES cells provide a highly suitable experimental model system for investigating the molecular mechanisms underlying the establishment of new methylation patterns. Okano is studying the consequences of conditional knockout and overexpression of methyltransferase genes on mouse development using ES cells and transgenic mice, in the hopes of uncovering links with tumorigenesis, congenital nervous system defects and abnormal cell differentiation.

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 as well as the roles of methylation in development, health, and regeneration.

De novo methylation is established in a spectrum of cells, including germ cell development and differentiation. Knockout mutations of Dmnt3a and Dmnt3b result in a spectrum of organic, neurological and spermatogenic defects. Dmnt3b mutants generally die by embryonic stage 16.5 due to widespread defects affecting organ development and general growth. Loss of Dmnt3a function produces less pronounced effects; embryogenesis appears normal, but most mutants die by the fourth week after birth and exhibit abnormal growth, intestinal motility and spermatogenesis. The demethylation of both genes results in developmental arrest at around E8.0, prior to somitogenesis, and early embryonic lethality by E9.5. These loss-of-function defects indicate that the establishment of the DNA methylation state plays an important part in many aspects of mouse development, an area the Okano lab will continue to explore.

Abnormal methylation has been implicated in a number of human congenital anomalies as well. ICF (Immunodeficiency-Centromeric instability-Facial anomalies) syndrome, which is characterized by immune deficiency, heterochromatin instability and defects in facial development, results from a mutation in the gene encoding Dmnt3b. A mutation in the gene encoding the methyl-cytosine binding protein, Mcf21, is also known to cause Reif syndrome, a neurological disorder that occurs almost entirely in females. In this form of mental retardation, development appears normal until the child is 6-18 months old, after which the child loses communication skills and develops disturbances in gait and use of the hands and slowed growth of the head.

As de novo methylation activity takes place mainly in embryonic stem (ES) cells, early postimplantation embryos and gametogenesis, ES cells provide a highly suitable experimental model system for investigating the molecular mechanisms underlying the establishment of new methylation patterns. Okano is studying the consequences of conditional knockout and overexpression of methyltransferase genes on mouse development using ES cells and transgenic mice, in the hopes of uncovering links with tumorigenesis, congenital nervous system defects and abnormal cell differentiation.

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 as well as the roles of methylation in development, health, and regeneration.

De novo methylation is established in a spectrum of organic, neurological and spermatogenic defects. Dmnt3b mutants generally die by embryonic stage 16.5 due to widespread defects affecting organ development and general growth. Loss of Dmnt3a function produces less pronounced effects; embryogenesis appears normal, but most mutants die by the fourth week after birth and exhibit abnormal growth, intestinal motility and spermatogenesis. The demethylation of both genes results in developmental arrest at around E8.0, prior to somitogenesis, and early embryonic lethality by E9.5. These loss-of-function defects indicate that the establishment of the DNA methylation state plays an important part in many aspects of mouse development, an area the Okano lab will continue to explore.

Abnormal methylation has been implicated in a number of human congenital anomalies as well. ICF (Immunodeficiency-Centromeric instability-Facial anomalies) syndrome, which is characterized by immune deficiency, heterochromatin instability and defects in facial development, results from a mutation in the gene encoding Dmnt3b. A mutation in the gene encoding the methyl-cytosine binding protein, Mcf21, is also known to cause Reif syndrome, a neurological disorder that occurs almost entirely in females. In this form of mental retardation, development appears normal until the child is 6-18 months old, after which the child loses communication skills and develops disturbances in gait and use of the hands and slowed growth of the head.

As de novo methylation activity takes place mainly in embryonic stem (ES) cells, early postimplantation embryos and gametogenesis, ES cells provide a highly suitable experimental model system for investigating the molecular mechanisms underlying the establishment of new methylation patterns. Okano is studying the consequences of conditional knockout and overexpression of methyltransferase genes on mouse development using ES cells and transgenic mice, in the hopes of uncovering links with tumorigenesis, congenital nervous system defects and abnormal cell differentiation.

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 as well as the roles of methylation in development, health, and regeneration.
The methylation of mammalian DNA plays an important role in determining gene expression. The number and locations of methyl tags provide molecular icons marking genes as inactive, or in some cases, active. Certain types of protein recognize and bind to the tagged DNA, affecting the expression patterns of the genes it encodes. During embryonic development, DNA methylation patterns are established in a sequence of steps involving both the removal of methyl tags at the preimplantation stage and the establishment of new sets of tags, a process known as de novo methylation, which occurs after implantation and again during gametogenesis. These methylation patterns can subsequently maintain and transmitted across generations by maintenance methylation.

Masaki Okano’s research concentrates on the mechanisms by which DNA methylation is established and maintained. Throughout development, focusing on the properties and activity of the Dnmt family of methyltransferases, which includes Dnmt1, Dnmt3a, and Dnmt3b. He particularly focuses on working out and clarifying the roles of de novo methyltransferases in embryogenesis and cell differentiation, seeing these enzymes as necessary agents in maintaining the proper balance between stability and plasticity in the expression of specific genes.

Dnmt3a and Dnmt3b
Methylation lays down developmentally significant gene expression patterns that tend not to alter once established. 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 as 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 genes are expressed in early embryonic and ES cells, suggesting that they function in the epigenetic reprogramming of DNA methylation states. Based on these findings, it is now known that Dnmt1 functions primarily in methylation maintenance, which is necessary for the stable inheritance of tissue-specific methylation patterns, but that Dnmt3a and Dnmt3b are the principal determinants in initiating DNA methylation. Recent work has also shown that isoforms of Dnmt3a and Dnmt3b function as maintenance methyltransferases in mouse embryonic stem cells, indicating a broader role for these proteins.

As de novo methylation activity takes place mainly in embryonic stem (ES) cells, early postimplantation embryos and gametogenesis, ES cells provide a highly suitable experimental model system for investigating the molecular mechanisms underlying the establishment of new methylation patterns. Okano is studying the consequences of conditional knockout and overexpression of methyltransferase genes on mouse development using ES cells and transgenic mice, in the hopes of uncovering links with tumorigenesis, congenital nervous system defects and abnormal cell differentiation.
By mechanisms still unknown, fertilization achieves the transformation of two highly specialized cells — a sperm and an unfertilized egg (oocyte) — to a single-cell embryo that can give rise to an entire individual. The first moments of this remarkable process are known as oocyte activation, an intricate orchestration of sub-cellular events that include all the checks and balances that presage healthy growth of a new embryo. Oocyte activation can be observed at the light microscope level as dramatic change in the morphology of the newly fertilized egg and provides an incredible read-out of the underlying molecular mechanisms. The Laboratory of Mammalian Molecular Embryology combines molecular and cellular biology with piezo-activated micromanipulation of mouse (Mus musculus) gametes and embryos to study oocyte activation and its developmental consequences.

This task is large, because beneath the membrane of a sperm head reside macromolecular complexes that include a nucleus (containing the paternal genome and associated proteins) and a surrounding cytoplasmic matrix, the perinuclear matrix (PNM). It has been estimated that the PNM comprises some 230 protein species. Because it is juxtaposed to the inner leaflet of ensheathing sperm head membranes, the PNM rapidly comes into contact with the oocyte cytoplasm at fertilization and is an immediate source of paternally-contributed molecules that potentially modulate development. However, owing in part to its size and complexity, its excoriation and disassembly at fertilization achieves the transformation of two highly specialized cells — a sperm and an unfertilized egg (oocyte) — to a single-cell embryo that can give rise to an entire individual. The first moments of this remarkable process are known as oocyte activation, an intricate orchestration of sub-cellular events that include all the checks and balances that presage healthy growth of a new embryo. Oocyte activation can be observed at the light microscope level as dramatic change in the morphology of the newly fertilized egg and provides an incredible read-out of the underlying molecular mechanisms. The Laboratory of Mammalian Molecular Embryology combines molecular and cellular biology with piezo-activated micromanipulation of mouse (Mus musculus) gametes and embryos to study oocyte activation and its developmental consequences.

### Interactions between gamete cytolysas

Microinjection of an isolated sperm head into an oocyte can result in the birth of normal young. Thus, the sperm head — the part of the head that lies beneath its membranes — apparently contains all of the paternal information sufficient for full development. Tony Perry is systematically elucidating interactions between sub-membrane sperm head components and the oocyte at fertilization, particularly during the moments soon after the sperm has entered the oocyte. The Perry lab’s interests center on attributing molecular identities to the proteins involved in these interactions and characterizing their function.

The Perry team’s analysis of sperm-oocyte interactions at fertilization begins with demembranated sperm that are subsequently exposed to standardized conditions that recapitulate key aspects of the oocyte cytoplasm. Proteins solubilized in this way can be injected into eggs, typically by piezo-activated micromanipulation, to probe their function. Such functional analysis is coupled to molecular analyses that span several disciplines. The proteins can be purified and identified. Perry’s team employs chromatographic and state-of-the-art 2D electrophoretic methods to this end. Antibodies raised against peptides identified in this way are used to localize the proteins before, during and after fertilization; antibody function can also be assayed by microinjection. Owing to dissimilarities between frog and mammalian oocytes and the paucity of material obtainable from the latter, genetic methods are used to identify mouse oocyte proteins that interact with the sperm proteins that have been characterized. Collectively, these studies hold the promise of enabling the identification of oocyte signaling pathways and processes that become operational at fertilization and establish what roles they play in subsequent development. Such roles may not be restricted to short-term development, as a growing body of evidence indicates that they have far-reaching consequences, even after the resulting adult is in old age.

### Novel methods of genetic modulation using gametes

Utilizing the integrated molecular, cellular, and embryological approach from the work in his laboratory, Perry expects to gain insights that will enable us to genetically engineer embryos in new ways. He has developed a novel method of genome manipulation known as metaphase II (mII) transgenesis. This efficient method of transgenesis works when oocytes (normally arrested at mit) are co-injected with a nucleus and transgene (tg) DNA. Typically, sperm heads are depleted of their membranes by detergent extraction prior to mixing with tg DNA and co-injection. This has several advantages over traditional methods of tg introduction; cloning and propagation in viral vectors is not required and the method lends itself to tgs in the megabase range, which is too large for lentiviral delivery. Perry and members of his lab are adapting the method to facilitate high throughput targeted knock-out and -down phyla-toxins. Developing these approaches is an important supplementary part of their work to facilitate the molecular dissection of sperm protein function in the context of embryos and whole animals.

<table>
<thead>
<tr>
<th>Protein ID</th>
<th>Role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>p30 Calmodulin BP</td>
<td>Role unknown</td>
<td>Leclerc and Goupil, 2000</td>
</tr>
<tr>
<td>PT32 WBP2, a c-Yes BP</td>
<td>Role unknown</td>
<td>Gong et al., 1997</td>
</tr>
<tr>
<td>Stat4 Jak-Stat signaling</td>
<td>Role unknown</td>
<td>Herrada &amp; Wolgemuth, 1997</td>
</tr>
<tr>
<td>c-Yes Protein tyrosine kinase</td>
<td>Role unknown</td>
<td>Leclerc and Goupil, 2002</td>
</tr>
<tr>
<td>Arp-T1 Actin-related</td>
<td>Role unknown</td>
<td>Heid et al., 2002</td>
</tr>
<tr>
<td>Arp-T2 Actin-related</td>
<td>Role unknown</td>
<td>Heid et al., 2002</td>
</tr>
<tr>
<td>Sup728</td>
<td>Role unknown</td>
<td>Aikawa and Ono, 2002</td>
</tr>
<tr>
<td>PknRF1</td>
<td>Lipophilic transport membrane</td>
<td>Role unknown</td>
</tr>
<tr>
<td>Start</td>
<td>Jak3 signaling, role unknown</td>
<td>Henders and Ngomoh, 1997</td>
</tr>
<tr>
<td>v-Src Protein tyrosine kinase</td>
<td>Role unknown</td>
<td>Leclerc and Goupil, 2002</td>
</tr>
<tr>
<td>MAP4K3, a Yes BP</td>
<td>Role unknown</td>
<td>Gong et al., 1997</td>
</tr>
<tr>
<td>p18</td>
<td>Cullin5BP1, role unknown</td>
<td>Leclerc and Goupil, 2008</td>
</tr>
</tbody>
</table>

To date, few PNM proteins have been described, and the function of almost none of them is known.
By mechanisms still unknown, fertilization achieves the transformation of two highly specialized cells—a sperm and an unfertilized egg (oocyte)—to a single-cell embryo that can give rise to an entire individual. The first moments of this remarkable process are known as oocyte activation, an intricate orchestration of sub-cellular events that include the checks and balances that preserve healthy growth of a new embryo. Oocyte activation can be observed at the light microscope level as dramatic change in the morphology of the newly fertilized egg and provides an incredible read-out of the underlying molecular mechanisms. The Laboratory of Mammalian Molecular Embryology combines molecular and cellular biology with piezo-actuated micromanipulation of mouse (Mus musculus) gametes and embryos to study oocyte activation and its developmental consequences.

This task is large, because beneath the membrane of a sperm head resides macromolecular complexes that include a nucleus (containing the paternal genome and associated proteins) and a surrounding cytoplasmic matrix, the perinuclear matrix (PNM). It has been estimated that the PNM comprises some 230 protein species. Because it is juxtaposed to the inner leaflet of enshrouding sperm head membranes, the PNM rapidly comes into contact with the oocyte cytoplasm at fertilization and is an immediate source of paternally-contributed molecules that potentially modulate development. However, owing in part to its size and complexity, its excoriation and disassembly at fertilization begins with demembranated sperm that are subsequently exposed to standardized conditions that recapitulate key aspects of the oocyte cytoplasm. Proteins solubilized in this way can be injected into eggs, typically by piezo-activated microinjection, to probe their function. Such functional analysis is coupled to molecular analyses that span several disciplines. The proteins can be purified and identified. Perry’s team employs chromatographic and state-of-the-art 2D electrophoretic methods to this end. Antibodies raised against peptides identified in this way are used to localize the proteins before, during and after fertilization; antibody function can also be assayed by microinjection. Owing to dissimilarities between frog and mammalian oocytes and the paucity of material obtainable from the latter, genetic methods are used to identify mouse oocyte proteins that interact with the sperm proteins that have been characterized. Collectively, these studies hold forth the promise of enabling the identification of oocyte signaling pathways and processes that become operational at fertilization and establish what roles they play in subsequent development. Such roles may not be restricted to short-term development, as a growing body of evidence indicates that they have far-reaching consequences, even after the resulting adult is in old age.

### Novel methods of genetic modulation using gametes

Utilizing the integrated molecular, cellular, and embryological approach from the work in his laboratory, Perry expects to gain insights that will enable us to genetically alter embryos in new ways. He has developed a novel method of genome manipulation known as metaphase II (MII) transgenesis. This efficient method of transgenesis works on oocytes (normally arrested at MII) co-injected with a nucleus and transgene (tg) DNA. Typically, sperm heads are depleted of their membranes by detergent extraction prior to mixing with tg DNA and co-injection. This has several advantages over lentiviral methods of tg delivery: introduction and propagation in viral vectors is not required and the method lends itself to tgs in the megabase range, which is too large for lentiviral delivery. Perry and members of his lab are adapting the method to facilitate high throughput targeted knock-out and -down pheno- typing. Developing these approaches is an important supplementary part of their work to facilitate the molecular dissection of sperm protein function in the context of embryos and whole animals.

<table>
<thead>
<tr>
<th>Protein id</th>
<th>Role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT32</td>
<td>WBP2, a c-Yes BP</td>
<td>Gong et al., 1997</td>
</tr>
<tr>
<td>PERF15</td>
<td>Lipophilic transport protein-related</td>
<td>Oko and Morales, 1994</td>
</tr>
<tr>
<td>SubH2Bv</td>
<td>Histone H2B variant Aul and Oko, 2002</td>
<td></td>
</tr>
<tr>
<td>Arp-T2</td>
<td>Actin-related, role unknown</td>
<td>Heid et al., 2002</td>
</tr>
<tr>
<td>Arp-T1</td>
<td>Actin-related, role unknown</td>
<td>Heid et al., 2002</td>
</tr>
<tr>
<td>s-Yes</td>
<td>Protein tyrosine kinase-related</td>
<td>Lenders and Grupol, 2002</td>
</tr>
<tr>
<td>PT22</td>
<td>Actin-related</td>
<td>Gong et al., 1997</td>
</tr>
<tr>
<td>pII</td>
<td>Calmodulin BP, role unknown</td>
<td>Lenders and Grupol, 2002</td>
</tr>
</tbody>
</table>


Molecular identities of PNM proteins

Interactions between gamete cytoplasm

Microinjection of an isolated sperm head into an oocyte can result in the birth of normal young. Thus, the sperm head — the part of the head that lies beneath its membranes — apparently contains all of the paternal information sufficient for full development. Tony Perry is systematically elucidating interactions between sub-membrane sperm head components and the oocyte at fertilization, particularly during the moments soon after the sperm has entered the oocyte. The Perry lab’s interests center on attributing molecular identities to the proteins involved in these interactions and characterizing them functionally.
Hiroshi Sasaki's research team investigates the network of signaling centers that work to induce normal development in mammals. The node, a central responsibility for induction of body parts in the mouse, is a particular focus of Sasaki's research. He has identified a number of transcription factors involved in the formation and function of the node in previous studies, and he remains intent on solving questions of how these centers are formed and how they set courses for the developing embryo to follow.

Activation of nodal differentiation
Foxa2 (also known as Hepatocyte Nuclear Factor 3[β], HNF3β) is a highly-conserved transcription factor required for the formation of embryonic signaling centers in the mouse. The Sasaki lab is engaged in an analysis of molecules that activate Foxa2 expression in both node and notochord. Members of the Sasaki team previously identified the key regulatory element of the Foxa2 enhancer, CS3, and have now determined that this element binds to a member of the TEAD/TEF family of proteins. Four transcription factors found in both mice and humans, which share a common DNA-binding domain, TEAD proteins are widely expressed in the mouse embryo and their activity is regulated by interaction with multiple transcription factors and co-activators. Complementary studies of TEAD activation showed that increases or decreases in TEAD activity resulted in corresponding increases or decreases in the area of Foxa2 expression. The Foxa2 enhancer element CS3 (which binds with TEAD) functions downstream of Wnt, a known node-inducing signal and the synergistic activation of Foxa2 by TEAD and Wnt has also been demonstrated in zebrafish, indicating that this pathway has been conserved at least in some other vertebrate taxa as well. A second signaling factor known to work in node induction, Nodal, has also been linked to TEAD activity. These recent findings suggest a new model to explain node induction in which TEAD complements the function of multiple activating co-factors from primary signaling centers by activating the enhancer element CS3 to induce Foxa2 expression.

headshrinker
Although a variety of mutations can cause the head to fail to develop properly, it is rare for embryos in which the head is entirely lacking to develop to full term. However, a limited number of such headless birth phenotypes have been reported, all of which have been linked to mutations in node-related genes, such as Lim1 and Otx2. The fortuitous discovery of a previously unknown headless phenotype in the mouse prompted Sasaki to investigate the genetic cause of this mutation, which he named headshrinker (hsk), and to study the gene's role in the induction of the head. Gene expression analysis revealed losses in the expression of important head-organizing genes in hsk mutants, resulting in the impaired function of the early-stage head organizer or the prechordal plate, a forebrain-inducing mesenchymal mass that forms at the anterior end of the developing embryo.

Northern blotting showed reduced levels in the expression of the mRNA for the gene Ssdp1 (a sequence-specific single-stranded-DNA-binding protein) in headshrinker mutants, and the researchers found that they could rescue the headless phenotype by using Ssdp1 transgenic mice engineered to express Ssdp1 alone showed almost no activity, but boosted the activity of both Lim1 and Otx2 on co-expression, and had the strongest effect when all three factors were present. In addition to the lethal loss of the head, hsk mutants exhibit a range of other developmental abnormalities, implicating Ssdp1 as a co-activator molecule with multiple and diverse embryonic roles. Sasaki continues to pursue a deeper understanding of the structure and function of signaling centers using an array of approaches and techniques. Other ongoing work in the lab is aimed at isolating cells from transgenic mice engineered to express LacZ in the node and notochord, which promises to speed the identification of new genes specifically expressed in those regions.
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 assoc.
iate in Atsushi Kuroiwa’s lab at Osaka University from 1986 to 1988, and in Bridig Hogan’s lab at Vanderbilt University. From 1988 to 1990, Hiroshi Sasaki continued to pursue a deeper understanding of the genetic cause of this mutation, which he named headshrinker (hsk), and to study the gene’s role in the induction of the head. Gene expression analysis revealed losses in the expression of important head-
organizing genes in hsk mutants, resulting in the impaired function of the late-stage head organizer or the prechordal plate, a fore/midbrain-inducing mesenchymal mass that forms at the anterior end of the developing embryo.

Northern blotting showed reduced levels in the expression of the mRNA for the gene Ssdp1 (a sequence-specific, single-stranded DNA-binding pro-
tein) in headshrinker mutants, and the researchers found that they could rescue the headless phenotype by using Ssdp1 cDNA. Ssdp1 hsk encodes a protein that binds with Ldb1, which itself binds LIM-
homeodomain proteins and other developmentally important transcription factors. Sasaki proposes a model for the interaction of these three co-factors in which Ssdp1 enhances the activation of target genes by the L1m-Ldb1 complex. In an analysis using a labeled reporter gene, Ssdp1 alone showed almost no activity, but boosted the activity of both L1m and Ldb1 on co-expression, and had the stron-
gest effect when all three factors were present. In addition to the lethal loss of the head, hsk mutants exhibit a range of other developmental abnormalities, implicative Ssdp1 as a co-activator molecule with multiple and diverse embryogenic roles.

Hiroshi Sasaki’s research team investigates the net-
work of signaling centers that work to induce normal development in mammals. The node, a center responsible for induction of body parts in the mouse, is a particular focus of Sasaki’s research. He has identified a number of transcription factors involved in the formation and the function of the node in previ-
ous studies, and he remains intent on solving ques-
tions of how these centers are formed, and how they set courses for the developing embryo to follow.
Promoting Program  
Creative Research Promoting Program

Asymmetric cell division and cell diversity

Asymmetric cell division is a process by which a single mother cell splits to generate daughter cells with discrete characters, which is the fundamental process by which cellular diversity is achieved, but remains imperfectly understood at the molecular level. Using the nematode C. elegans as a model system, Hitoshi Sawa is engaged in the study of the genetic and molecular interactions that underlie asymmetric division, and the related phenomenon of cellular polarity, in which the contents of individual cells are distributed unequally, giving distinct characters to daughter cells on division. He seeks also to resolve the ways in which transcription-regulating complexes integrate the intracellular signaling networks that collaborate to help determine cell fate.

Factors in asymmetric T cell division

Additional work has also focused on T cell hypodermal cells, which in normal development divide asymmetrically to produce non-identical daughter cells of neural or hypodermal fates. Recent work by the Sawa team has shown that three genes, psa-3, ceh-20 and nob-1 (homologs of Meis, Pbx and Hox, respectively) are involved in this asymmetric T mitosis, as mutations in any of these genes result in the abnormal loss of asymmetry in cell divisions, in which the posterior daughters of the T cell are hypodermal rather than neural in character.

Investigations into the role of PSA-3 uncovered that expression of PSA-3 is stronger in the posterior T cell daughter, a pattern dependent on a consensus binding site within its promoter region for the transcriptional factor POP-1/TCF, which has previously been reported to be required for asymmetric T cell division, suggesting that the PSA-3 gene is a direct target of Wnt signaling and functions as a cell fate determinant in asymmetric cell division. The asymmetry of PSA-3/Meis expression is also perturbed in lin-44(ju173) and lin-17(ju17) mutants, corroborating the argument for a downstream role for psa-3 in the Wnt pathway.

Expression of psa-3/Meis in the T cell lineage is also markedly reduced in nob-1/hox and ceh-20/pbx mutants, indicating that, NOB-1/Hox and CEN-20/Pbx also act in the regulation of psa-3/Meis expression. Taken together, these data indicate that the expression of psa-3/Meis is controlled by binary mechanisms; Wnt signaling establishes cellular polarity, while the Hox-Pbx complex regulates positional identity. In other metazoans, both Meis and Pbx are known to bind directly as co-factors to Hox, raising the possibility that 20/Pbx and NOB-1/Hox may form a ternary complex with PSA-3/Meis. This complex is predicted to antagonize anterior or posterior polarity signals by C. elegans. Cell 87:717-26 (1999).

Expression of PSA-3/Meis in the T cell lineage is also markedly reduced in nob-1/hox and ceh-20/pbx mutants, indicating that, NOB-1/Hox and CEN-20/Pbx also act in the regulation of psa-3/Meis expression. Taken together, these data indicate that the expression of psa-3/Meis is controlled by binary mechanisms; Wnt signaling establishes cellular polarity, while the Hox-Pbx complex regulates positional identity. In other metazoans, both Meis and Pbx are known to bind directly as co-factors to Hox, raising the possibility that 20/Pbx and NOB-1/Hox may form a ternary complex with PSA-3/Meis. This complex is predicted to antagonize anterior or posterior polarity signals by C. elegans. Cell 87:717-26 (1999).

Asymmetric cell division and tissue polarity

A pathway homologous to the Wnt cascade exists in C. elegans, and it has been suggested that a number of downstream factors corresponding to many of the most prominent Wnt signaling components also play roles in roundworm asymmetric cell division. POP-1, the C. elegans homolog of TCF/LEF-1, is a transcription factor acting downstream of Wnt, has recently been shown to be required for asymmetric T cell division. In the canonical Wnt pathway, TCF/LEF-1 is a target of β-catenin, but the direct involvement of β-catenin in C. elegans asymmetric cell division has never clearly been demonstrated. Researchers in the Sawa lab found that β-catenin mutants show defects in asymmetric T cell division, similar to those observed in lin-17 mutants, suggesting that the asymmetric cell division is controlled by β-catenin.
Asymmetric cell division and cell diversity

The nematode C. elegans as a model system, Hitoshi Sawa is engaged in the study of the genetic and molecular interactions that underlie asymmetric division, and the related phenomenon of cellular polarity. In the contents of individual cells are distributed unequally, giving distinct characteristics to daughter cells on division. He seeks also to resolve the ways in which transcriptional cues integrate the differentiation and cell fate decision.

T cell polarity

The Wnt pathway represents one of the most highly conserved and widely adapted signaling systems in biology, playing developmentally critical roles in the regulation of cell fate, proliferation and polarity in species from worm to human. Wnt signaling, in fact, transpires along multiple divergent routes. In the canonical Wnt pathway in Drosophila (the species in which it was first described), Wnt binds to the transmembrane receptor Frizzled, which activates a series of molecules and molecular complexes within the cell, thereby preventing the degradation of β-catenin and allowing it to accumulate and signal transcription factors that act on the pathway’s ultimate downstream gene targets. The effects of lin-44 (a Wnt homolog) on asymmetric division in the nematode, the Sawa team ectopically expressed and examined its effects on the division of T cells, which form a diverse lineage of hypodermal and neuronal cell diversity. In normal development, the anterior daughter of the parent T cell produces neuronal cells. In lin-44 mutants, however, the opposite is often the case, with anterior daughters frequently giving rise to neuronal cells, and posterior daughters generating cells in the hypodermal lineage. This phenotype is attributable to a reversal in parent cell polarity, which occurs in about 70% of lin-44 mutants. Misexpression of lin-44 at the anterior of the T cell in lin-44 mutants caused a significant enhancement of the reversal phenotype: 97% of such mutants showed switching of fates in T cell daughters. These results demonstrate that the orientation of T cell polarity is regulated by the LIN-44 signal.

In vitro studies show that expression of PSA-3/Mais in the T cell lineage is also markedly reduced in nob-1/hox and ceh-20/pbx mutants, indicating that, NOB-1/Hox and CEH-20/Pbx also act in the regulation of PSA-3/Mais expression. Taken together, these data indicate that the expression of PSA-3/Mais is controlled by binary mechanisms; Wnt signaling establishes cellular polarity, while the Hox-Pbx complex regulates positional identity. In other metazoans, both Meis and Pbx are known to bind directly as co-factors to Hox, raising the possibility that 20/Pbx and NOB-1/Hox to determine the asymmetric cell fates.

Factors in asymmetric T cell division

Additional work has also focused on T cell division. Cells in normal development divide asymmetrically to produce non-identical daughter cells of neural or hypodermal fate. Recent work by the Sawa team has shown that three genes, psa-3, ceh-20 and nob-1 (homologs of Meis, Pbx and Hox, respectively) are involved in this asymmetric T mitosis, as mutations in any of these genes result in the abnormal loss of asymmetry in cell divisions, in which the posterior daughters of the T cell are hypodermal rather than neural in character.

Investigations into the role of PSA-3 uncovered that expression of PSA-3 is stronger in the posterior T cell daughter, and PSA-3 expression is dependent on a consensus binding site. Within its promoter region for the transcriptional factor POP-1/TCF, which has previously been reported to be required for asymmetric T cell division, suggesting that PSA-3 gene is a direct target of Wnt signaling and functions as a cell fate determinant in asymmetric cell division. The asymmetry of PSA-3/Mais expression is also perturbed in lin-44/Wnt and lin-17/Frizzled mutants, corroborating the argument for a downstream role for psa-3 in the Wnt pathway.

Expression of psa-3/Mais in the T cell lineage is also markedly reduced in nob-1/Hox and ceh-20/Pbx mutants, indicating that, NOB-1/Hox and CEH-20/Pbx also act in the regulation of psa-3/Mais expression. Taken together, these data indicate that the expression of psa-3/Mais is controlled by binary mechanisms; Wnt signaling establishes cellular polarity, while the Hox-Pbx complex regulates positional identity. In other metazoans, both Meis and Pbx are known to bind directly as co-factors to Hox, raising the possibility that 20/Pbx and NOB-1/Hox to determine the asymmetric cell fates.

Expression of PSA-3/Mais is directly induced by POP-1/TCF. PSA-3/Mais forms a ternary complex with CEH-20/Pbx and NOB-1/Hox, lending support to the model in which PSA-3/Mais functions as a cell fate determinant by forming a complex with CEH-20/Pbx and NOB-1/Hox in asymmetric cell division of the T cell.
Interactions between genes at the network level are of fundamental importance in instructing the development and function of multicellular organisms. While the characterization of genomes at the level of the solitary gene remains an important challenge in many yet unsequenced organisms, the analysis of how genes function in networks is now in increasing demand for those genomes that are already available. In the post-genome era, scientists seek to understand the roles of genes in evolution through comparative genomics studies as well as to characterize genes in isolation and understand their functions in the context of interactive networks, a field of investigation known as functional genomics.

Asako Sugimoto has adopted the nematode Caenorhabditis elegans as an experimental model to take advantage of its tractability to systematic functional analysis of its genome using unique high-throughput screening techniques. By studying the interactions between the approximately 19,000 genes predicted for C. elegans, the Sugimoto research team seeks to identify the means by which sets of 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. 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.

**RNAi-based profiling of gene function**

The wild-type nematode is built from precisely 959 somatic cells, yet this simple organism exhibits a wide range of the specialized cell types, such as muscles and neurons, that characterize more highly derived species. And, thanks to the complete knowledge of the lineage of every cell in the C. elegans body, the differentiation pathway of every one of those cells can be followed from its origin in the fertilized egg to its role in the fully-grown adult. The amenability of this worm’s genome to reverse-genetic techniques has also served to make it one of the standard models 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 enabled systematic high-throughput studies of gene suppression to be conducted more rapidly than ever before possible.

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 has started to construct a database in which the resulting phenotypes are sorted by developmental outcome. To date, nearly 6,000 phenotypes have been cataloged, and it was found that loss of function in more than 25% of these genes resulted in lethal, morphologically aberrant sterile phenotypes. Many lethal phenotypes result in developmental arrest and death at very early stages, which typically prevents the study of the underlying gene function in later development. By exposing worms to RNAi-inducing dsRNA at the L1 stage of larval development, Sugimoto is able to study the post-embryonic function of genes that are lethal when knocked down in embryos. This is critically important to the understanding of genetic networks in post-embryonic development, as more than one out of every ten genes analyzed by the lab so far is essential for embryogenesis. Sugimoto’s studies to date show that approximately 50% of all such genes play post-embryonic roles as well, supporting the concept that a great number of genes play multiple roles at different stages in development.

**Phenotype analysis**

The data sets produced by high-throughput phenotype analyses can be formidably large and unwieldy, and the lack of standardized descriptive terms and categories can limit the accessibility to the wealth of information they contain. To improve the ease-of-use and distributability of her RNAi phenotype results, Sugimoto has developed a taxonomic system and nomenclature to enable the precise and consistent description of embryonic and post-embryonic phenotypes using a checklist of more than 50 identifying traits, such as abnormalities in cell division, cellular differentiation, organogenesis, morphogenesis, growth, and movement. She plans to make these RNAi phenotype profiles available to the C. elegans research community, in the hopes of establishing a universal taxonomy for phenotype analysis. Phenotypes described using this system can be subjected to a hierarchical clustering analysis, which makes it possible to categorize genes based on relatedness between phenotypes. The genes clustered by this method are likely to be involved in the same developmental process, thus this analysis will provide pivotal information to uncover genetic networks involved in the regulation of development. Members of the Sugimoto team are now utilizing these RNAi analysis data to investigate developmental processes such as microtubule dynamics in mitosis, morphogenesis, and programmed cell death.
Promoting Program Creative Research Promoting Program

Asako Sugimoto

Asako Sugimoto received her B. Sc. degree from the Department of Biophysics and Biostatistic at the University of Tokyo (1987), and her Ph. D. from the same institution in 1992. She worked as a postdoctoral fellow in Joel Rothman’s laboratory at 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 2000, pursuing concurrent work as a Junior Science and Technology Corporation RES- TO researcher from 1997 to 2000. She was appointed Team leader at the RIKEN CDB in 2001.

team leader at the RIKEN TECHNOLOGY CORPORATION PRES-
japan Science and Technology Corporation.
artificial role of the C. elegans Arp2/3 complex in cell migra-
tion, organogenesis, morphogenesis, and programmed cell death.

Phenotype analysis

The data sets produced by high-throughput phenotype analyses can be formidable large and unwieldy, and the lack of standardized descriptive terms and categories can limit the accessibility to the wealth of information they contain. To improve the value, ease-of-use and distributability of her RNAi phenotyping results, Sugimoto has developed a taxonomic system and nomenclature to enable the precise and consistent description of embryonic and post-embryonic phenotypes using a checklist of more than 50 identifying traits, such as abnormalities in cell division, cellular differentiation, organogenesis, morphogenesis, growth, and movement. She plans to make these RNAi phenotype profiles available to the C. elegans research community, in the hopes of establishing a universal taxonomy for phenotype analysis. Phenotypes described using this system can be subjected to hierarchical clustering analysis, which makes it possible to categorize genes based on relatedness between phenotypes. The genes clustered by this method are likely to be involved in the same developmental process, thus this analysis will provide pivotal information to uncover genetic networks involved in the regulation of development. Members of the Sugimoto team are now utilizing these RNAi analysis data to investigate developmental processes such as morphotube dynamics in mitosis, morphogenesis, and programmed cell death.

Sugimoto has developed a taxonomic system and nomenclature to enable the precise and consistent description of embryonic and post-embryonic phenotypes.

Interactions between genes at the network level are of fundamental importance in instructing the development and function of multicellular organisms. While the characterization of genes at the level of the solitary gene remains an important challenge in many yet unsequenced organisms, the analysis of how genes function in networks is now in increasing demand for those genomes that are already available. In the post-genome era, scientists seek to understand the roles of genes in evolution through comparative genomics studies as well as to characterize genes in isolation and understand their functions in the context of interactive networks, a field of investigation known as functional genomics.

functional genomics

Asako Sugimoto has adopted the nematode Caenorhabditis elegans as an experimental model to take advantage of its tractability to systematic functional analysis of its genome using unique high-throughput screening techniques. By studying the interactions between the approximately 19,000 genes predicted for C. elegans, the Sugimoto research team seeks to identify the means by which sets of 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. 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.

RNAi-based profiling of gene function

The wild-type nematode is built from precisely 959 somatic cells, yet this simple organism exhibits a wide range of the specialized cell types, such as muscles and neurons, that characterize more highly derived species. And, thanks to the complete knowledge of the lineage of every cell in the C. elegans body, the differen
tial pathway of every one of those cells can be followed 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 standard 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 enabled systematic high-throughput studies of gene suppression to be conducted more rapidly than ever before possible.

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 has started to construct a database in which the resulting phenotypes are sorted by developmental outcome. To date, nearly 6,000 phenotypes have been categorized, and it was found that loss of function in more than 25% of these genes resulted in lethal, morphologically altered and sterile phenotypes. Many lethal phenotypes result in developmental arrest and death at very early stages, which typically prevents the study of the underlying genetic function in later development. By exposing worms to RNAi-inducing dsRNA at the L1 stage of larval development, Sugimoto is able to study the post-embryonic function of genes that are lethal when knocked down in embryos. This is critically important to the understanding of genetic networks in post-embryonic development, as more than one out of every ten genes analyzed by the lab so far is essential for embryogenesis. Sugimoto’s studies to date show that approximately 50% of all such genes play post-embryonic roles as well, supporting the concept that a great number of genes play multiple roles at different stages in development.

Asako Sugimoto

Asako Sugimoto has also served to make it one of the standard 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 enabled systematic high-throughput studies of gene suppression to be conducted more rapidly than ever before possible.

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 has started to construct a database in which the resulting phenotypes are sorted by developmental outcome. To date, nearly 6,000 phenotypes have been categorized, and it was found that loss of function in more than 25% of these genes resulted in lethal, morphologically altered and sterile phenotypes. Many lethal phenotypes result in developmental arrest and death at very early stages, which typically prevents the study of the underlying genetic function in later development. By exposing worms to RNAi-inducing dsRNA at the L1 stage of larval development, Sugimoto is able to study the post-embryonic function of genes that are lethal when knocked down in embryos. This is critically important to the understanding of genetic networks in post-embryonic development, as more than one out of every ten genes analyzed by the lab so far is essential for embryogenesis. Sugimoto’s studies to date show that approximately 50% of all such genes play post-embryonic roles as well, supporting the concept that a great number of genes play multiple roles at different stages in development.

Asako Sugimoto

Asako Sugimoto has also served to make it one of the standard 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 enabled systematic high-throughput studies of gene suppression to be conducted more rapidly than ever before possible.

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 has started to construct a database in which the resulting phenotypes are sorted by developmental outcome. To date, nearly 6,000 phenotypes have been categorized, and it was found that loss of function in more than 25% of these genes resulted in lethal, morphologically altered and sterile phenotypes. Many lethal phenotypes result in developmental arrest and death at very early stages, which typically prevents the study of the underlying genetic function in later development. By exposing worms to RNAi-inducing dsRNA at the L1 stage of larval development, Sugimoto is able to study the post-embryonic function of genes that are lethal when knocked down in embryos. This is critically important to the understanding of genetic networks in post-embryonic development, as more than one out of every ten genes analyzed by the lab so far is essential for embryogenesis. Sugimoto’s studies to date show that approximately 50% of all such genes play post-embryonic roles as well, supporting the concept that a great number of genes play multiple roles at different stages in development.
Epithelial-mesenchymal transitions in somites

Somites comprise both epithelial and mesenchymal cells, two cell types that vividly demonstrate the range of possibilities in cellular function and morphology. Epithelial cells, which in adults form the skin and the linings of many organs, adhere to each other tightly and have polarized structures with distinct basal and apical sides. By comparison, mesenchymal cells are less rigidly structured and form looser aggregations. However, these two cell types are by no means isolated or independent from one another; indeed, a great many developmental and physiological phenomena rely on interactions between epithelium and mesenchyme. An extreme example of their inter-relatedness is the ability of cells of one type to convert into the other, in a process known as epithelial-mesenchymal transition. Such transitions (which can proceed in either an epithelial-to-mesenchymal or the reverse direction) are not only essential to normal embryonic development and organogenesis, but also play roles in wound healing and the pathogenesis of cancer.

After the boundaries of a somite have been established, the mesenchymal cells in the border region are involved in epithelial-mesenchymal transitions in epiblast cells. These findings suggest that the mesenchymal-to-epithelial transition that occurs during somitogenesis 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. This research is ongoing and new findings are expected to further our understanding of the mechanisms involved in these transitions.

Yoshiko Takahashi’s research involves molecular and cellular analyses of segmentation and somitogenesis in the chick embryo, which is invaluable for the insights they provide into how populations of cells are able to cleave at specific boundary lines and reorganize into highly ordered and repetitive patterns. In the early chick embryo, two strips of unsegmented paraxial 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, directed by the inductive activity of a putative regulatory region Takahashi has termed the ‘segmeter.’ Recent work in her lab is aimed at elucidating transitions in cell structure and type that take place in nascent somites once their initial boundaries have been established.

Pattern formation, the process by which the border zones that define germ layers and tissue fields are established, is strictly regulated to occur at specific sites and stages in the developing embryo. This close coordination prevents cells from differentiating inappropriately, and enables the establishment of complex and specialized tissues and organs in the embryo and the adult body. One type of pattern formation involves the regimented establishment of periodically alternating bands, or segments, of differentiated cells along the anterior-posterior body axis. The results of this process of segmentation can be observed in repeated orderly structures such as vertebrae, ribs and spinal ganglia. In vertebrates, one form of segmentation takes place in the embryonic region known as the ‘somitic mesoderm,’ and involves the formation of transient segment-organizing bodies called somites.
Epithelial-mesenchymal transitions in somites

Somites comprise both epithelial and mesenchymal cells, two contrasting cell types that vividly demonstrate the range of possibilities in cellular function and morphology. Epithelial cells, which in adults form the skin and the linings of many organs, adhere to each other tightly and have polarized structures with distinct basal and apical sides. In contrast, mesenchymal cells are less rigidly structured and form looser aggregations. However, these two cell types are by no means isolated or independent from one another; indeed, a great many developmental and physiological phenomena rely on interactions between epithelium and mesenchyme. An extreme example of their inter-relatedness is the ability of cells of one type to convert into the other, in a process known as epithelial-mesenchymal transition. Such transitions (which can proceed in either an epithelial-to-mesenchymal or the reverse direction) are not only essential for normal embryonic development and organogenesis, they also play roles in wound healing and the pathogenesis of cancer.

After the boundaries of a somite have been established, the mesenchymal cells in the border region undergo a transition, acquiring the polarized structure and properties of epithelial cells. This results in a somite body in which a core of mesenchymal cells is circumscribed by an outer layer of cells of epithelial character. The Takahashi lab seeks to clarify the molecular mechanisms that regulate this epithelial-mesenchymal transition, and is concentrating on the roles played by members of the Rho family of GTPase proteins in this process. These proteins, notably Rho, Rac1 and Cdc42, are known to play roles in cytoskeletal rearrangement and cell locomotion in vitro, cycling between GDP-bound inactive and GTP-bound active states, and triggering downstream effectors linked to the cytoskeleton when activated.

Now, studies in the Takahashi lab are beginning to reveal additional functions for Rho family members in the context of somite development. 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 roles seem to be at least partially specific to somites; neither Cdc42 nor Rac1 was found to be involved in epithelial-mesenchymal transitions in epiblast cells. These findings suggest that the mesenchymal-epithelial transition that occurs during somitogenesis 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. She now seeks to further characterize the induction of the activity of these Rho family members in the earliest stages of the establishment of the somite by segmenter activity.

Takahashi emphasizes that what drew her to science and maintains her interest are the opportunities and challenges it offers to those willing and able to learn from natural phenomena. Her interests are diverse and far-ranging; she chooses to pursue avenues that others may have overlooked, and encourages her students and colleagues to do the same.
The experimental cloning of animals has a history that extends back more than fifty years, to when Briggs and King successfully produced tadpoles by transplanting the nuclei from embryonic cells into frogs’ eggs whose own nuclei had been removed. At the time, cloning was used not as a technique to be studied for its immediate application, but as a means of testing a fundamental question of reproductive biology: whether the processes of fertilization and later development, in which the body’s cells become more specialized and functionally distinct, involve a loss of genetic information, or whether all cells retain the full set of genetic code, even after differentiation has proceeded. The success of these initial experiments in cloning by nuclear transfer demonstrated conclusively that cells do maintain intact genomes even after differentiation, as the genetic code in a specialized cell’s nucleus is sufficient to instruct an egg into which it is transplanted to give rise to a normal individual.

Although questions of whether genetic information is lost or irreversibly altered during development were laid to rest, new questions arose to take their place. For Teruhiko Wakayama, the most intriguing issue raised by the ability of animals to be cloned using the nuclei of specialized somatic cells (which form the body and, unlike sperm and eggs, cannot normally produce a new individual), is that of reprogramming, the processes by which the genome receives new sets of coding instructions enabling it to order the development of all the cells in a new individual while remaining intact and fundamentally unchanged in each of those cells. The Wakayama lab studies mammalian cloning and fertilization with the same basic goal as drove Briggs and King in their nuclear transfer studies a half century ago: to answer central questions in the biology of animal reproduction.

Cloning efficiencies

In all species and in all experimental methods tested to date, cloning by nuclear transfer has consistently low efficiency rates — generally below 5% of enucleated eggs fertilized by nuclear transfer go on to develop into live-born offspring. Many hypotheses have been proposed to explain the inefficiency of this procedure, while accounting for the fact that cloning is not altogether impossible. It has been suggested that the process of removing the nucleus from an oocyte (an unfertilized egg) or the absence of chromosomal information during the several hours in which the egg is missing a nucleus may somehow damage or cause the loss of factors that would normally act to reprogram the genetic information in the fertilizing (or transplanted) cell’s nucleus. To test this possibility, Wakayama re-ordered conventional cloning methodol- ogy by first transferring nuclei from cumulus cells into oocytes whose own nuclei were still present, and only then removing the mitotic spindle derived from the native nucleus. These experiments resulted in the generation of live offspring at a rate of efficiency similar to that of standard cloning by nuclear transfer, providing evidence that tends to counter the hypothesis that the temporary absence of a nucleus is responsible for the poor development of NT oocytes.

Scientists in the Wakayama lab have adopted similar approaches in the study of fertilization — experiments that involve the substitution of components or the reordering of natural sequences of events to test for specific biological function. In nature, fertilization occurs after a mature spermatozoon fuses with and activates an oocyte, but some laboratory techniques achieve fertilization by artificially activating the egg and then injecting a sperm, which is an incompletely matured sperm cell. Such techniques are known to be less efficient than fertilization using mature spermatozoa, but the reason for this lower developmental competency remains unknown. The Laboratory for Genomic Reprogramming is now comparing in vitro fertilization using spermatozoa and spermatids under controlled conditions in an effort to identify the differences between these stages in the developing sperm cell. Future research will look at changes in epigenetic modifications to the sperm genome over time as a possible explanation for their disparate potentials.

New technologies

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. Oocytes and fertilized eggs are also extremely labile, and must likewise be stored cryogenically. But these requirements tend to limit the access of germ cells to researchers unequipped with liquid nitrogen facilities, preventing the spread of the technology and the development of research using these cells. Wakayama hopes to develop new, less expensive and less technically demanding methods for the storage and maintenance of germ cells for experimental use. Recent tests using a modified commercial available culture medium showed that under the right conditions spermatids can be preserved for long periods, up to 70 days, at 4 degrees Cel- sius, a temperature that can be maintained using ordinary and inexpensive refrigeration equipment. This new preservation method opens up opportunities to the right conditions spermatids can be preserved for long periods, up to 70 days, at 4 degrees Cel- sius, a temperature that can be maintained using ordinary and inexpensive refrigeration equipment. This new preservation method opens up opportunities to

Oocytes (large, upper left) and a somatic (renal, bottom center) spindle follow- ing nuclear transfer.
The experimental cloning of animals has a history that extends back more than fifty years, to when Briggs and King successfully produced tadpoles by transplanting the nuclei from embryonic cells into frogs’ eggs whose own nuclei had been removed. At the time, cloning was used not as a technique to be studied for its immediate application, but as a means of testing a fundamental question of reproductive biology: whether the processes of fertilization and later development, in which the body’s cells become more specialized and functionally distinct, involve a loss of genetic information, or whether all cells retain the full set of genetic code, even after differentiation has proceeded. The success of these initial experiments in cloning by nuclear transfer demonstrated conclusively that cells do maintain intact genomes even after differentiation, as the genetic code in a specialized cell’s nucleus is sufficient to instruct an egg into which it is transplanted to give rise to a normal individual.

Although questions of whether genetic information is lost or irreversibly altered during development were laid to rest, new questions arose to take their place. For Teruhiko Wakayama, the most intriguing issue raised by the ability of animals to be cloned using the nuclei of specialized somatic cells (which form the body and, unlike sperm and eggs, cannot normally produce a new individual), is that of reprogramming, the processes by which the genome receives new sets of coding instructions enabling it to order the development of all the cells in a new individual while retaining intact and fundamentally unchanged in each of those cells. The Wakayama lab studies mammalian cloning and fertilization with the same basic goal as Briggs and King in their nuclear transfer studies a half century ago: to answer central questions in the biology of animal reproduction.

Cloning efficiencies

In all species and in all experimental methods tested to date, cloning by nuclear transfer has consistently low efficiency rates — generally below 5% of enucleated eggs fertilized by nuclear transfer go on to develop into live-born offspring. Many hypotheses have been proposed to explain the inefficiency of this procedure, while accounting for the fact that cloning is not altogether impossible. It has been suggested that the process of removing the nucleus from an oocyte (an unfertilized egg) or the absence of chromosomal information during the several hours in which the egg is missing a nucleus may somehow damage or cause the loss of factors that would normally act to reprogram the genetic information in the fertilizing (or transplanted) cell’s nucleus. To test this possibility, Wakayama re-ordered conventional cloning methodology by first transferring nuclei from cumulus cells into oocytes whose own nuclei were still present, and only then removing the mitotic spindle derived from the native nucleus. These experiments resulted in the generation of live offspring at a rate of efficiency similar to that of standard cloning by nuclear transfer, providing evidence that tends to counter the hypothesis that the temporary absence of a nucleus is responsible for the poor development.

Many hypotheses have been proposed to explain cloning’s inefficiency, while accounting for the fact that it is not altogether impossible

Scientists in the Wakayama lab have adopted similar approaches in the study of fertilization — experiments that involve the substitution of components or the re-ordering of natural sequences of events to test for specific biological function. In nature, fertilization occurs after a mature spermatozoan fuses with and activates an oocyte, but some laboratory techniques achieve fertilization by artificially activating the egg and then injecting a spermatozoon, which is an incompletely maturated sperm cell. Such techniques are known to be less efficient than fertilization using mature spermatozoa, but the reason for this lower developmental competency remains unknown. The Laboratory for Genomic Reprogramming is now comparing in vivo fertilization using spermatozoa and spermatids under controlled conditions in an effort to identify the differences between those stages in the developing sperm cell. Future research will look at changes in epigenetic modifications to the sperm genome over time as a possible explanation for their disparate potentials.

New technologies

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. Oocytes and fertilized eggs are also extremely labile, and must likewise be stored cryogenically. But these requirements limit the access of germ cells to researchers unequipped with liquid nitrogen facilities, preventing the spread of the technology and the development of research using these cells. Wakayama hopes to develop new, less expensive and less technically demanding methods for the storage and maintenance of germ cells for experimental use. Recent tests using a modified commercially available culture medium showed that under the right conditions spermatids can be preserved for long periods, up to 70 days, at 4 degrees Celsius, a temperature that can be maintained using ordinary and inexpensive refrigeration equipment. This new preservation method opens up opportunities to the sperm genome over time as a possible explanation for their disparate potentials.

Researchers in the Wakayama lab have adopted similar approaches in the study of fertilization — experiments that involve the substitution of components or the re-ordering of natural sequences of events to test for specific biological function. In nature, fertilization occurs after a mature spermatozoan fuses with and activates an oocyte, but some laboratory techniques achieve fertilization by artificially activating the egg and then injecting a spermatozoon, which is an incompletely maturated sperm cell. Such techniques are known to be less efficient than fertilization using mature spermatozoa, but the reason for this lower developmental competency remains unknown. The Laboratory for Genomic Reprogramming is now comparing in vivo fertilization using spermatozoa and spermatids under controlled conditions in an effort to identify the differences between those stages in the developing sperm cell. Future research will look at changes in epigenetic modifications to the sperm genome over time as a possible explanation for their disparate potentials.

New technologies

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. Oocytes and fertilized eggs are also extremely labile, and must likewise be stored cryogenically. But these requirements limit the access of germ cells to researchers unequipped with liquid nitrogen facilities, preventing the spread of the technology and the development of research using these cells. Wakayama hopes to develop new, less expensive and less technically demanding methods for the storage and maintenance of germ cells for experimental use. Recent tests using a modified commercially available culture medium showed that under the right conditions spermatids can be preserved for long periods, up to 70 days, at 4 degrees Celsius, a temperature that can be maintained using ordinary and inexpensive refrigeration equipment. This new preservation method opens up opportunities to the sperm genome over time as a possible explanation for their disparate potentials.

Many hypotheses have been proposed to explain cloning’s inefficiency, while accounting for the fact that it is not altogether impossible

Scientists in the Wakayama lab have adopted similar approaches in the study of fertilization — experiments that involve the substitution of components or the re-ordering of natural sequences of events to test for specific biological function. In nature, fertilization occurs after a mature spermatozoan fuses with and activates an oocyte, but some laboratory techniques achieve fertilization by artificially activating the egg and then injecting a spermatozoon, which is an incompletely maturated sperm cell. Such techniques are known to be less efficient than fertilization using mature spermatozoa, but the reason for this lower developmental competency remains unknown. The Laboratory for Genomic Reprogramming is now comparing in vivo fertilization using spermatozoa and spermatids under controlled conditions in an effort to identify the differences between those stages in the developing sperm cell. Future research will look at changes in epigenetic modifications to the sperm genome over time as a possible explanation for their disparate potentials.

New technologies

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. Oocytes and fertilized eggs are also extremely labile, and must likewise be stored cryogenically. But these requirements limit the access of germ cells to researchers unequipped with liquid nitrogen facilities, preventing the spread of the technology and the development of research using these cells. Wakayama hopes to develop new, less expensive and less technically demanding methods for the storage and maintenance of germ cells for experimental use. Recent tests using a modified commercially available culture medium showed that under the right conditions spermatids can be preserved for long periods, up to 70 days, at 4 degrees Celsius, a temperature that can be maintained using ordinary and inexpensive refrigeration equipment. This new preservation method opens up opportunities to the sperm genome over time as a possible explanation for their disparate potentials.
mammalian germ cell biology

A case could be made for the statement that the ultimate role of all of the other types of cells in the body is to ensure that germ cells—spERM and eggs—are able to fulfill their task of conveying genetic information from one generation to the next. But despite this centrality, many aspects of germ cell biology have been, and remain to this day, poorly understood. There has been, however, progress in deepening this understanding, particularly in regard to the mechanisms by which germ cells are specified and segregated from the larger population of somatic cells early in development. This is achieved by different means in different species. In some animals, germ cell fate is predetermined from the very onset of development, in others, including all mammals, all cells in the early embryo have the potential to take on a germ fate, but only a small percentage of them actually do.

Mitinori Saitou studies the ways in which germ cell fate is specified in mammalian embryonic development, using the mouse as a representative model. He joined the CDB from Azin Surani’s lab at the Wellcome Trust/Cancer Research UK Institute, where he worked on the single cell analysis of primordial germ cells, research that ultimately yielded a number of molecules that act to distinguish cells on the pathway to differentiation into germ cells. The first of these molecules, fragile, is the product of a gene found to be strongly and specifically expressed in cells on route to assuming a germ cell fate, the result of inductive signaling from cells lying outside the embryo proper. A second such protein, Stella, acts even more specifically, and is expressed only in a core subset of fragile-expressing primordial germ cells. The co-expression of fragile and Stella serves to distinguish germ cells from the somatic cell community in the developing embryo, and their activity is thought to play a part in the gene network that maintains germ cell characteristics and suppresses those of the somatic lineage. Single cell analysis has also revealed that repression of Hox genes and maintenance of genes linked to pluripotency, such as Oct4, are the two major events that occur during germ cell lineage-restriction.

Although progress has been made, much about the specification of the germine has yet to be explained. The identification of fragile and Stella now offers a means to identify incipient germ cells, but the initial molecular trigger for germ cell determination and key (epi-)genetic properties that determined germ cells acquire are still unknown. The Saitou lab plans to develop comprehensive gene expression analysis at the single cell level to understand the cellular mechanisms that underlie this process, and to identify the external factors that instruct a pluripotent cell to take on a germ cell fate. The lab also hopes to develop technologies to maintain immature germ cells in vitro, and to induce their maturation in vivo, thereby enabling oocyte-mediated gene transfer techniques.

Recent large-scale efforts in genome-sequencing and expression analysis have produced an embarrassment of riches for life science researchers—biological data can now be accessed in quantities that are far greater than were available even a few years ago. This burgeoning set of raw data has not, however, necessarily led to equally explosive advances in the understanding of the relation between its component parts. The need for integration has set the stage for the advent of systems biology, in which discrete biological processes and phenomena are approached as complex, interactive, active systems. Hiroki Ueda sees systems biology as a multi-scale process, beginning with the identification and analysis of individual gene components and their networked interactions, and leading to the ability to control existing systems and design new ones based on an understanding of structure and underlying principles.

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

To address complex and dynamic biological systems such as the circadian clock, it is necessary to make comprehensive and precise measurements of the system’s dynamics and to work out the organization of its underlying gene network. The Ueda lab has conducted a genome-wide screen and statistical analysis of gene expression to work out the clock-controlled genes that are rhythmically expressed in the central (suprachiasmatic nucleus; SCN) and peripheral (liver) circadian clocks. This phase of the study required the development of a genome-wide promot- er database, which the Ueda lab will make available to all researchers at the CDB. Subsequent phases involved determining gene expression start sites across the entire genome, predicting the regulatory sequences involved in time-specific transcription, and studying their actual functions in vivo using a high-throughput real-time monitoring system for luciferase-tagged transgenes. Analysis of the transcriptional regulation of gene expression in the morning, daytime, evening and night periods revealed a gene network comprising sixteen inter-regulating activators and inhibitors of time-linked gene expression.

The initial success of the systems approach to the mammalian circadian clock has been encouraging, and the Ueda lab now seeks to apply similar genome-wide, high-throughput technologies to more involved and elaborated developmental processes. Guiding that research will be Einstein’s (and the system biologist’s) dictum to “Make everything as simple as possible, but not simpler.”

systems biology

Gene expression profiles of BMP4 negative single PCGs and neighboring somatic cells

Publications


Authors


A case could be made for the statement that the ultimate role of all of the other types of cells in the body is to ensure that germ cells — sperm and eggs — are able to fulfill their task of conveying genetic information from one generation to the next. But despite this centrality, many aspects of germ cell biology have been, and remain to this day, poorly understood. There has been, however, progress in deepening this understanding, particularly in regard to the mechanisms by which germ cells are specified and segregated from the larger population of somatic cells early in development. This is achieved by different means in different species. In some animals, germ cell fate is predetermined from the very onset of development, in others, including all mammals, all cells in the early embryo have the potential to take on a germ cell fate, but only a small percentage of them actually do.

Mitinori Saitou studies the ways in which germ cell fate is specified in mammalian embryonic development, using the mouse as a representative model. He joins the CDB from Azim Surani’s lab at the Wellcome Trust/Cancer Research UK Institute, where he worked on the single cell analysis of primordial germ cells, research that ultimately yielded a number of molecules that act to distinguish cells on the pathway to differentiation into germ cells. The first of these molecules, fragilis, is the product of a gene found to be strongly and specifically expressed in cells on route to assuming a germ cell fate, the result of inductive signaling from cells lying outside the embryo proper. A second such protein, stella, acts even more specifically, and is expressed only in a core subset of fragilis-expressing primordial germ cells. The co-expression of fragilis and stella serves to distinguish germ cells from the somatic cell community in the developing embryo, and their activity is thought to play a part in the gene network that maintains germ cell characteristics and suppresses those of the somatic lineage. Single cell analysis has also revealed that repression of Hox genes and maintenance of genes linked to pluripotency, such as Oct4, are the two major events that occur during germ cell lineage-restriction.

Despite progress that has been made, much about the specification of the germine has yet to be explained. The identification of fragilis and stella now offers a means to identify incipient germ cells, but the initial molecular trigger for germ cell determination and key (epi-)genetic properties that determined germ cells acquire are still unknown. The Saitou lab plans to develop comprehensive gene expression analysis at the single cell level to search for the molecular mechanisms that underlie this process, and to identify the external factors that instruct a pluripotent cell to take on a germ cell fate. The lab also hopes to develop tools to maintain immature germ cells in vitro and to induce their maturation in vitro, thereby enabling coculture mediated gene transfer techniques.

Recent large-scale efforts in genome-sequencing and expression analysis have produced an embarrassment of riches for life science researchers - biological data can now be accessed in quantities that are far greater than were available even a few years ago. This burgeoning set of raw data has not, however, necessarily led to equally explosive advances in the understanding of the relationships between its component parts. The need for integration has set the stage for the advent of systems biology, in which discrete biological processes and phenomena are approached as complex, interactive, and active systems. Hiroti Ueda sees systems biology research as a multi-stage process, beginning with the identification and analysis of individual gene components and their networked interactions, and leading to the ability to control existing systems and design new ones based on an understanding of structure and underlying principles.

The Ueda lab has taken the mammalian circadian clock as a relatively simple and self-contained initial model for the study of a biological system. In addition to its advantages as a basic research model, the function of the circadian clock is intimately connected to its advantages as a basic research model, and its dysregulation is linked to the onset and symptomatology of numerous human diseases, and its dysregulation is linked to the onset and symptomatology of numerous human diseases, including sleep disorders. An improved understanding of the mechanisms that control the transition to the very promising to provide biomedical and clinical investigators with a powerful new arsenal to attack these conditions.

To address complex and dynamic biological systems such as the mammalian circadian clock, it is necessary to make comprehensive and precise measurements of the system’s dynamics and to work out the organization of its underlying gene network. The Ueda lab has conducted a genome-wide screen and statistical analysis of gene expression to work out the clock-controlled genes that are rhythmically expressed in the central (suprachiasmatic nucleus; SCN) and peripheral (liver) circadian clocks. This phase of the study required the development of a genome-wide promoter database, which the Ueda lab will make available to all researchers at the CDB. Subsequent phases of this study involved determining gene expression start sites across the entire genome, predicting the regulatory sequences involved in time-specific transcription, and studying their actual functions in vivo using a high-throughput real-time monitoring system for luciferase-lagged transgenes. Analysis of the transcriptional regulation of gene expression in the morning, daytime, evening and night periods revealed a gene network comprising sixteen inter-regulating activators and inhibitors of time-linked gene expression.

The initial success of the systems approach to the mammalian circadian clock has been encouraging, and the Ueda lab now seeks to apply similar genome-wide, high-throughput technologies to more involved and elaborated developmental processes. Guiding that research will be Einstein’s (and the systems biologist’s) dictum to “Make everything as simple as possible, but not simpler.”
The supporting laboratories offer technical services, develop new technology, and conduct independent research projects. Their services are available to all CDB research groups and teams.
Supporting Laboratories

The supporting laboratories offer technical services, develop new technology, and conduct independent research projects. Their services are available to all CDB research groups and teams.
Cellular morphogenesis

Cellular morphology is defined by interactions involving mechanical and osmotic stresses from the cell's external environment, its permeable membrane, its viscous cytosolic content, and an internal framework comprising pre-stressed tubular struts, tensional cables and a lattice-like filamentous cortex. Microtubules are one important and extensively studied component of this cytoskeletal structure, acting both as internal stress-bearing braces and pathways to guide intracellular transport. A second critical form-giving element, actin filaments, are most prevalent in cellular peripheries, where they provide a stable but regulable meshwork enabling the cell to maintain its structural integrity while responding to external and internal stimuli through shape alterations and movements.

Actin filaments maintain cell structural integrity while responding to external and internal stimuli

The behavior of actin is intimately linked to the activity of a group of related small GTPases proteins known as the Rho family, which is characterized by the actin-regulatory functions of its three primary members, Rho, Rac and Cdc42. The spatial and temporal localization of Rho-family proteins is known to be important in the structural organization of cells in culture. Shigenobu Yonemura is working to develop technologies to allow the precise determination of the localization of these proteins in cultured cells and tissues within specific biological contexts.

Cleavage plane determination

The final stages of mitotic cell division involve a process known as cytokinesis, in which the parent cell cleaves in two, partitioning cytoplasmic elements and genetic information into each of the daughter cells. Cytokinesis begins with the appearance of a cleavage furrow at the cell surface, a process which is known to be specified by microtubules in the mitotic apparatus, and which is essential to ensuring that each progeny cell receives one and only one set of chromosomes. However, the question of which complement of mitotic apparatus microtubules — astral or central — spatially determines this cleavage remains unresolved. Yonemura studied Rho accumulation at the putative furrow site, taking this as an index of cleavage plane determination signaling, and analyzed the roles of both microtubule organizations by selectively disrupting each. The study showed that both astral and central microtubules are able to transmit determinant signals to the cell cortex, a finding that helps explain how astral microtubules that extend to the cell cortex can play an important role in cleavage plane determination in larger cells, such as oocytes, in which central microtubules are located more remotely from the cortex, while being dispensable in the same process in smaller cells in which central microtubules are located more proximally to the cell periphery.

Actin fibers and the adherens junction

The main zone of contact between a cell and its neighbors is called the adherens junction, which is defined by the selective binding of transmembrane adhesion molecules that are themselves anchored to molecular complexes located within each cell's interior. In fibroblastic cells in culture, bundles of actin filaments that run perpendicularly to the plasma membrane are connected to the components of the adherens junction, a phenomenon that is also observed in epithelial cell adhesion. In many cells, the mature adherens junction forms a continuous belt, although during the process of its formation it passes through a stage characterized by discrete, spot-like adhesions. The Yonemura lab used NRK cells, a type of non-polarized fibroblast which forms spot-like junction zones, to study the role of actin fibers in adherens junction formation. In this cell line, the adherens junction and its associated actin bundles can be disrupted and reconstituted by removing or adding serum to the culture medium. Using this system, he revealed that the activity of the small GTPase, Rho, is required both for the formation of the adherens junction and of actin bundles. The study showed that disruption of actin bundling affects the integrity of the adherens junction, indicating the importance of Rho-actin svnaling in this form of cell-cell adhesion.

ER protein recognition-site structure

ER proteins, named for three representative members of the family, ezrin, radixin and moesin, act as links between actin filaments and the plasma membrane. Yonemura and colleagues studied the crystal structure of a functional domain of radixin to identify the structural basis for adhesion molecule recognition by one ER protein, radixin. Radixin plays a role in the formation of microvilli, cellular extensions in which actin filaments are closely associated with the plasma membrane. The importance of radixin in sites of bile secretion is suggested by the finding that mice lacking radixin, which is the dominant ER protein in the liver, show severely impaired hepatic microvilli formation, resulting in a phenotype similar to human congenital hyperbilirubinemia, a rare autosomal recessive liver disorder. This work provides the first insights into the structural bases for the recognition between an ER protein and an adhesion molecule, and may further serve as an experimental model for the study of molecular processes involved in bile secretion.

In addition to its full-time research efforts, the Yonemura lab offers services in support of electron microscopy, services available to all research staff at the CDB. By providing fluorescence and electron microscopic visualizations as well as instruction in techniques, the team hopes to help ensure the quality, fidelity and ease-of-interpretation of visualization data generated at the Center.

NRK cells at cytokinesis. Rho (magenta) accumulates at the equatorial cell cortex depending on microtubule organizations (green).

NRK cells contacting each other through adherens junctions (magenta) where actin filament bundles (green) are tightly connected.
Cleavage plane determination

The final stages of mitotic cell division involve a process known as cytokinesis, in which the parent cell cleaves in two, partitioning cytoplasmic elements and genetic information into each of the daughter cells. Cleptosis begins with the appearance of a cleavage furrow at the cell surface, a process which is known to be specified by microtubules in the mitotic apparatus, and which is essential to ensuring that each progeny cell receives one or only one set of chromosomes. However, the question of which complement of mitotic apparatus microtubules — astral or central — spatially determines this cleavage remains unresolved. Yonemura studied Rho accumulation at the putative furrow site, taking this as an index of cleavage plane determination signaling, and analyzed the roles of both microtubule organizations by selectively disrupting each. The study showed that both astral and central microtubules are able to transmit determinant signals to the cell cortex, a finding that helps explain how astral microtubules that extend to the cell cortex can play an important role in cleavage plane determination in larger cells, such as oocytes, in which central microtubules are located more remotely from the cortex, while being dispensable in the same process in smaller cells in which central microtubules are located more proximally to the cell periphery.

Actin fibers and the adherens junction

The main zone of contact between a cell and its neighbors is called the adherens junction, which is defined by the selective binding of transmembrane adhesion molecules that are themselves anchored to molecular complexes located within each cell’s interior. In fibroblastic cells in culture, bundles of actin filaments that run perpendicular to the plasma membrane are connected to the components of the adherens junction, a phenomenon that is also observed in epithelial cell adhesion. In many cells, the mature adherens junction forms a continuous belt, although during the process of its formation it passes through a stage characterized by discrete, spot-like adhesions. The Yonemura lab used NRK cells, a type of non-polarized fibroblast cell line, to study the role of actin filaments in adherens junction formation. In this cell line, the adherens junction and its associated actin bundles can be disrupted and reconstituted by removing or adding serum to the cell culture medium. Using this system, the team hoped to help ensure the quality, fidelity and ease-of-interpretation of visualization data generated at the Center.

ERM protein recognition-site structure

ERM proteins, named for three representative members of the family, ezrin, radixin and moesin, act as links between actin filaments and the plasma membrane. Yonemura and colleagues studied the crystal structure of a functional domain of radixin to identify the structural basis for adhesion molecule recognition by one ERM protein, radixin. Radixin plays a role in the formation of microvilli, cellular extensions in which actin filaments are closely associated with the plasma membrane. The importance of radixin in sites of bile secretion is suggested by the finding that mice lacking radixin, which is the dominant ERM protein in the liver, show severely impaired hepatic microvilli formation, resulting in a phenotype similar to human congenital hyperbilirubinemia, a rare autosomal recessive liver disorder. This work provides the first insights into the structural bases for the recognition between an ERM protein and an adhesion molecule, and may further serve as an experimental model for the study of molecular processes involved in bile secretion.

In addition to its full-time research efforts, the Yonemura lab offers a unique support to science, in the form of electron microscopy, services available to all research staff at the CDB. By providing fluorescence and electron microscopic visualizations as well as instruction in techniques, the team hopes to help ensure the quality, fidelity and ease-of-interpretation of visualization data generated at the Center.
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 are unique to each species. This approach has led to a deeper understanding of physiology, genetics and development, and has contributed to the establishment of the mouse as the most widely used model organism in science today. 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 a rich array of services related to the generation of experimental mice to labs within the CDB and around Japan. In its role as a CDB support laboratory, the LARGE team produces transgenic and knockout mouse models to the specifications of scientists working in a wide range of genetic, embryological and biomedical research projects, maintaining the highest quality standards and rapid turnover to ensure fast and easy access to researchers working within the Center and throughout the country. In addition to these core functions, the LARGE staff provides a number of other services, such as cloning by nuclear transfer and cryopreservation of mouse zygotes and sperm. The lab also performs a number of maintenance and logistical functions, such as the specific pathogen free (SPF) housing and processing and distribution of animals.

In 2003, the LARGE team started to expand its services and initiate new programs, notably the generation of target vectors from sequence information alone, and the independent production of novel genetically-modified constructs, a drive that generates on the order of 40-50 new mutant strains per year. Such strains serve as research platforms with the potential to provide new insights into a range of important research problems, from the developmental mechanisms of organ development to the genetic bases of human disease. The lab will also function as part of Japan’s system of Mouse Embryo Banks, with a special emphasis on producing, storing and cataloging embryos for use in developmental biology and regenerative medical research.

publications


The CDB Sequencing Laboratory 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 ranging from under one week for medium-scale projects to one month for larger jobs, at rates of up to 1,920 samples per day. Smaller requests are fulfilled 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.

In addition to DNA sequencing, the lab also offers a full range of DNA microarray services, from the amplification of target DNA and the preparation of probe cDNA, to microarray image data analysis. 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. The lab also stores and distributes DNA resources, making clones available to all members of the CDB staff.
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 are unique to each species. The mouse, one of the most important and widely used model organisms in science today, is prized for its amenability to genetic manipulation, its high level of homology with humans, and the trove of data regarding its physiology, genetics, and development that has accumulated over nearly one century of intensive scientific research.

The Laboratory for Animal Resources and Genetic Engineering (LARGE) provides an important suite of services related to the generation of experimental mice to labs within the CDB and around Japan. In its role as a CDB support laboratory, the LARGE team produces transgenic and knockout mouse models to the specifications of scientists working in a wide range of genetic, embryological, and biomedical research projects, maintaining the highest quality standards and rapid turnaround to ensure fast and easy access to researchers working within the Center and throughout the country.

In addition to DNA sequencing, the lab also offers a full range of DNA microarray services, from the amplification of target DNA and the preparation of probe CDNA, to microarray image data analysis. 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.

The lab also stores and distributes DNA resources, making clones available to all members of the CDB staff.

In 2003, the LARGE team started to expand its services and initiate new programs, notably the generation of novel genetically-modified constructs, a drive that generated a steady stream of new mutant strains per year. Such strains serve as research platforms with the potential to provide new insights into a range of important research problems, from the developmental mechanisms of organ development to the genetic bases of human disease. The lab will also function as part of Japan’s system of Mouse Embryo Banks, with a special emphasis on producing, storing, and cataloging embryos for use in developmental biology and regenerative medical research.

### Publications


Animal Facility

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

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

Research Aquarium

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

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

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

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

Research Aquarium

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

The aquarium includes seven climate-controlled rooms geared to providing optimal environments for the handling and breeding of freshwater and marine species, and utilizes reverse osmotic technology to maintain consistent tank-water purity. In addition to the commonly used zebrafish and African clawed frog, the facility also houses specimens from more novel models used in evolutionary development research, such as the lamprey, Lampetra japonica.
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 21st century. The organization and operations of the CDB were consigned to RIKEN, which coordinates the construction management and administration of the Center.

CDB Staff (December 2002)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research scientists</td>
<td>101</td>
</tr>
<tr>
<td>Research associates</td>
<td>3</td>
</tr>
<tr>
<td>Technical staff</td>
<td>105</td>
</tr>
<tr>
<td>Assistants</td>
<td>25</td>
</tr>
<tr>
<td>Visiting researchers</td>
<td>9</td>
</tr>
<tr>
<td>Collaborative scientists</td>
<td>30</td>
</tr>
<tr>
<td>Student trainees</td>
<td>47</td>
</tr>
<tr>
<td>Part time staff</td>
<td>53</td>
</tr>
<tr>
<td>Research promotion division</td>
<td>43</td>
</tr>
<tr>
<td>Other</td>
<td>65</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>511</td>
</tr>
</tbody>
</table>

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 serves as an unbiased review board for CDB research activities.

Facility Expenses
- Energy expenses
- Maintenance

Research Management Expenses
- Personnel expenses

Research Expenses
- FY2003
  - 762
  - 2732
  - 1827

Graduate School Affiliates
- 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 Western Japan, providing students with opportunities for hands-on laboratory benchwork and lectures on topics in development and regeneration. The center also has also accepted 50 students from other universities as research associates 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 8 graduate students working in CDB labs.

Scientific Exchange Programs
- The CDB is also engaged in programs of cooperation with research organizations in other parts of Japan and around the world. This activity is based primarily on cooperation between individual research laboratories. These scientific exchange agreements facilitate processes such as the hosting and dispatch of research staff for international collaborative research, the joint sponsorship of scientific meetings and the sharing of intellectual property produced in collaborations.
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 21st century. The organization and operations of the CDB were consigned to RIKEN, which coordinates the construction management and administration of the Center.

The Center for Developmental Biology employed a total of 511 people as of December 2002, with 101 research scientists, 3 research associates, 105 technical staff, 25 assistants, 9 visiting researchers, 30 collaborative scientists, 47 student trainees, 53 part-time staff, 43 research promotion division staff, and 65 other staff. 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 serves as an unbiased review board for CDB research activities.

The Institutional Review Board includes representatives from local academic, research and lay organizations as well as CDB research staff, and meets regularly to review and discuss the ethical and social implications of programs and investigations being conducted at the CDB. The results of the Board's discussions are submitted to the Center Director and taken into consideration when planning research activities.

The Graduate School Affiliates program at the CDB is designed to encourage young scientists to participate in advanced research projects as they prepare for careers in the life sciences. The CDB has collaborative educational programs with a number of graduate schools in Western Japan, providing students with opportunities for hands-on laboratory benchwork and lectures on topics in development and regeneration. The center has also accepted 50 students from other universities as research associates 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 8 graduate students working in CDB labs.

The CDB is also engaged in programs of cooperation with research organizations in other parts of Japan and around the world. This activity is based primarily on cooperation between individual research laboratories. These scientific exchange agreements facilitate processes such as the hosting and dispatch of research staff for international collaborative research, the joint sponsorship of scientific meetings and the sharing of intellectual property produced in collaborations.
Mitsuko Kosaka heads the Research Unit for Cell Plasticity, which seeks to determine the limits and possibilities in the differentiative potential of somatic stem cells. Their work focuses on differentiation in the iris of the eye, a population of pigmented cells that contains stem cells that show an intriguing plasticity, or ability to assume more than one cellular fate. The Kosaka lab will investigate the mechanisms by which stem cells in the iris can be induced to give rise to cells of other types, such as photoreceptive neural cells. This work is of great clinical promise in the treatment of vision loss due to nerve damage, as cells from the iris are readily obtainable and may one day be used as an autologous source of retinal replacement cells if they can be steered reliably to take up a neural fate. Recent findings from the lab suggesting that iris stem cells express genes characteristic of highly undifferentiated pluripotent stem cells also offer new avenues for exploration for researchers studying the genetic regulation of differentiative potency.

Hideki Taniguchi, M.D., Ph.D.
Unit Leader

The Research Unit for Organ Regeneration, headed by Hideki Taniguchi, focuses on studies of the differentiative and regulatory mechanisms underlying endodermal tissues and organs in the digestive system, which include liver, pancreas, intestine and salivary glands. By analyzing stem cells isolated from each of these tissue types, the Taniguchi lab seeks to confirm the existence of "endodermal stem cells" that enable the high levels of cellular plasticity seen in digestive organs. Using cell sorting technologies, Taniguchi is working to characterize these putative endodermal stem cells by sorting and analyzing cells from the livers and other digestive organs of fetal mice. The identification of molecular markers specific to such cells will make it possible to isolate them by flow cytometry and to pursue further investigations into their functional roles in the development of the endoderm-derived digestive system. The ability to identify and isolate stem cells capable of giving rise to endodermal lineage tissue types would also be of enormous potential value in the development of regenerative medical applications for the treatment of diabetes.

In a concurrent project, Taniguchi is working to develop a system for isolating and guiding the differentiation of stem cells from the pancreas of adult mice, which is aimed at establishing the foundations for cell replacement therapies using insulin-producing pancreatic beta cells grown in vitro.
Leading Project in Regenerative Medicine

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

Organ Regeneration

The Research Unit for Organ Regeneration, headed by Hideki Taniguchi, focuses on studies of the differentiative and regulatory mechanisms underlying endodermal tissues and organs in the digestive system, which include liver, pancreas, intestine and salivary glands. By analyzing stem cells isolated from each of these tissue types, the Taniguchi lab seeks to confirm the existence of "endodermal stem cells" that enable the high levels of cellular plasticity seen in digestive organs. Using cell sorting technologies, Taniguchi is working to characterize these putative endodermal stem cells by sorting and analyzing cells from the livers and other digestive organs of fetal mice. The identification of molecular markers specific to such cells will make it possible to isolate them by flow cytometry and to pursue further investigations into their functional roles in the development of the endoderm-derived digestive system. The ability to identify and isolate stem cells capable of giving rise to endodermal lineage tissue types would also be of enormous potential value in the development of regenerative medical applications for the treatment of diabetes.

In a concurrent project, Taniguchi is working to develop a system for isolating and guiding the differentiation of stem cells from the pancreas of adult mice, which is aimed at establishing the foundations for cell replacement therapies using insulin-producing pancreatic beta cells grown in vitro.

Cell Plasticity

Mitsuko Kosaka heads the Research Unit for Cell Plasticity, which seeks to determine the limits and possibilities in the differentiative potential of somatic stem cells. Their work focuses on differentiation in the iris of the eye, a population of pigmented cells that contains stem cells that show an intriguing plasticity, or ability to assume more than one cellular fate. The Kosaka lab will investigate the mechanisms by which stem cells in the iris can be induced to give rise to cells of other types, such as photoreceptive neural cells. This work is of great clinical promise in the treatment of vision loss due to nerve damage, as cells from the iris are readily obtainable and may one day be used as an autologous source of retinal replacement cells if they can be steered reliably to take up a neural fate. Recent findings from the lab suggesting that iris stem cells express genes characteristic of highly undifferentiated pluripotent stem cells also offer new avenues for exploration for researchers studying the genetic regulation of differentiative potency.

In 2003, the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) inaugurated a number of Leading Projects to drive research into areas identified as having great potential to make contributions to the nation’s economy, environment, social climate or health and welfare. One such project was introduced to promote research leading to applications for stem cells in regenerative medicine.
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.

### 2003 Seminars

<table>
<thead>
<tr>
<th>Date</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003.1.15</td>
<td>Hox genes and brainstem patterning</td>
<td>Filippo M. Rijli</td>
</tr>
<tr>
<td>2003.1.16</td>
<td>Neural patterning in vertebrate embryogenesis</td>
<td>Nobuo Iwashita</td>
</tr>
<tr>
<td>2003.1.17</td>
<td>Left-right asymmetry in the zebrafish forebrain</td>
<td>Marnie Halper</td>
</tr>
<tr>
<td>2003.1.27</td>
<td>Patterning formation in mesodermal area by FGF2</td>
<td>Tatsuro Fukatsu-Shiraneo</td>
</tr>
<tr>
<td>2003.1.29</td>
<td>To branch or not to branch: MAPLE controls dendritic Arbor branching and lineage restriction in the Drosophila peripheral nervous system</td>
<td>Toshio Suda</td>
</tr>
<tr>
<td>2003.2.21</td>
<td>Wnt signaling in development and disease</td>
<td>Randell T. Moon</td>
</tr>
<tr>
<td>2003.3.11</td>
<td>The new revolution in developmental biology: Expansion and reconciliation</td>
<td>Scott Gilbert</td>
</tr>
<tr>
<td>2003.3.27</td>
<td>Imaging cell movements that pattern the vertebrate embryo</td>
<td>Scott Fraser</td>
</tr>
<tr>
<td>2003.3.27</td>
<td>Induction and evolution of the neural crest</td>
<td>Marienne Bronner Fraser</td>
</tr>
<tr>
<td>2003.3.27</td>
<td>Functional expression cloning of Noggin, a gene controlling cytokine independent ES cell self-renewal</td>
<td>Ian Chambers</td>
</tr>
<tr>
<td>2003.4.22</td>
<td>Differentiation of embryonic stem cells into hepatocytes: biological functions and therapeutic applications</td>
<td>Tatsuro Ochiya</td>
</tr>
<tr>
<td>2003.5.13</td>
<td>Dynamics of cellular interactions during T cell recognition in 3-dimensional tissue reconstituted by tissue-mimetic microarray</td>
<td>Sheen-Teo Huh</td>
</tr>
<tr>
<td>2003.5.14</td>
<td>BMP-3a and BMP-3 function as different destabilising factors in Xenopus embryos</td>
<td>Shin-ichi Nakamura</td>
</tr>
<tr>
<td>2003.5.21</td>
<td>Building brains from embryonic stem cells, Naive cultures offer greatest potential</td>
<td>Hidemasa Katoh</td>
</tr>
<tr>
<td>2003.5.22</td>
<td>Considerations in microscopy experimental design and data analysis</td>
<td>Ali P. Zarefi</td>
</tr>
<tr>
<td>2003.6.18</td>
<td>The role of myosin VI in the spermatogenesis of Drosophila</td>
<td>Naoyuki Tatsukawa</td>
</tr>
<tr>
<td>2003.6.23</td>
<td>Cell surface molecules and molecular mechanisms of signaling pathways mediated by target of rapamycin</td>
<td>Kazuhiro Yoneyama</td>
</tr>
<tr>
<td>2003.7.9</td>
<td>Diversity of CNTF-produdhkin family in the brain</td>
<td>Takashi Yagi</td>
</tr>
<tr>
<td>2003.7.9</td>
<td>Membrane raft: Molecular mechanisms and real-time imaging</td>
<td>Abhijit Naskar</td>
</tr>
<tr>
<td>2003.7.8</td>
<td>Ontogeny of the hematopoietic system</td>
<td>M. Bruno Pedruz</td>
</tr>
<tr>
<td>2003.7.17</td>
<td>PKA and TGF2 in disease, development and evolution</td>
<td>Veronica van Heyningen</td>
</tr>
<tr>
<td>2003.7.19</td>
<td>Querocentric hematopoietic stem cells in the niche</td>
<td>Toshio Suzuki</td>
</tr>
<tr>
<td>2003.8.4</td>
<td>Manipulating mouse genome from single gene knockout to chromosome engineering</td>
<td>Toshimi Nishiyama</td>
</tr>
<tr>
<td>2003.8.5</td>
<td>Genetic regulation of retinal interneuron differentiation</td>
<td>Ashida Osaki</td>
</tr>
<tr>
<td>2003.8.6</td>
<td>Cell contact of neural stem cells and CNS neurons</td>
<td>Mayuki Ozawa</td>
</tr>
<tr>
<td>2003.8.7</td>
<td>Regulation of spindle orientation by centrosome and APC tumor suppressor</td>
<td>Yukiko M. Yamashita</td>
</tr>
<tr>
<td>2003.8.8</td>
<td>A protein interaction matrix for an integrin adhesion complex within C. elegans musculature</td>
<td>Hiroshi Qadota</td>
</tr>
<tr>
<td>2003.8.26</td>
<td>Improved spermatogenesis in mice lacking Cat1</td>
<td>Takayoshi Yamamoto</td>
</tr>
<tr>
<td>2003.8.11</td>
<td>Mechanisms controlling heart and blood vessel growth</td>
<td>Kenneth Walsh</td>
</tr>
<tr>
<td>2003.9.12</td>
<td>Genomic approaches for functional annotation of the mammalian genome</td>
<td>John Hogemueck</td>
</tr>
<tr>
<td>2003.9.19</td>
<td>Genetic analysis of mouse triumbrach and limb development</td>
<td>Seew-Lee Ang</td>
</tr>
<tr>
<td>2003.9.29</td>
<td>Fibronectin requirement in branching morphogenesis</td>
<td>Takahiko Sakai</td>
</tr>
<tr>
<td>2003.10.3</td>
<td>Chemical modulation of embryonic development and morphogenesis</td>
<td>James K. Chen</td>
</tr>
<tr>
<td>2003.10.10</td>
<td>Hox-in leukemia and as avenues to hematopoietic stem cell expansion</td>
<td>R. Keith Humphries</td>
</tr>
<tr>
<td>2003.10.14</td>
<td>Characterization of key modulators of the FGF signal transduction pathway and their role in axis polarity</td>
<td>Michael Tsang</td>
</tr>
<tr>
<td>2003.10.14</td>
<td>Patterning neurogenesis in the zebrafish embryo</td>
<td>Moriyuki Ishi</td>
</tr>
<tr>
<td>2003.10.16</td>
<td>Endoderm as a source of patterning information for early chick embryogenesis</td>
<td>Gary Schiermeyer</td>
</tr>
<tr>
<td>2003.10.23</td>
<td>Structure and function of Slx5 protein: A conserved repression function in developmental signalling pathways</td>
<td>Marko Anyai</td>
</tr>
<tr>
<td>2003.10.24</td>
<td>Regulation of lampbrush and spatial patterns of gene expression in Dicyostelium</td>
<td>William Loomis</td>
</tr>
<tr>
<td>2003.10.27</td>
<td>Phosphogenic conservation of a cis-regulating gene that controls polarized expression of Sonic hedgehog (shh) in limb buds</td>
<td>Toshikiko Shintani</td>
</tr>
<tr>
<td>2003.10.29</td>
<td>Shivering out animal’s code and map</td>
<td>Naoyuki Uchida</td>
</tr>
<tr>
<td>2003.11.14</td>
<td>Molecular mechanisms specifying muscle identity and myosin number in the Drosophila adult</td>
<td>K. VladyRagavan</td>
</tr>
<tr>
<td>2003.11.18</td>
<td>Genes of the neural progenitors and their regional diversities through regulation of Scl2</td>
<td>H. Kodoh</td>
</tr>
<tr>
<td>2003.11.20</td>
<td>Nuclear interpretation of the Omp morphogen in Drosophila melanogaster</td>
<td>Markus Affolter</td>
</tr>
<tr>
<td>2003.11.26</td>
<td>Genes that regulate the formation and regeneration of skeletal muscle</td>
<td>Margaret Buckingham</td>
</tr>
<tr>
<td>2003.11.27</td>
<td>Patterning and growth in Drosophila limb development</td>
<td>Koosje Basler</td>
</tr>
<tr>
<td>2003.12.2</td>
<td>Secondary rearrangement of Tolf alpha chain in vivo shaping and reshaping of cell morphology</td>
<td>Osumi Kurosawa</td>
</tr>
<tr>
<td>2003.12.3</td>
<td>Proteins involved in nuclear scaffold formation of ESCs by separation is required for marine spermatogenesis</td>
<td>Nobuo Kudo</td>
</tr>
<tr>
<td>2003.12.5</td>
<td>Function and regulation of cyclin-dependent, a leucine-zipper complex, in cytokinesis</td>
<td>Masanori Mochida</td>
</tr>
<tr>
<td>2003.12.9</td>
<td>Eph receptors and ephrins in neural map development</td>
<td>Masao Nakamoto</td>
</tr>
<tr>
<td>2003.12.15</td>
<td>Establishment of the ventral tube plan in relation to body formation</td>
<td>Mikako Tanaka</td>
</tr>
<tr>
<td>2003.12.16</td>
<td>Wnt-mediated axon guidance at the edge of Drosophila</td>
<td>Shinya Yashiki</td>
</tr>
<tr>
<td>2003.12.26</td>
<td>Hox gene function in neural crest development</td>
<td>Mamoru Ichi</td>
</tr>
</tbody>
</table>
## 2003 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.

<table>
<thead>
<tr>
<th>Date</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003.3.22</td>
<td>lameness and immunity in the zebrafish embryo</td>
<td>Toshiyuki Ito</td>
</tr>
<tr>
<td>2003.3.11</td>
<td>Endodermal as a source of patterning information for early chick embryos</td>
<td>Gary Schorlemmer</td>
</tr>
<tr>
<td>2003.3.10</td>
<td>Structure and function of oocyte proteins: A conserved repressive function in developmental signaling pathways</td>
<td>Marko Araki</td>
</tr>
<tr>
<td>2003.3.8</td>
<td>Wnt-mediated axon guidance at the midline of Zebrafish</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.3.7</td>
<td>BMP-2 and BMP-4 function as different dorso-anteroventral factors in Xenopus embryos</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.3.6</td>
<td>Building brains from embryonic stem cells, rare cultures offer greatest potential</td>
<td>Tomoko Ueno</td>
</tr>
<tr>
<td>2003.3.5</td>
<td>ESP proteins: A conserved repressive function in developmental signaling pathways</td>
<td>Marko Araki</td>
</tr>
<tr>
<td>2003.3.4</td>
<td>The role of myosin VI in the spatiotemporal control of C. elegans</td>
<td>Toshihiko Shiroishi</td>
</tr>
<tr>
<td>2003.3.3</td>
<td>Total cross-section of neural stem cells and CD1 markers</td>
<td>Hidemi Ishikawa</td>
</tr>
<tr>
<td>2003.3.2</td>
<td>Cell migrations in the vertebrate body plan in relation to limb formation</td>
<td>Masahiro Nakamoto</td>
</tr>
<tr>
<td>2003.3.1</td>
<td>Regulation of the formation and regeneration of skeletal muscle</td>
<td>Marian Zaki</td>
</tr>
<tr>
<td>2003.2.26</td>
<td>Patterning and growth in Drosophila limb development</td>
<td>Konrad Basler</td>
</tr>
<tr>
<td>2003.2.19</td>
<td>Patterning neurogenesis in the zebrafish embryo</td>
<td>Osamu Yamamoto</td>
</tr>
<tr>
<td>2003.2.18</td>
<td>Eph receptors and ephrins in neural map development</td>
<td>Marko Araki</td>
</tr>
<tr>
<td>2003.2.17</td>
<td>Genetic analysis of mouse forebrain and midbrain development</td>
<td>Siew-Lan Ang</td>
</tr>
<tr>
<td>2003.2.16</td>
<td>Mechanisms controlling heart and blood vessel growth</td>
<td>Hisato Yamamoto</td>
</tr>
<tr>
<td>2003.2.15</td>
<td>Wnt-mediated axon guidance at the midline of Zebrafish</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.2.14</td>
<td>BMP-2 and BMP-4 function as different dorso-anteroventral factors in Xenopus embryos</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.2.13</td>
<td>Building brains from embryonic stem cells, rare cultures offer greatest potential</td>
<td>Tomoko Ueno</td>
</tr>
<tr>
<td>2003.2.12</td>
<td>The role of myosin VI in the spatiotemporal control of C. elegans</td>
<td>Toshihiko Shiroishi</td>
</tr>
<tr>
<td>2003.2.11</td>
<td>Total cross-section of neural stem cells and CD1 markers</td>
<td>Hidemi Ishikawa</td>
</tr>
<tr>
<td>2003.2.10</td>
<td>Cell migrations in the vertebrate body plan in relation to limb formation</td>
<td>Masahiro Nakamoto</td>
</tr>
<tr>
<td>2003.2.9</td>
<td>Patterning and growth in Drosophila limb development</td>
<td>Konrad Basler</td>
</tr>
<tr>
<td>2003.2.8</td>
<td>Patterning neurogenesis in the zebrafish embryo</td>
<td>Osamu Yamamoto</td>
</tr>
<tr>
<td>2003.2.7</td>
<td>Eph receptors and ephrins in neural map development</td>
<td>Marko Araki</td>
</tr>
<tr>
<td>2003.2.6</td>
<td>Genetic analysis of mouse forebrain and midbrain development</td>
<td>Siew-Lan Ang</td>
</tr>
<tr>
<td>2003.2.5</td>
<td>Mechanisms controlling heart and blood vessel growth</td>
<td>Hisato Yamamoto</td>
</tr>
<tr>
<td>2003.2.4</td>
<td>Wnt-mediated axon guidance at the midline of Zebrafish</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.2.3</td>
<td>BMP-2 and BMP-4 function as different dorso-anteroventral factors in Xenopus embryos</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.2.2</td>
<td>Building brains from embryonic stem cells, rare cultures offer greatest potential</td>
<td>Tomoko Ueno</td>
</tr>
<tr>
<td>2003.2.1</td>
<td>The role of myosin VI in the spatiotemporal control of C. elegans</td>
<td>Toshihiko Shiroishi</td>
</tr>
<tr>
<td>2003.1.31</td>
<td>Total cross-section of neural stem cells and CD1 markers</td>
<td>Hidemi Ishikawa</td>
</tr>
<tr>
<td>2003.1.30</td>
<td>Cell migrations in the vertebrate body plan in relation to limb formation</td>
<td>Masahiro Nakamoto</td>
</tr>
<tr>
<td>2003.1.29</td>
<td>Patterning and growth in Drosophila limb development</td>
<td>Konrad Basler</td>
</tr>
<tr>
<td>2003.1.28</td>
<td>Patterning neurogenesis in the zebrafish embryo</td>
<td>Osamu Yamamoto</td>
</tr>
<tr>
<td>2003.1.27</td>
<td>Eph receptors and ephrins in neural map development</td>
<td>Marko Araki</td>
</tr>
<tr>
<td>2003.1.26</td>
<td>Genetic analysis of mouse forebrain and midbrain development</td>
<td>Siew-Lan Ang</td>
</tr>
<tr>
<td>2003.1.25</td>
<td>Mechanisms controlling heart and blood vessel growth</td>
<td>Hisato Yamamoto</td>
</tr>
<tr>
<td>2003.1.24</td>
<td>Wnt-mediated axon guidance at the midline of Zebrafish</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.1.23</td>
<td>BMP-2 and BMP-4 function as different dorso-anteroventral factors in Xenopus embryos</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.1.22</td>
<td>Building brains from embryonic stem cells, rare cultures offer greatest potential</td>
<td>Tomoko Ueno</td>
</tr>
<tr>
<td>2003.1.21</td>
<td>The role of myosin VI in the spatiotemporal control of C. elegans</td>
<td>Toshihiko Shiroishi</td>
</tr>
<tr>
<td>2003.1.20</td>
<td>Total cross-section of neural stem cells and CD1 markers</td>
<td>Hidemi Ishikawa</td>
</tr>
<tr>
<td>2003.1.19</td>
<td>Cell migrations in the vertebrate body plan in relation to limb formation</td>
<td>Masahiro Nakamoto</td>
</tr>
<tr>
<td>2003.1.18</td>
<td>Patterning and growth in Drosophila limb development</td>
<td>Konrad Basler</td>
</tr>
<tr>
<td>2003.1.17</td>
<td>Patterning neurogenesis in the zebrafish embryo</td>
<td>Osamu Yamamoto</td>
</tr>
<tr>
<td>2003.1.16</td>
<td>Eph receptors and ephrins in neural map development</td>
<td>Marko Araki</td>
</tr>
<tr>
<td>2003.1.15</td>
<td>Genetic analysis of mouse forebrain and midbrain development</td>
<td>Siew-Lan Ang</td>
</tr>
<tr>
<td>2003.1.14</td>
<td>Mechanisms controlling heart and blood vessel growth</td>
<td>Hisato Yamamoto</td>
</tr>
<tr>
<td>2003.1.13</td>
<td>Wnt-mediated axon guidance at the midline of Zebrafish</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.1.12</td>
<td>BMP-2 and BMP-4 function as different dorso-anteroventral factors in Xenopus embryos</td>
<td>Shinya Nakamura</td>
</tr>
<tr>
<td>2003.1.11</td>
<td>Building brains from embryonic stem cells, rare cultures offer greatest potential</td>
<td>Tomoko Ueno</td>
</tr>
</tbody>
</table>
The Origin and Formation of Multicellular Systems

The CDB held its first annual symposium at the nearby Portopia Hotel. The meeting drew more than 200 speakers and attendees from around the world, with lectures, poster presentations and lively discussion on the origins of metazoan biology, the fundamental processes of development and regeneration and the formation of higher-order structures, such as neural networks.

The Symposium was organized into five sessions over three days, with full and short talks by 28 distinguished speakers.

Monday, March 24
Session 1
The Origin of Multicellular Systems
9:00 am 9:40 am Peter W Holland, Oxford University
9:40 am 10:00 am Hitoshi Suga, Kyoto University
10:00 am 10:40 am Toczko King, University of Wisconsin
10:40 am 11:20 am Yoko Watanabe, National Science Museum, Japan
11:30 am 12:10 pm Peter Devreotes, Johns Hopkins University
Session 2
Germ Cells, Stem Cells and Regeneration
1:30 pm 2:10 pm Judith C Kimble, University of Wisconsin-Madison
2:10 pm 2:50 pm Allan Spradling, Carnegie Institution of Washington
3:10 pm 3:30 pm Yuzo Niki, Ibaraki University
3:30 pm 4:10 pm Christopher Wyse, Cincinnati Children’s Hospital
8:00 pm 8:40 pm Shin-ichi Nishikawa, RIKEN CDB
8:40 pm 9:00 pm Ian Chambers, University of Edinburgh
9:00 pm 9:40 pm Kiyokazu Agata, RIKEN CDB

Tuesday, March 25
Session 3
Cellular Polarization and Asymmetric Division
9:00 am 9:40 am David Ish-Horowicz, Cancer Research UK
9:40 am 10:00 am Antony A Pyman, Max Planck Institute
10:00 am 10:40 am Yuh-Nung Jan, UCSF
10:40 am 11:20 am Fumio Matsuzaki, RIKEN CDB
11:30 am 12:10 pm Tadashi Uemura, Kyoto University
Session 4
Intercellular Signaling and Morphogenetic Cell Behavior
1:30 pm 2:10 pm Hiroshi Hamada, Osaka University
2:10 pm 2:50 pm Edward M DeRobertis, UCLA
2:50 pm 3:30 pm Olivier Pourquie, Stowers Institute for Medical Research
3:30 pm 4:10 pm Yasunio Sawai, Kyoto University
4:10 pm 4:50 pm Yuko Ozawa, University of Tokyo
4:50 pm 5:10 pm Elaine Fuchs, The Rockefeller University
5:10 pm 5:50 pm Tom Curran, St Jude Children’s Research Hospital
6:00 pm 6:30 pm 2003 RIKEN CDB Symposium

Wednesday, March 26
Session 5
Cellular Basis for Neuronal Circuit Formation
9:00 am 9:40 am Hajime Fujisawa, Nagoya University
9:40 am 10:20 am Peter Mombaerts, The Rockefeller University
10:40 am 11:20 am Christine E Holt, Cambridge University
11:20 am 12:00 pm Masami Kinoshita, UC Berkeley
The CDB held its first annual symposium at the nearby Portopia Hotel. The meeting drew more than 200 speakers and attendees from around the world, with lectures, poster presentations and lively discussion on the origins of metazoan biology, the fundamental processes of development and regeneration and the formation of higher-order structures, such as neural networks.

The Symposium was organized into five sessions over three days, with full and short talks by 28 distinguished speakers.

**Monday, March 24**

**Session 1**

**The Origin of Multicellular Systems**

9:00 am 9:40 am 
Peter W Holland, Oxford University

9:40 am 10:00 am 
Hitoshi Suga, Kyoto University

10:00 am 10:40 am 
Mozee King, University of Wisconsin

10:50 am 11:20 am 
Yoko Watanabe, National Science Museum, Japan

11:30 am 12:10 pm 
Peter Devreotes, Johns Hopkins University

**Session 2**

**Germ Cells, Stem Cells and Regeneration**

1:30 pm 2:10 pm 
Judith C Kiem, University of Wisconsin-Madison

2:10 pm 2:50 pm 
Allan Spradling, Carnegie Institution of Washington

3:30 pm 4:10 pm 
Christopher Wylie, Cincinnati Children’s Hospital

8:00 pm 8:40 pm 
Shigehiro Ikawa, RIKEN CDB

8:40 pm 9:20 pm 
Jen Chamberlin, University of Edinburgh

9:00 pm 9:40 pm 
Kiyokazu Agata, RIKEN CDB

**Tuesday, March 25**

**Session 3**

**Cellular Polarization and Asymmetric Division**

3:50 pm 4:10 pm 
Yoshinori Ohara, Kyoto University

4:10 pm 4:30 pm 
Naoto Ikeda, RIKEN CDB

4:30 pm 5:10 pm 
Elaine Fuchs, The Rockefeller University

5:10 pm 5:50 pm 
Tom Curran, St Jude Children’s Research Hospital

**Session 4**

**Intercellular Signaling and Morphogenetic Cell Behavior**

1:30 pm 2:10 pm 
Hiroshi Harada, Osaka University

2:10 pm 2:50 pm 
Eckhard Ciechanover, UCLA

2:50 pm 3:30 pm 
Oliver Pourquie, Stowers Institute for Medical Research

3:50 pm 4:10 pm 
Yasunori Sohara, Kyoto University

4:10 pm 4:50 pm 
Yukio Otsu, University of Tokyo

4:30 pm 5:10 pm 
Elaine Fuchs, The Rockefeller University

5:10 pm 5:50 pm 
Tom Curran, St Jude Children’s Research Hospital

**Wednesday, March 26**

**Session 5**

**Cellular Basis for Neuronal Circuit Formation**

9:00 am 9:40 am 
Hiroshi Osumi, RIKEN CDB

9:40 am 10:00 am 
Peter Montminy, The Rockefeller University

10:40 am 11:20 am 
Christine E Holt, Cambridge University

11:20 am 12:00 pm 
Norimichi Tauchi, UC Berkeley

On The Cover

Terminal branch of the Drosophila trachea. A single terminal cell that is polyploid expands numerous cytoplasmic tubules with intracellular lumen (tracheoles) over the surface of the larval gut.

PHOTO: Shigeo Hayashi