

RIKEN Center for Developmental Biology
2006 Annual Report

RIKEN Center for Developmental Biology 2006 Annual Report

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The Center for Developmental Biology was founded in April 2000 under the auspices of the Millennium Project research initiative launched by former Prime Minister Keizo Obuchi. The Millennium Projects were established to drive research in the fields of information technology, environmental science and the study of aging, areas of vital importance to both Japan and the world in the 21st century. The organization and operations of the CDB were consigned to RIKEN, which coordinates the construction, management and administration of the Center. The Millennium Project has now concluded its five-year term, but the CDB continues in its mission of exploring and developing a better understanding of the mechanisms of development and regeneration, and establishing the scientific basis of regenerative medicine.

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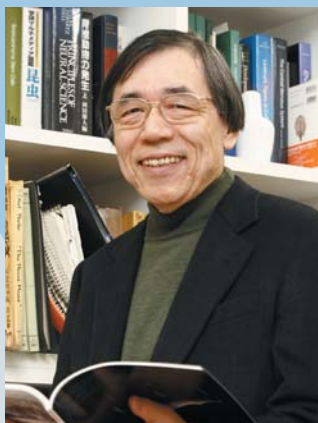
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RIKEN Center for Developmental Biology



With the new year already dawning, a look back at 2006 reveals what was certainly the most productive year in the history of the RIKEN Center for Developmental Biology. The quality and diversity of the research achievements over the past twelve months seems to confirm that CDB labs have risen to the challenge of first five years of the Center's career—to shed light on the fundamental mechanisms at work in development and regeneration and to offer new hope to a world awaiting the advent of regenerative medical therapies by helping to build a solid, broader and deeper foundation for that field through basic biological research.

Even a glance at the research highlights presented in this 2006 issue of the CDB Annual Report shows that the Center's research programs were at their most active ever, with articles published in major scientific journals on topics including cell cycle regulation in the oocyte; a series of reports relating to asymmetric cell division and its role in fate determination; transcriptional feedback in the mammalian circadian clock; and new functions for RNAi machinery in chromatin remodeling. A number of investigators developed new technologies that will contribute to research in labs around the world by improving the efficiency of PCR at the single cell level and providing a method for labeling individual neurons with photo-inducible proteins. Advances were made in biomedically important areas as well, from the identification of a gene implicated in the progression of a childhood brain cancer, to the development of a new, clinically safe method for growing embryonic stem cells in culture.

At the institutional level, the CDB entered a new stage of growth, participating even more actively in international organizations and meetings in developmental biology and related fields. Our 2006 Annual Symposium on new strategies and concepts in the "logic of development" drew researchers from around the world to participate in three days

of intensive discussions on the latest findings and ideas on the architecture of developmental pathways, networks and systems. And starting in 2006, members of the CDB staff (including myself) now play roles in the administration of the International Society of Developmental Biologists (ISDB; <http://www.developmental-biology.org/>) as well as a number of regional organizations, including the Asia-Pacific Developmental Biology Network and the Asia Reproductive Biotechnology Society. We are honored to have the privilege to serve the international research community by participating in the efforts of these organizations to promote the study of development and related fields, particularly by supporting students and junior scientists in their work.

One of the greatest advantages for scientists working at the CDB has to be the tremendous concentration of talent and expertise in a research environment providing extensive technical services and support. The past year saw an expansion of the core facilities, equipment and technical support available to CDB researchers with the addition of a mass spectrometry unit and an increased range of services in genomic sequencing and microarray analysis. With cornerstone support services in mutant mouse generation and electron microscopy and an advanced lab animal facility and aquarium, the Center provides many of the most commonly used research technologies and services onsite, enabling scientists to spend more time on their research, as well as opening up new opportunities for international collaboration.

On the domestic front, the Center's rigorous employment and performance review policies, which emphasize autonomy, independent evaluations and encouraging positive mobility, have helped make the CDB an incubator of talent in the field, with many former CDB student trainees and research scientists moving on to positions in prestigious universities and research institutes in Japan and around the world. The decision to adopt an alternative to the

tenure model in a country that has traditionally favored lifetime employment for academic and research positions was a risky one, so it is doubly gratifying to see so many CDB alumni move onward and upward in their careers.

For aspiring scientists in the youngest generations, as well as the public at large, the CDB continues to develop and distribute publications, educational materials and news releases on its research and the fields of development, regeneration and regenerative medicine. To mark the conclusion of the Millennium Project, under the auspices of which the Center was first established, the CDB produced a 100-page commemorative book detailing (in Japanese) the fruits of that investment by the national government and outlining some of the questions that will define the future of the discipline. More than one thousand members of the general public turned out for our annual Open House event and approximately 150 graduate and medical school students joined the CDB's yearly intensive lecture program last summer. Through programs such as these, we seek to share the wonder and importance of developmental biology with experts and non-specialists alike, focusing especially on reaching out to the young in the hopes of building an appreciation for and excitement about the mysteries of the generation of animal forms. As a publicly funded institution, we make the data for all CDB-produced materials available bilingually and free of charge on our website at www.cdb.riken.jp.

As always, the opportunity to reflect on the past also occasions the urge to look ahead, and with support from CDB laboratories and administrative staff, I look forward to meeting new challenges in research and scientific governance in the years ahead. I hope that you find this issue of the Annual Report informative and stimulating, and wish you all the best in the year ahead.

Masatoshi TAKEICHI

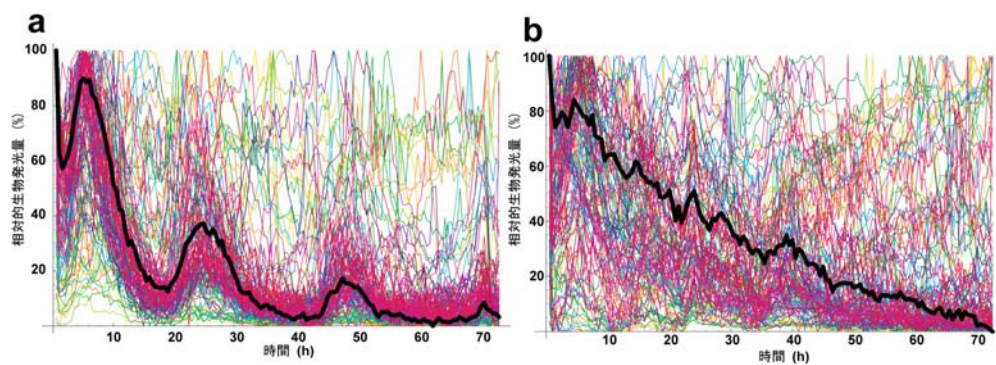
Director, RIKEN Center for Developmental Biology



Standing the test of time

Transcriptional feedback in the mammalian clock

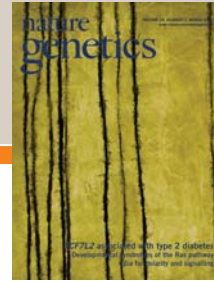
Researchers studying the cyanobacterium, *Synechococcus elongatus*, gave the circadian biology community something of an unexpected wakeup call in 2005 when they published their findings on how this primitive organism keeps time. Most classical models intended to describe the activity of biological clocks suggest that the autoregulatory mechanism relies on transcriptional negative feedback. In a simplified version of this clockwork, one set of genes acts to inhibit the transcription or translation of a second set, whose function is, somewhat paradoxically, to activate the first; this mechanism is believed to be universal to all circadian biology. The *S. elongatus* study, however, showed otherwise, for the molecular clocks in these unicellular creatures happily kept ticking away even when transcriptional regulation was factored out. This single report prompted a re-examination of what had been up to that point an article of faith, in which scientists found themselves scrambling to substantiate that feedback repression is indeed one of the mainsprings of the circadian clock in other species.



Circadian rhythm is lost in cells carrying mutations in biological clock genes.

That hunt for direct evidence paid off with the results of a thoroughgoing joint study by members of the Laboratory for Systems Biology (Hiroki R. Ueda; Team Leader) and colleagues in several American labs, including the Scripps Research Institute, which conclusively demonstrated the requirement for transcriptional feedback repression in the mouse biological clock function. The work, published in *Nature Genetics*, involved the meticulous dissection of the murine timekeeping network and showed that CRY and PER proteins regulate their own expression by repressing the formation of a functional complex by the transcriptional activators CLOCK and BMAL1, which themselves were suspected to form a complex that binds to promoters of CRY and PER.

To verify the feedback repressor model, the collaborators first identified mutations in the CLOCK and BMAL1 genes that showed resistance to inhibition by the Cry-encoded protein, CRY1. This desensitization was traced to defects in a pair of regions, one in the CLOCK PAS domain, the other in the C-terminus of BMAL1. Interestingly, mutation of both genes together showed a combinatorial effect; the double mutants were much less responsive to repression by CRY1 than either of the mutations on its own. Subsequent biochemistry indicated that CRY1 is less able to bind to CLOCK/BMAL1 complexes in which either or both of the constituent proteins contained a mutation. Additional findings involving the other putative set of transcriptional repressors, the PER proteins, tended to confirm that physical interaction between CRY and PER and the CLOCK/BMAL1 complex is responsible for the normal inhibitory effect, and that this interaction is interfered with in mutant CLOCK/BMAL1 complexes. Such mutations had a crossover effect as well, as CRY-insensitive CLOCK/BMAL1 phenotypes showed a loss of circa-



Sato T K, Yamada R G, Ukai H, Baggs J E, Miraglia L J, Kobayashi T J, Welsh D K, Kay S A, Ueda H R and Hogenesch J B. Feedback repression is required for mammalian circadian clock function. *Nat Genet* 38:312-9 (2006).

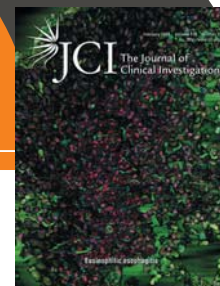
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dian rhythmicity in *Per2* and *Bmal1* promoter activity. Population- and single-cell-level monitoring of the cellular rhythms of mammalian cells carrying the mutations in question reinforced the importance of CRY-mediated repression; at both scales, normal circadian patterns were disrupted.

This latest work from the Ueda lab bolsters the old case for the centrality of negative feedback in the form of a transcriptional repressor in the regulation of the mammalian cellular rhythms. Of course, it is likely that there are additional components to this intricate clockwork that continue to cycle even when transcriptional feedback is stopped (as is the case in *S. elongatus*), but we know now that these alone are insufficient to keep our cells running on schedule. Perhaps more importantly, an improved understanding of the body's molecular clock might some day lead to therapies for individuals whose daily rhythms are out of sync. "If we could find a way to control these circadian regulatory mechanisms," Ueda notes, "it might open up new treatments for common medical problems such as sleep disorders, which could stand as a real benefit for people on non-standard wake-and-sleep schedules, such as those working irregular shifts."



Tlx blazes a trail for blood vessel growth



Uemura A, Kusuvara S, Wiegand S J, Yu R T and Nishikawa SI. Tlx acts as a proangiogenic switch by regulating extracellular assembly of fibronectin matrices in retinal astrocytes. *J Clin Invest* 116:369-77 (2006).

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The mouse provides a useful model for studying developmental angiogenesis in a mammal, as the vascular development in its retina occurs postnatally, following an influx of astrocytes induced by the retinal neurons. These astrocytes secrete factors that initiate vasculogenesis, such as VEGF, as well as fibronectin, an extracellular protein that forms a trellis-like meshwork along which the nascent vascular sprout crawls. While the hypoxia-inducible regulation of VEGF has been well characterized in general, the mechanisms by which astrocytes respond to oxygen debt and satiation have largely been a mystery.



Yuri SHIMIZU, Akiyoshi UEMURA

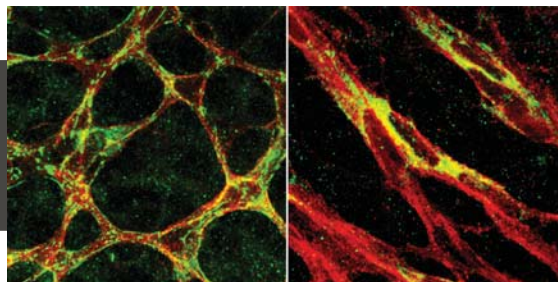
In a study published in *The Journal of Clinical Investigation*, Akiyoshi Uemura and colleagues in the laboratory for Stem Cell Biology (Shin-Ichi Nishikawa; Group Director), Regeneron Pharmaceuticals and the Salk Institute (USA), reported that astrocyte behavior is mediated by a molecular switch that alters fibronectin assembly in response to changes in the cell's oxygen supply. This regulator, Tlx, is a transcription factor that is expressed specifically in proangiogenic retinal astrocytes and which down-regulates dramatically on contact with blood vessels.

Curious about the function of the Tlx gene product, Uemura found that, in *Tlx*^{-/-} mutants, while astrocyte proliferation was only moderately affected, the astrocyte network failed to form in the retina and, as a result, blood vessels were entirely absent. The group conjectured that this may be due to defects in either cell motility or adhesion which prevent the advance of the growing vasculature, a

hypothesis that was supported by their observation that ectopic vessels derived from the hyaloid vasculature (a fetal vascular system in the eye) that were attracted to astrocyte-rich areas of the retina failed to align themselves along the extant astrocyte network on entering the mutant retina. Their speculation was proven right when they checked fibronectin expression and found that the null mutants' astrocytes produced only scarce amounts of this scaffold-building protein, unlike the profuse networks laid down in wild type.

"There are a number of human visual impairments, including diabetic retinopathies and infant R.O.P. (retinopathy of prematurity) which are related to defects in the vasculature," comments Uemura. "It's exciting to think that this work on a developmental system might contribute to what we know about vessel growth in the human retina, and that this knowledge of the role of the astrocytic scaffold might one day lead to a therapy."

In contrast to the filamentous extracellular deposition of fibronectin proteins (green) around the astrocyte networks (red) in normal retinas (left), fibronectin proteins were absent in the extracellular spaces, but retained in the cytoplasm of retinal astrocytes in Tlx knockout mice (right).



Olfactory bulb development follows *Fez*

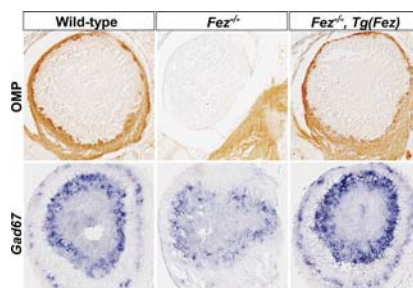
Hirata T, Nakazawa M, Yoshihara S, Miyachi H, Kitamura K, Yoshihara Y and Hibi M, Zinc-finger gene *Fez* in the olfactory sensory neurons regulates development of the olfactory bulb non-cell-autonomously. *Development* 133:1433-43 (2006).

Journal cover image reproduced with permission of the Company of Biologists.



Working out the means by which structurally complex, multi-component tissues and organs are formed is one of the central questions in developmental biology, and one of especial interest to the field of neurodevelopment, where complexity is taken to new levels in the organization of the vertebrate brain. That remarkable convolution is evident in the mammalian olfactory bulbs, two structures that jut rostrally from the forebrain to receive inputs from axons extending from neurons in the epithelium of the nose.

Tsutomu Hirata and colleagues in the Laboratory for Vertebrate Axis formation (Masahiko Hibi; Team Leader) reported direct genetic evidence that the development of the olfactory bulb is regulated extrinsically by signals from the sensory neurons of the olfactory epithelium. Their article, which appeared in the journal *Development*, explains how the gene *Fez* guides axons from the olfactory epithelium to innervate the bulbs during the embryonic development of the mouse.



Expression of OMP protein and *Gad67* transcripts, which mark axons of olfactory sensory neurons and interneurons, respectively, in wild-type, *Fez*-deficient (*Fez*^{-/-}), and *Fez*^{-/-} mice in which *Fez* expression was rescued under the control of the olfactory sensory neuron-specific promoter (*Fez*^{-/-}; Tg(*Fez*)). *Fez* is required in olfactory sensory neurons for axon targeting and layer formation of olfactory bulbs.

The team was first clued to a possible role for *Fez* by its pattern of expression, which shows that it (along with its relative, *Fez*-like) is expressed specifically in forebrain and olfactory sensory neurons. On testing for function by generating *Fez*-deficient mice, Hirata et al. found that while heterozygotes appeared normal, homozygous mutants died soon after birth. Strikingly, the *Fez*^{-/-} animals exhibited a range of defects in the formation of the olfactory bulb, including failure of olfactory sensory neurons to reach their destination in the bulbs and size reductions and disorganized layer formation of the olfactory bulbs themselves. These mice also showed aberrant rostral migration of the interneuron progenitors that normally emanate from the subventricular zone of the lateral ganglionic eminence in the telencephalon. The team next used plasmid-mediated expression of the gene to determine whether *Fez* in the olfactory sensory neurons controls the formation of the olfactory bulb non-cell-autonomously, and found that the transgene was sufficient to rescue the phenotype, in full or in part.

The means by which the sensory neuronal axons direct the migration of interneurons is particularly interesting, as the two do not come into physical contact during the earliest stages of bulb development. It remains to be seen whether the effect is dependent on a diffusible guidance signal capable of bridging the distance between sensory and inter-neurons, or secondary to a defective wiring of the olfactory sensory and projection neurons in the olfactory bulbs caused by loss of *Fez* function.



Masato NAKAZAWA, Masahiko HIBI

Next-generation PCR

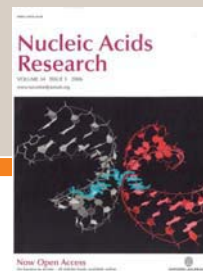
Establishment of a reliable single-cell microarray methodology

When scientists want to discern the properties and function of a given type of cell, its gene expression profile provides an invaluable source of information. Thankfully, complete genomic sequences are already available for many of the most commonly studied model organisms, making it possible to track expression level across the entire genome using microarrays. This technology, however, typically demands the availability of as much as several micrograms of RNA, which can only be obtained from a large number of cells, a requirement that problematizes its use in the analysis of smaller groups of, or individual, cells.

The demand for large quantities of faithfully amplified mRNAs from a single cell has now become fulfillable, thanks to a virtuoso set of refinements to the polymerase chain reaction (PCR) method developed by Mitinori Saitou (Team Leader, Laboratory for Mammalian Germ Cell Biology; Researcher, PRESTO program, Japan Science and Technology Agency), Kazuki Kurimoto, Yukihiro Yabuta (Laboratory for Mammalian Germ Cell Biology) and colleagues in the CDB. And, in a proof-of-principle demonstration published in the same article in *Nucleic Acids Research*, the team showed that the technique can be used to characterize individual cells from the inner cell mass of the blastocyst, revealing that this developmentally important population actually comprises two distinct groups of cells.

The high-density oligonucleotide microarray analyses performed in this research involve a number of preparatory steps, first of which is the high-fidelity amplification of cDNAs complementary to the full set of messenger RNAs transcribed by a cell. Of the extant methods for amplification, PCR, with its exponential yields, is the most suited to the tiny amounts of mRNA obtainable from single cells, but a previously reported single-cell PCR method tends to produce by-products as the result of the non-specific amplification of misused primer-derivatives, has been dogged by problems of bias and misrepresentation, and often fails to yield mRNAs of reproducible quality. Here, as with so many other amplification strategies, the question of how to boost signal without doing the same for noise remains fundamental.

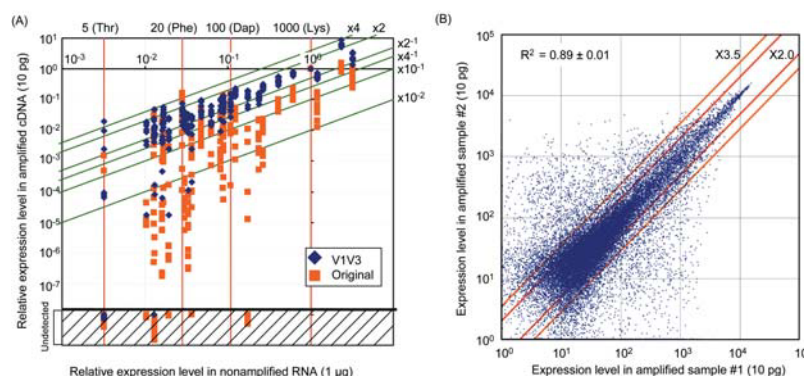




Kurimoto K, Yabuta Y, Ohinata Y, Ono Y, Uno K D, Yamada R G, Ueda H R and Saitou M. An improved single-cell cDNA amplification method for efficient high-density oligonucleotide microarray analysis. *Nucleic Acids Res* 34:e42 (2006).

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Kurimoto et al's achievement was to overcome the shortcomings of PCR without sacrificing its amplificatory power, by a wedding of attributes of both exponential and linear strategies. The team first obtained RNAs expressed in embryonic stem cells and diluted them to a single-cell level. They then made quantitative comparisons of the pre- and post-amplification levels of 23 genes. They found that by eliminating unreacted primers at an early stage, and by limiting the number of cycles of amplification in the first round to 20, they were able to reduce distortion and the generation of by-products dramatically. These analyses also identified a pair of primers, V1 and V3, that, when used in combination, produced much greater efficiencies over single-primer schemes by allowing the retention of directionality of cDNA amplification products. This two-primer strategy now makes it possible to linearly amplify the complementary RNAs used in microarray experiments from a single strand of DNA. These, and the team's many other refinements have resulted in a method of PCR that provides better representation, reproducibility and coverage than ever before, with single-cell accuracies of 97% true-positive detection of transcripts present as 20 or more copies per cell, and 93% for those expressed at levels of as low as 5 copies.



Performance of the new cDNA amplification method. (A) Representative and reproducible amplification of single-cell cDNAs by the new method (V1V3) compared to the original one. Results of 10 independent amplifications (10 pg) are plotted against the nonamplified control (1 μ g). (B) Scatter plots of microarray data obtained from two independently amplified samples from 10 pg ES cellular RNA. R^2 values were calculated for probes detected reproducibly in pairwise comparisons.

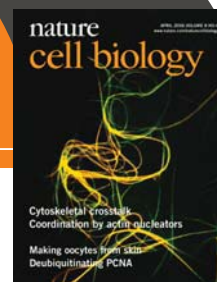
Eager to prove the utility of the new strategy, Kurimoto et al. performed microarray analyses of individual cells from the inner cell mass of day 3.5 mouse embryos. Even this first proof-of-concept experiment yielded new insights, when the authors found that the inner cell mass, which was thought to be homogeneous at this stage of development, actually comprises cells expressing genes characteristic of a pair of more differentiated populations, primitive endoderm (PE) and epiblast. This represents the first demonstration that the morphological segregation of PE and epiblast at day 4.5 may in fact have its underpinnings in changes in gene expression a full day earlier in embryogenesis.

By enabling the analysis of genome wide expression patterns at the single-cell level, the methodological advancements described in the Saitou report are poised to stand as a watershed in the study of a broad range of biological questions. Thanks to this technology, early developmental processes and the mechanisms of fate determination in somatic stem cell systems, phenomena that have long eluded explication, now await study at single-cell resolution.

Got your back

Muraoka O, Shimizu T, Yabe T, Nojima H, Bae Y K, Hashimoto H and Hibi M, Sizzled controls dorso-ventral polarity by repressing cleavage of the Chordin protein. *Nat Cell Biol* 8:329-38 (2006).

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Sizzled protects Chordin in dorsal-ventral patterning

During early development, the back of the embryo contends with the belly for territory. In this competition, the dorsal region defends itself by secreting antagonists to bone morphogenetic proteins, or Bmps, which act as ventralizing factors. The gradient that results as ventral Bmp signaling weakens in the face of cancellation by repressors from the dorsal region plays a central role in establishing the dorsal-ventral axis. One such BMP antagonist, Chordin, is itself subject to a form of counterattack, as it can be cleaved by Bmp1/Tolloid metalloproteinases, rendering it much less effective in neutralizing Bmp. Many of the ventral-side players in this claim-staking have been identified in zebrafish, but to date only a pair of genes whose loss

of function results in ventralization is known: the gene encoding Chordin, and a second, which codes for a zebrafish ortholog of Secreted Frizzled (Sizzled), but their modes of operation remain unknown.

In a study led by Osamu Muraoka of the Laboratory for Vertebrate Axis Formation (Masahiko Hibi; Team Leader), that question was resolved with the finding that Sizzled protects Chordin from cleavage by Bmp1a and Tolloid-like1 (Tll1). The team reported in *Nature Cell Biology* that Sizzled acts as kind of bodyguard to Chordin, enabling it to antagonize the ventralizing effects of other Bmps.

Muraoka et al. looked at the means by which Sizzled works in a Chordin-specific manner and found that higher levels of intact Chordin and lower levels of the cleaved protein in embryos co-expressing *sizzled*, suggesting that its product somehow stabilizes Chordin against cleavage by metalloproteinases. They next examined whether Sizzled interacts directly with either Tolloid-like1 or Bmp1a, both of which cleave Chordin *in vitro*, and found that it bound both, with a higher affinity for Bmp1a. Tests using truncated and chimerized versions of the Sizzled protein revealed that the critical domain is a cysteine-rich stretch that includes the same amino acid as is mutated in the *ogon* phenotype.

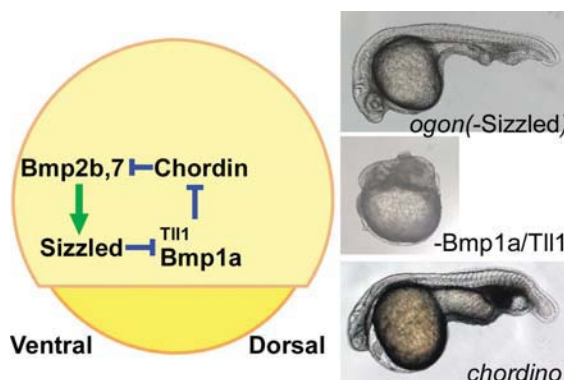
The picture of regulatory crosstalk that emerges is unexpectedly complex. Ventrally expressed *sizzled* keeps Bmp1a and Tolloid-like1 in check, freeing Chordin to antagonize other Bmps and dorsalize its side of the embryo, while at the same time generating the moderate Bmp activity needed for the development of ventral and lateral tissues.

The picture of regulatory crosstalk that emerges is unexpectedly complex. Ventrally expressed *sizzled* keeps Bmp1a and Tolloid-like1 in check, freeing Chordin to antagonize other Bmps and dorsalize its side of the embryo, while at the same time generating the moderate Bmp activity needed for the development of ventral and lateral tissues.



Osamu MURAOKA

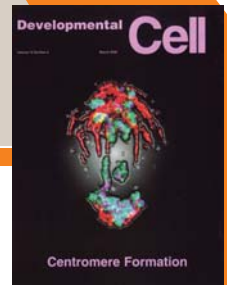
Schematic representation of the mechanism by which Sizzled inhibits Bmp signals. The phenotypes of embryos lacking Sizzled, Bmp1a/Tolloid-like1, or Chordin are shown on the right side



For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.

Off to one side

Goldstein B, Takeshita H, Mizumoto K and Sawa H. Wnt signals can function as positional cues in establishing cell polarity. *Dev Cell* 10:391-6 (2006).
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Determination of cell polarity by Wnt signaling

Cellular polarity is a criterion in many forms of asymmetric cell division, the process by which a cell divides to produce daughter cells of different types. Communication between cells often dictates where, when and how cells become polarized and subsequently divide, but the means by which these molecular communiqués actually get the work done has remained obscure. The question of whether the Wnt signaling pathway functions as an essential positional cue in establishing cell polarity has been a particular teaser.



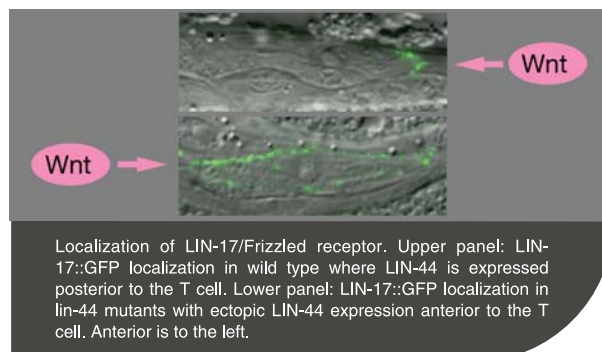
Hitoshi SAWA, Kota MIZUMOTO, Hisako TAKESHITA

In a study published in *Developmental Cell*, Hitoshi Sawa (Team Leader; Laboratory for Cell Fate Decision) and colleagues from Kobe University and at the University of North Carolina at Chapel Hill (USA) revealed that Wnt signals can determine the polarity of both embryonic and postembryonic cells in the nematode *C. elegans*.

The team looked first at the four-cell stage nematode development, in which messages from one signaling cell to its responding

neighbor (the EMS cell) result in the responder's polarization, thereby enabling it to divide asymmetrically. Two factors expressed in the signaling cell, MOM-2 and MES-1, were known to play roles both in the mitotic spindle orientation and polarization of the EMS cell itself, and to send one of its daughters down the path to an endodermal fate. They devised a series of tests in which a pair of altered signaling cells (one lacking MOM-2, the other lacking MES-1) were placed on either side of a responding cell allowing them to study the effects on endoderm development. When they placed the Wnt factor MOM-2 and the (non-Wnt) MES-1 cells in different positions relative to each other, they found that the orientation of the mitotic spindle was consistently oriented in line with the source of the Wnt signal.

Turning next to the adult, they ectopically expressed another Wnt factor, LIN-44, and examined the effects on asymmetric cell division of T cells in the worm's tail. In cells anterior to T cells, the authors observed strong enhancement of the polarity reversal phenotype in *lin-44* mutants. Once more, the position of the Wnt signal source was shown to play a critical role in determining cellular polarity. Sawa et al further showed that the Wnt signal determines the polarized localization of the Wnt receptor LIN-17/Frizzled in the T cell, indicating that its polarizing effect in these cells is direct.

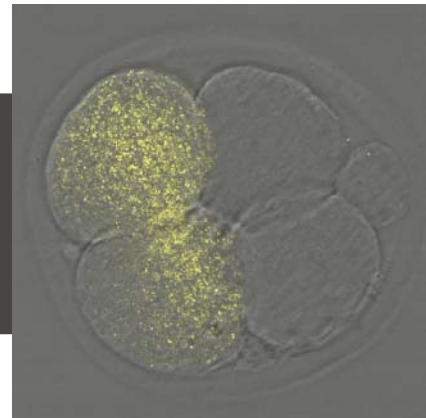


Localization of LIN-17/Frizzled receptor. Upper panel: LIN-17::GFP localization in wild type where LIN-44 is expressed posterior to the T cell. Lower panel: LIN-17::GFP localization in *lin-44* mutants with ectopic LIN-44 expression anterior to the T cell. Anterior is to the left.

Cytostatic factor after a century's search

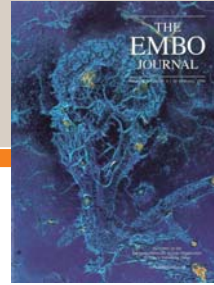
The signal for embryonic development to begin is given within seconds after a fertilizing sperm fuses with an oocyte, but what prevents the egg from jumping the gun and starting a solo developmental program before the sperm has arrived? This question is an old one: in 1911, Frank Lillie asserted that, "The nature of the inhibition that causes the need for fertilization is a most fundamental problem." In the early 1970s, Masui and Markert showed that the division of cells in embryonic frogs could be stopped by injecting cytoplasm from an unfertilized egg. They concluded that the activity responsible for this developmental arrest includes a "cytostatic factor" (CSF) that prevents oocytes from dividing prematurely, but the molecular identity of CSF has remained enigmatic ever since. Studies have suggested a plethora of CSF candidates, including Mos and Emi1, but results have been inconclusive and the search has continued.

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



In an article published in *The EMBO Journal*, Shisako Shoji and colleagues from the Laboratory of Mammalian Molecular Embryology (Tony Perry; Team Leader) reported that the conclusions of the classic Masui-Markert experiment of do not hold entirely true in mice, despite the fact that over the years its findings in amphibians had become firmly established *frogma*. Prompted by this incongruity, the team developed an alternative approach to the problem, one that utilized RNA interference (RNAi) to remove selected oocyte transcripts and proteins, allowing them to attack the problem systematically on a molecule-by-molecule basis. Surprisingly, depletion of the previous CSF candidates Emi1 or Mos had little or no effect. But when they knocked down the oocyte component, Emi2, they found that oocytes clearly progressed through the cell cycle without arresting at meiotic metaphase II (mII), the phase in which the mature oocyte normally remains prior to its activation at fertilization. When the group looked more closely at older, non-manipulated oocytes, they found that Mos almost entirely disappeared autonomously, even though the oocytes remained at mII; RNAi was not necessary.

The group found that Emi2 is necessary not only to establish mII arrest, but also to sustain it. Emi2 removal causes cell cycle progression with unexpected embryonic degeneration, suggesting that it links the meiotic cell cycle with cytokinesis (cell division, which in this case occurs asymmetrically to produce a single cell embryo plus a second polar body). Following this thread, Shoji et al. next showed that Emi2 works through Cdc20, an adaptor of the anaphase-promoting complex (APC). The APC is a multiprotein complex that works by blocking the activity of the cyclin-containing kinase (MPF), whose presence is ultimately responsible for maintaining the state of mII arrest prior to fertilization. It appears that Emi2 arrests the oocyte at mII by inhibiting the degradation of MPF, and that it achieves this by binding to Cdc20.



Shoji S, Yoshida N, Amanai M, Ohgishi M, Fukui T, Fujimoto S, Nakano Y, Kajikawa E and Perry A C. Mammalian Emi2 mediates cytoskeletal arrest and transduces the signal for meiotic exit via Cdc20. *Embo J* 25:834-45 (2006).

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Satoko FUJIMOTO, Manami AMANAI, Anthony C. F. PERRY, Naoko YOSHIDA, Shisako SHOJI

The team then looked at both parthenogenetically activated oocytes and those activated by fertilization and found that MPF was eliminated in a Cdc20-dependent manner. "This is probably the first formal demonstration that the signaling responsible for MPF ablation has molecular components common to both parthenogenesis and fertilization," Perry notes, "which is reassuring given the application of parthenogenetic activation in nuclear transfer and other current research."

If, as the findings of Shoji et al. indicate, Emi2 is indeed the long-sought CSF in mammals, a new question arises: How does fertilization cause the inactivation of Emi2? Answers to this question promise to shed light not only on mechanisms in fertilization but carcinogenesis as well, since tantalizing new evidence is beginning to suggest that the two processes are linked.



Vertebrate Body Plan

Shinichi AIZAWA Ph. D.

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

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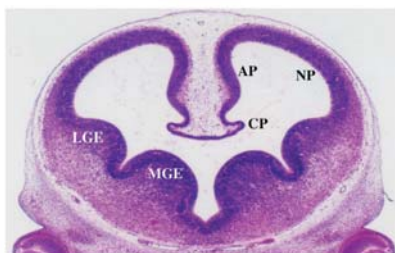
Kurokawa, D., Takasaki, N., Kiyonari, H., Nakayama, R., Kimura, C., Matsuo, I., and Aizawa, S. Regulation of *Otx2* expression and its functions in mouse epiblast and anterior neuroectoderm. *Development*, 131, 3307-3317 (2004).

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

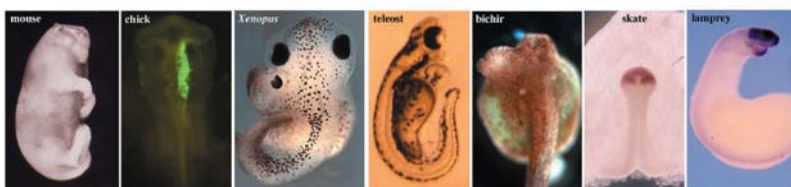
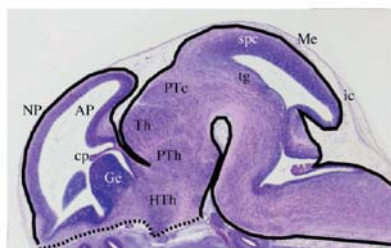
The head is the most highly layered of the body's structures and its development begins with anterior-posterior axis formation. This A-P axis patterning is one of the most primary phenomena in animal development, and the emergence of the vertebrate lineage has been accompanied by

widespread adaptations to this fundamental rule, as evidenced by the diversity of mechanisms by which vertebrate taxa achieve the common ends of axis formation and jaw induction.

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



Regions of the vertebrate brain



Vertebrate embryos used in our research

Stem Cell Translational Research

Takayuki ASAHARA M. D., Ph. D.

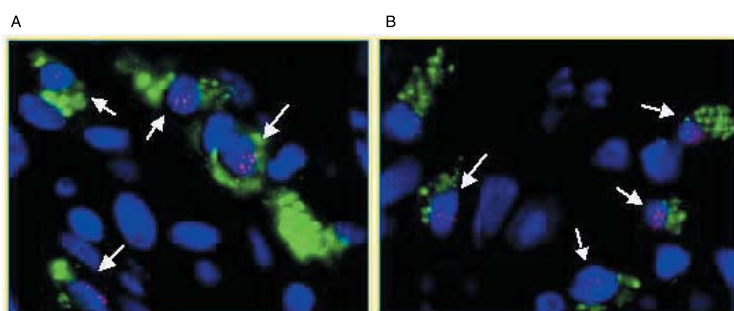
Takayuki Asahara received his M. D. from Tokyo Medical College in 1984, and performed residencies in cardiology and emergency medicine. He worked as a research fellow in cardiology at the Tokyo Medical College Hospital from 1989 to 1993 before moving to a fellowship in cardiovascular research at St.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



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

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

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



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

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Publications

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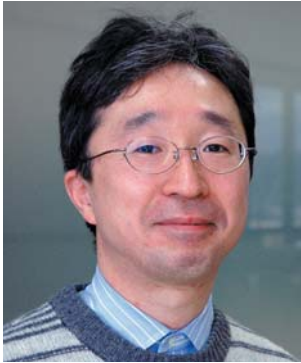
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Neuronal Differentiation and Regeneration

Hideki ENOMOTO M. D., Ph. D.

Hideki Enomoto received his M. D. from the Chiba University School of Medicine in 1988, and his Ph. D. from the same institution in 1996 for his work in the molecular cloning of the human *DAN* gene. After serving as the Chief of the Department of Pediatric Surgery, Matsudo Municipal Hospital in Chiba, Japan, he moved to the Washington University School of Medicine, St. Louis in 1997, to work as a research associate studying the physiological roles of the GDNF family of ligands in neural development and function. He returned to Japan to take a position as a team leader at the CDB in 2002.

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Vohra B PS et al. Differential gene expression and function analysis implicates novel mechanisms in enteric nervous system precursor migration and neurogenesis. *Dev Biol* 298: 259-71 (2006).

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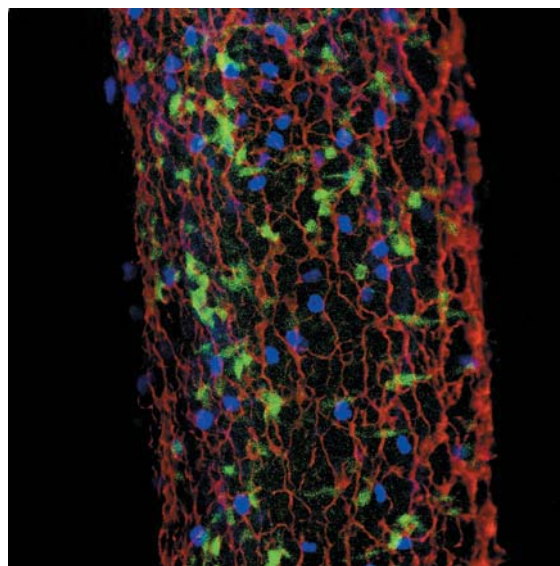
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The architecture of the neural system, which underlies much of the dynamism of animal life, is frequently described as complex, but closer examination reveals great beauty as well. The construction of this exquisite and elaborate structure involves the birth, growth and death of countless individual cells and is regulated by intercellular communication and cooperative activity.

Our laboratory is interested in questions fundamental to the development of the nervous system: determining the specific extracellular signals and cell autonomous routines that participate in the processes of neural development, and resolving the means by which diverse families of signaling molecules coordinate and interact at the molecular and cellular levels. To address these questions, we focus on a class of signaling molecules known as neurotrophic factors, most particularly, the GDNF Family Ligands (the GFLs). This family of neurotrophic factors includes four known mem-

bers: GDNF (for Glial cell line Derived Neurotrophic Factor), Neurturin, Artemin and Persephin. The GFLs signal via receptor complexes formed by the RET receptor tyrosine kinase and one of four co-receptors, GRF α 1-4. In vitro, these four receptors show differential affinities for specific GFLs. GFL signaling has been shown to affect neuronal growth and migration as well as axon guidance, and defects in this signaling system have been implicated in a number of congenital health problems.

Our efforts will focus on the analysis of the physiological roles of neurotrophic factors, which we hope will lead to an improved understanding of the molecular bases of neural development, differentiation and pathology, and ultimately to potential clinical applications in the treatment of nervous system trauma and disease.



■ Developing gut of conditional GFR α 1-knockout embryos stained with anti-class III β tubulin (red) and anti-phospho-histone H3 (blue) antibodies. In this preparation, cells in which GFR α 1 is conditionally inactivated are identifiable as GFP-positive cells (green).

Neural Network Development

Chihiro HAMA Ph. D.

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

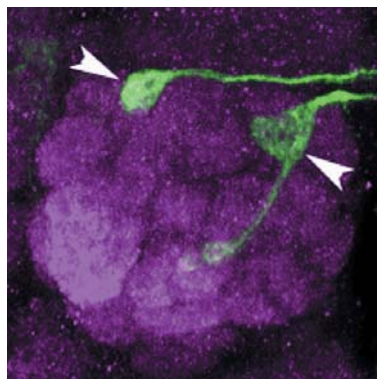


One of the most tantalizing questions in the field of neurobiology is how neural circuits of exquisite complexity are generated during nervous system development. Human brains consist of 10^{10} neurons, and each neuron projects an axon that extends along a predetermined pathway before finally finding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

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

To study the questions of how this variety of ORNs is generated and how ORN axons are specifically targeted to the correct glomerular positions, we have isolated a number of mutations that impair the projection of ORN axons into glomeruli. Through the analysis of one of the mutations, we have demonstrated that Notch signaling is involved in the diversification of ORNs. Systematic clonal analysis reveals that a cluster of ORNs housed in each sensillum are differentiated into two classes, depending on the level of Notch activity in their sibling precursors. Notably, ORNs in different classes segregate their axonal projections into distinct domains in the antennal lobes. In addition, both OR expression and axonal targeting of ORNs are specified according to their Notch-mediated identities. Thus, Notch signaling contributes to the diversification of ORNs, regulating multiple developmental events that establish the olfactory map in *Drosophila*.

We will continue to analyze other mutations, seeking to understand more completely the molecular mechanisms underlying olfactory development including specific axonal targeting and stereotyped glomerular organization. An appreciation of basic mechanisms uncovered in the *Drosophila* brain may also help us to explain how the elaborate wiring of the human brain is established during development.



■ Subsets of olfactory receptor neurons specifically project their axons into two out of 50 glomeruli in the brain.

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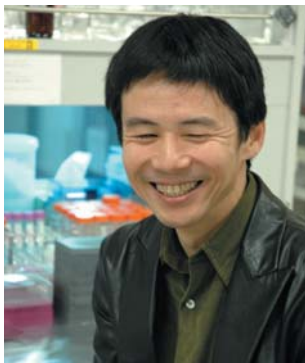
Publications

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Morphogenetic Signaling

Shigeo HAYASHI Ph. D.

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

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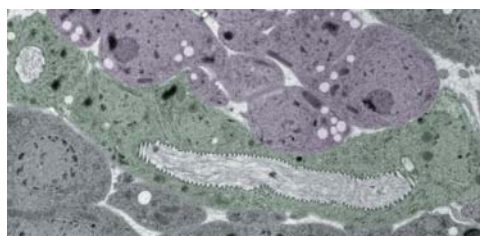
The main research interest in my lab focuses on the mechanisms by which cell-cell and tissue-to-tissue interactions are modulated during embryonic morphogenesis. Our strategy is to identify intercellular signaling systems and intracellular transducers that control cell-cell and tissue-tissue interactions. Our recent work includes identification of Dpp signaling as a key modulator of integrin-mediated cell adhesion between embryonic and extraembryonic epidermis (Wada et al., 2007), the role of the tissue specific expression of a laminin isoform in mediating interaction between wing imaginal disc and respiratory system (trachea, Inoue and Hayashi 2007), and the identification of IKK epsilon as a regulator of actin-dependent cell morphogenesis (Oshima et al, 2006).

A range of vital developmental processes, including the ability to build complex structures through selective cell adhesion, to move through the body, and to form epithelial tissues, rely on interactions between cells, and between cells and the extracellular matrix. The means by which cells are able to communicate, congregate and work together to build a body is a central question in the study of morphogenesis, the focus of research in our laboratory.

The *Drosophila* tracheal system is a respiratory organ that delivers air directly to tissues through a network of finely branched epithelial tubes. Tracheal development begins by invagination of ectodermal epithelium that subsequently forms branches with specific types of cells migrating in stereotyped patterns. The branching patterns and cell fate are instructed by external cues, including

FGF, WG and Dpp. We are studying the roles of these signaling molecules in the specification and migration of tracheal branches, as well as the mechanisms that coordinate cell movement and cell adhesion. We additionally use 4D confocal imaging of GFP-labeled embryos to study the dynamism of cell and organelle movement in living organisms. Using combinations of GFP markers and transcriptional enhancers of cell-specific expression, we have been able to capture movements of tracheal cells at resolutions sufficient to image cytoskeletal organization and cell adhesion structures in single cells.

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

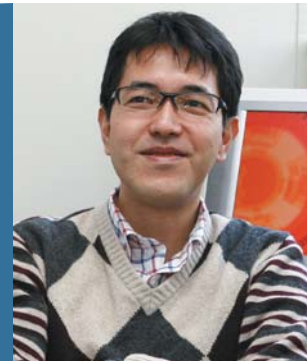


■ Transmission electron micrograph of *Drosophila* embryo at stage 16. The wing imaginal disc (magenta) tightly attaches to tracheal branches (green). This tissue association requires integrin/laminin-mediated cell adhesion and is essential for posterior directed movement of the wing disc.

Vertebrate Axis Formation

Masahiko HIBI M. D., Ph. D.

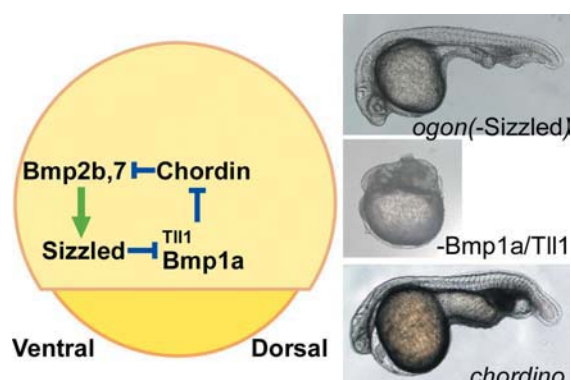
Masahiko Hibi received his M. D. from Hiroshima University School of Medicine in 1988, and his Ph. D. from the Osaka University Institute for Molecular and Cellular Biology in 1992. From 1992 to 1995, he worked as a postdoctoral fellow in Michael Karin's lab in the University of California, San Diego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



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

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

Neural patterning and neurogenesis as a model of cell fate determination (a process that is linked to axis formation) is also a question of interest to our team. Neuronal tissues are generated in a step-wise manner in vertebrates; these steps include neural induction, AP patterning and neurogenesis. In amphibian and teleost embryos, the neuroectoderm is initially induced by BMP inhibitors, which are generated by the dorsal organizer. The induced neuroectoderm is initially anterior in character and is subsequently subjected to posteriorization and regionalization. Accordingly, the central nervous system becomes highly ordered along the AP axis—regionalized into forebrain, midbrain, hindbrain and spinal cord compartments in a head-to-tail direction. We have found that two groups of genes play important roles in the AP patterning of neural tissue. The zinc-finger genes *Fez* and *Fez1* are expressed in the anterior forebrain and control the AP patterning of forebrain by repressing the caudal forebrain fate, while the *caudal*-related genes *cdx1a* and *cdx4* are expressed in the posterior neural tissue and control the formation of posterior spinal cord by repressing anterior fate. We are also working to investigate the mechanisms that establish the complex structure of the cerebellum.



■ Sizzled protects Chordin, a Bmp antagonist, by inhibiting the Toll-like family metalloproteinases Bmp1a/Toll-like1 (Tll1), which cleave and inactivate Chordin (left panel). The phenotypes of embryos lacking Sizzled, Bmp1a/Toll-like1, or Chordin are shown on the right side.

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Publications

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Cell Lineage Modulation

Toru KONDO Ph. D.

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

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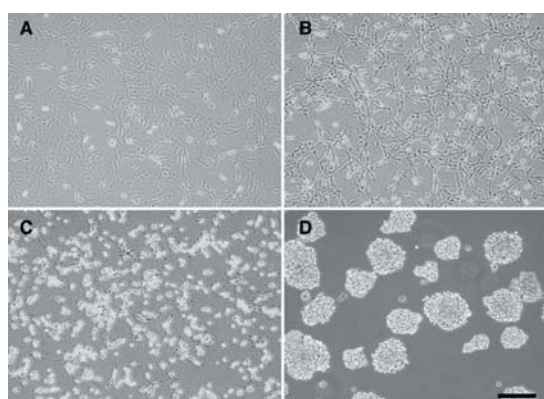
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Mankind has long wondered whether it would be possible to regenerate body tissues and structures that have been lost. Recent research has shown that even the adult body contains a diverse range of tissue-specific somatic stem cells that serve to maintain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

Research in our lab seeks to develop a better understanding of the molecular mechanisms involved in the reacquisition of "stemness" (stem cell characteristics) using the dedifferentiation of oligodendrocyte progenitor cells (OPCs), which are abundantly present in the central nervous sys-

tem, into neural stem-like cells, knowledge which it is hoped may lead to new techniques for the generation of neural stem cells. We are also interested in studying the characteristics of stem-like cells found in malignant tumors, with an eye toward the potential development of novel anti-cancer therapies.



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

Evolutionary Morphology

Shigeru KURATANI Ph. D.

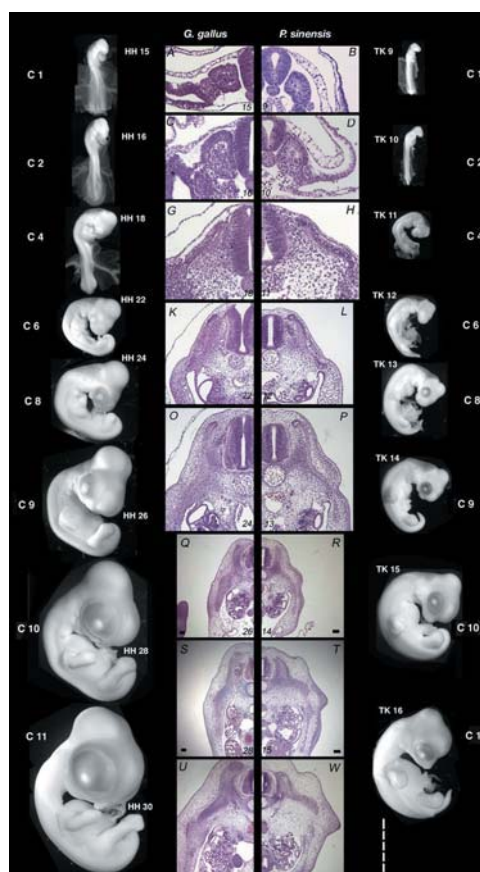
Shigeru Kuratani received his masters and Ph. D. from the Kyoto University Department of Zoology. He spent the period from 1988 to 1991 working in experimental embryology in the Department of Anatomy at the Medical College of Georgia before moving to the Biochemistry Department at the Baylor College of Medicine, where he was engaged in molecular embryological research. He returned to Japan in 1994 to take a position as associate professor at the Institute of Medical Embryology and Genetics in the Kumamoto University School of Medicine. He moved to Okayama University to assume a professorship in the Department of Biology in 1997, where he remained until he was appointed team leader at the CDB. He was appointed group director in 2005.



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

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

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



■ Embryonic developmental stages of chicken (left) and turtle (*Pseudemys*; right)

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Sensory Development

Raj LADHER Ph. D.

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

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Manley G. A. and Ladher R. K. Phylogeny and Evolution of Ciliated mechanoreceptors Springer *Handbook of the Senses* (Elsevier) ed. P. Dallos (2006)

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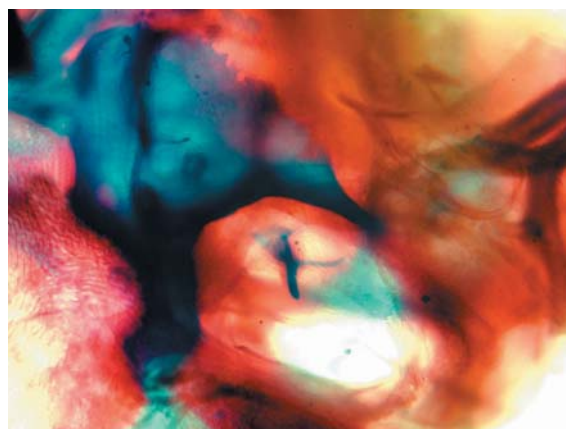
Organogenesis is a monumental undertaking. From situating a given organ at its correct location, to confirming that it is correctly engineered and constructed, to ensuring the necessary functional connections with other organs, the embryo has a daunting task before it; indeed, perhaps we should be surprised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

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

tion of the otic placode, and in better understanding the cellular, molecular and embryological process responsible for this elegantly engineered organ of hearing.

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

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



■ Alizarin Red (bone) and Alcian Blue (cartilage) staining of the 14 day old chick head. In contrast to the three bones found in mammals, the chicken middle ear consists of only one bone, the columella.

Cell Asymmetry

Fumio MATSUZAKI Ph. D.

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

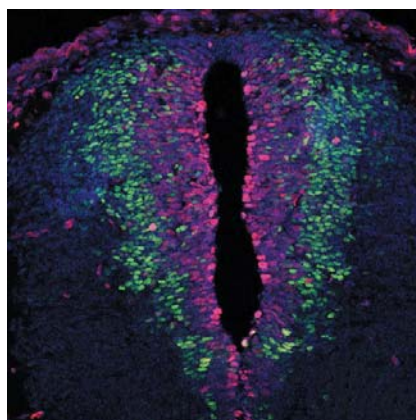


Our group seeks to explore the molecular mechanisms underlying the organization of cells into highly ordered structures in the developing brain, where neural stem cells generate a large number of neurons of different fates at appropriate points in time. Asymmetric cell division is thought to play an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

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

number of fundamental aspects of the asymmetric division of neuroblasts, such as the mechanisms responsible for asymmetrically sorting cellular components to the cortex, maintaining the neuroblast's cell polarity, and producing the smaller daughter GMC. We are addressing these issues using genetic screens for mutations affecting the asymmetric division of neuroblasts.

The vertebrate brain comprises a considerably larger number of neurons arranged in a vastly more complex functional network than that in *Drosophila*. In both vertebrate and fly, however, huge numbers of neural cells are generated from a relatively small number of neural stem cells. Previous work has shown that neural progenitor cells divide both asymmetrically and symmetrically to produce descendant neurons. Vertebrate homologs have been found for most of the components acting in the asymmetric division of *Drosophila* neuroblasts, but the modes and roles of asymmetric divisions in vertebrate neurogenesis remain incompletely understood. Furthermore, little is yet known about how asymmetric division contributes to neuronal fate determination. We are investigating the problems of how the property and division modes of neural stem cells are involved in neuronal fate decisions and in organizing the cellular architecture of the vertebrate brain.



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

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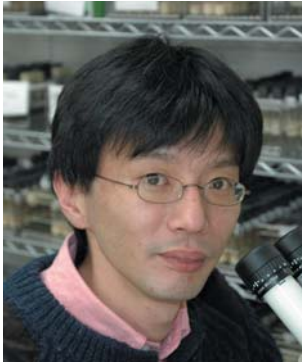
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Germline Development

Akira NAKAMURA Ph. D.

Akira Nakamura received both his baccalaureate and his Ph. D. from the University of Tsukuba. He spent a year as a post-doctoral fellow at the same institution before moving to the Department of Biology at McGill University in Montreal in 1995 to work as a post-doc under Paul Lasko. He returned to Japan in 1997 as a research associate at the University of Tsukuba. He was appointed assistant professor in the university's Gene Research Center and Institute of Biological Sciences in 2000, and began a three-year term as a PRESTO researcher in the Japan Science and Technology Corporation (JST) in December 2001. He was appointed CDB team leader in March 2002.

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Publications

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

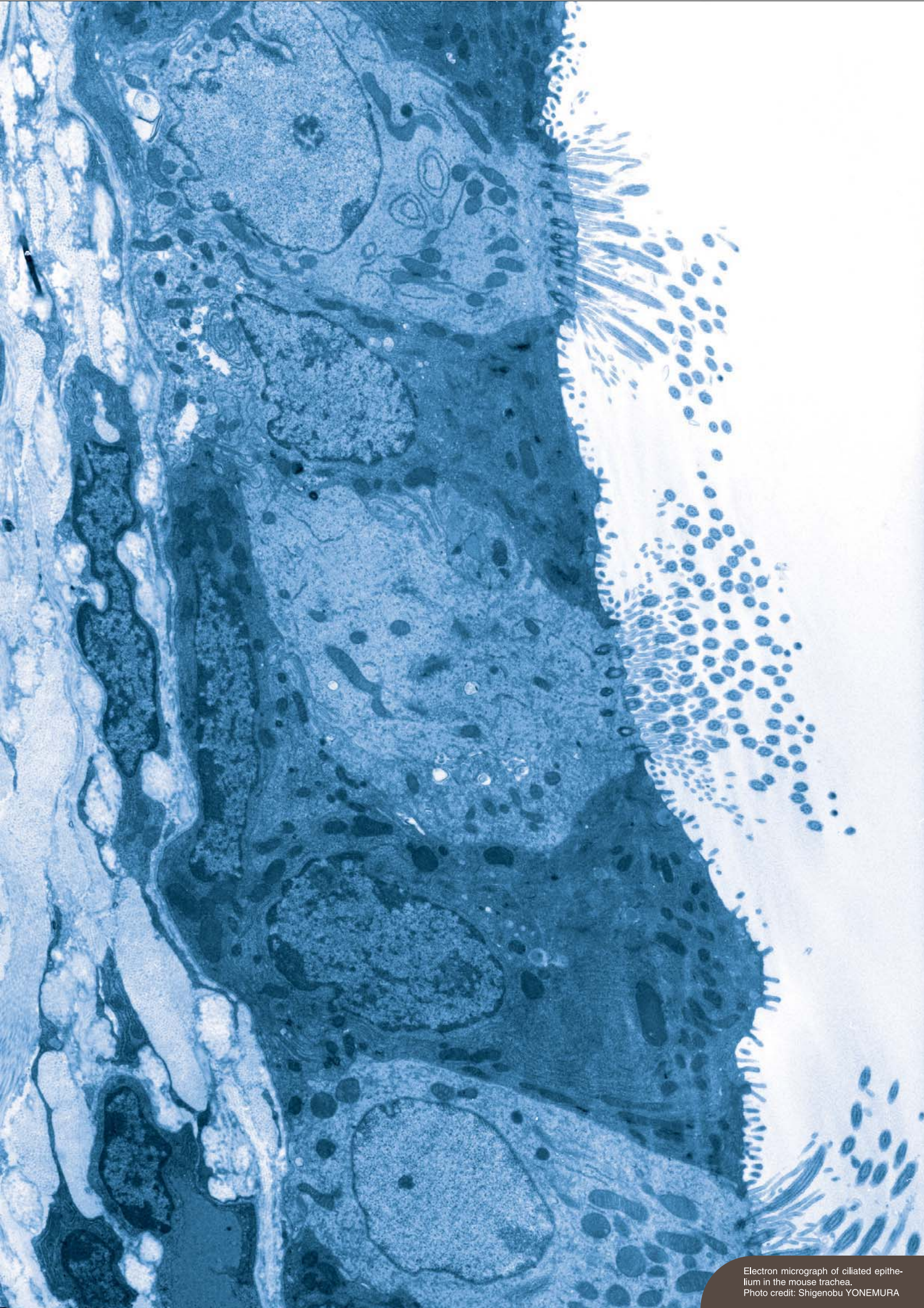
One area of particular interest is the mechanism of translational repression in germline development. In one example of this critically important form of regulation, the translation of the RNA for the mater-

nal gene *oskar*, which functions in embryonic patterning and the formation of germline cells in *Drosophila*, is repressed during its transport to the posterior pole of the oocyte. We are now studying the function of several proteins, such the recently identified factor, Cup, which regulates the repression of *oskar* translation during its localization to the oocyte posterior. In another parallel project, we are focusing on the roles of Wunen2 and *polar granule component (pgc)*, which are known to function in lipid signaling and global transcriptional regulation in germline cells during embryogenesis, respectively.

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



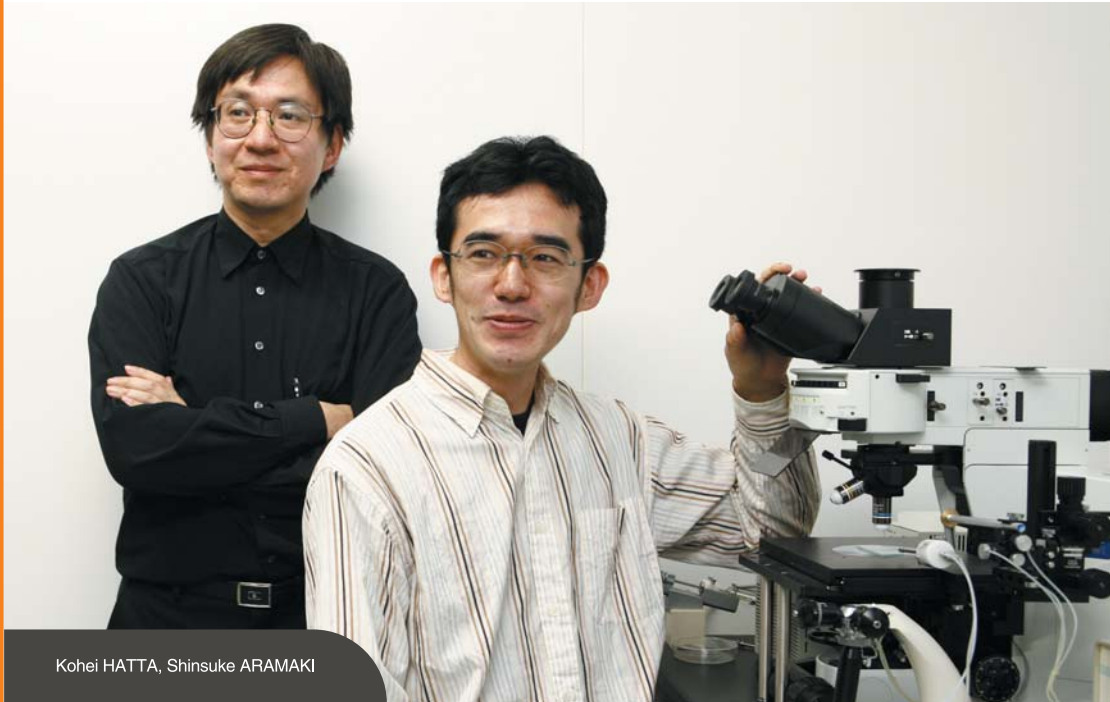
■ Migrating germ cells (blue) in stage 10 *Drosophila* embryo



Electron micrograph of ciliated epithelium in the mouse trachea.
Photo credit: Shigenobu YONEMURA

Protein plays peek-a-boo

Dronpa GFP sheds new light on neural networks



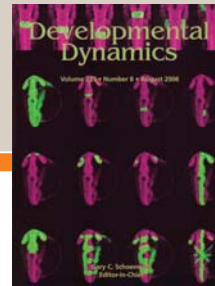
Kohei HATTA, Shinsuke ARAMAKI

Green fluorescent protein (GFP) and its multicolored fluorescent relatives are among the most commonly used tools used in biological visualization. Recent interest, however, has focused on developing alternatives to GFP in which fluorescence output can be adjusted, or converted, as an aid to more localized or precise studies of protein movement within a cell. Such technology is of particular value for the discrimination individual neurons in maze-like neural networks.

In a study by Shinsuke Aramaki and Kohei Hatta of the Laboratory for Vertebrate Body Plan (Shinichi Aizawa; Group Director) the use of such visualization was elevated to a new plane through the use of Dronpa, a GFP with a unique and striking photosensitivity. On excitation by wavelengths of light of about 490 nm, the Dronpa protein undergoes photobleaching and ceases to glow; but, remarkably, its powerful fluorescence is restored following irradiation at around 400 nm. In this study, published in *Developmental Dynamics*, Aramaki and Hatta took advantage of this novel set of photochemical properties for the first time in visualizing cellular-level structures in living zebrafish embryos.

Aramaki and Hatta initially showed that Dronpa can be expressed in zebrafish embryos by injecting them with Dronpa mRNA, which produced green fluorescence 5 hours post-fertilization. This fluorescence was then bleached away using a strong laser. The fluorescence was then reactivated using an UV or violet laser. The robust nature of this technique, with the brightness of the activated Dronpa not exhibiting any significant decay, allowed for the sequential labeling of structures such as rhombomeres. A further advantage of using Dronpa under these conditions was the ability to imprint a variety of patterns on embryos expressing the fluorescent protein, which were captured in a beautiful series of images under confocal microscopy.

After establishing the feasibility of using Dronpa in zebrafish embryos, the authors, through a series of novel techniques, were also able to analyze the anatomy of neural networks. Perhaps most importantly, they succeeded in visualizing a number of overlapping individual neurons that had previously proved



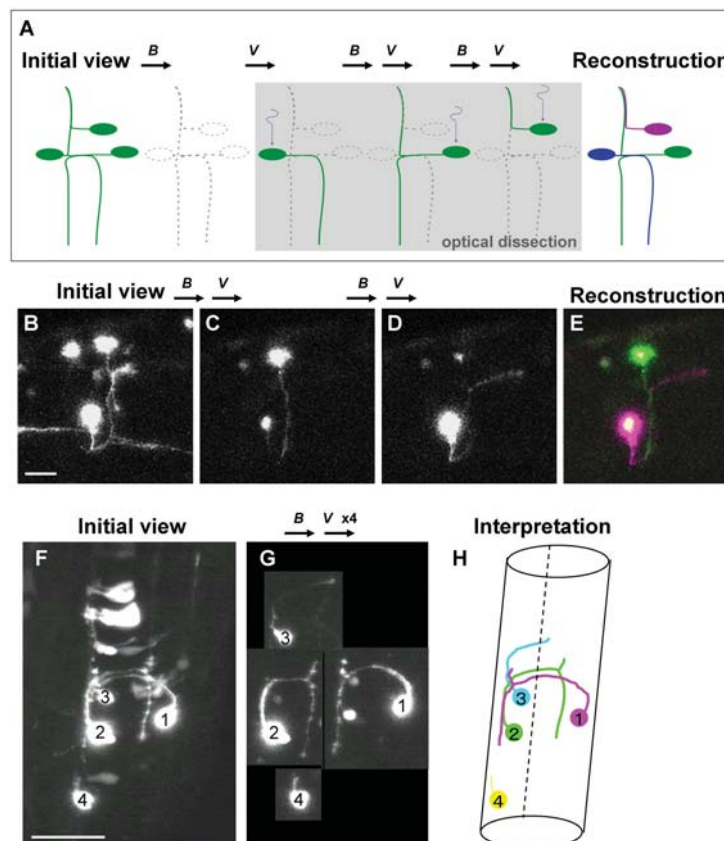
Aramaki S and Hatta K. Visualizing neurons one-by-one in vivo: optical dissection and reconstruction of neural networks with reversible fluorescent proteins. *Dev Dyn* 235:2192-9 (2006).

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difficult to view in isolation. Focusing on neurons found in complex wiring patterns, they dissected neural networks to the single cell level following injection of a UAS-Dronpa construct. In these transgenic fish, irradiation followed by a reactivation of the green fluorescence highlighted the entire morphology of each neuron as the protein diffused into the neurites. A subsequent merger of these isolated neuronal images enabled the reconstruction of neural networks in which single neurons were effectively "color-coded."

Further application of the technique included an attempt to locate the position of neuronal somata from visible axons by retrograde labeling, in which Dronpa was first eliminated from the area of interest by irradiation, followed by a further irradiation of the axon of interest. On attempting to obtain multiple bright images, however, Aramaki and Hatta found that the relatively high intensity required to eliminate Dronpa at 488 nm often resulted in the attenuation of signal. In an attempt to overcome this, they decided to irradiate neurons of interest each time, or after every few times, to illuminate then to a sufficient brightness when scanning for observation. This ultimately resulted in images with considerably stronger fluorescence. The authors also used two-photon confocal microscopy, which made it possible for Dronpa to be activated by a longer wavelength of laser light to illuminate cells buried deep in tissue while avoiding activating neighboring cells in the light path.

Hatta's interest in Dronpa was first piqued when a fellow CDB researcher suggested it to him following a presentation he gave in 2004. "It wasn't easy to adjust the conditions to allow us to switch the fluorescence on and off in vivo", he admits, "so I was very pleasantly surprised to see that it finally worked so well in zebrafish embryos." The success of Aramaki and Hatta's use of Dronpa has large-scale and high-throughput potential, as the repetitive cycle of photobleaching, reactivation and visualization lends itself to automation. The non-invasive highlighting of specific structures and organs by Dronpa will also assist in their dissection or sorting for use in transplantation or gene-expression experiments.



Visualization of individual neurons: optical dissection and reconstruction of neural networks using Dronpa.

Asymmetry assured

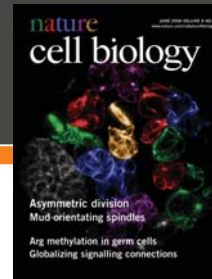
Mud orients mitotic spindle with cell polarity during neuroblast division

Imagine a globe on which snow only fell near the North Pole, with none around the South. If you cut this sphere in two along the equator, one of the hemispheres would get all the snow, while the other would be left with none. But if you sectioned it longitudinally, each would receive a half share. A similar decision is faced by polarized cells when they divide. The axis of cell division determines whether factors distributed unequally within the parent cell are apportioned to both or only one of the cell's immediate progeny. This dichotomy of symmetric and asymmetric cell division is critical to many developmental processes, such as the construction of the brain, for the set of proteins inherited by a cell via cytokinesis can be nearly as important in determining the cell's properties as the genetic information transferred during mitosis.

In a study published in *Nature Cell Biology*, Yasushi Izumi and colleagues working in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) reported that the Mud (mushroom body defect) protein coordinates the orientation of the mitotic spindle with cell polarity during asymmetric division of neuroblasts in the fruitfly, *Drosophila*. "This is the first time anyone has been able to show a general mechanism behind the coupling of cell polarity and axis of division," notes Matsuzaki, "which is exciting, as it may help us develop new insights into the means by which cells switch between proliferative and differentiative modes of division." These findings, which were made with support from the Japan Science and Technology Corporation CREST program and in collaboration with the Institute for Medical Radiation and Cell Research at the University of Wuerzburg (Germany), are of particular interest due to the highly evolutionarily conserved nature of the Mud protein, which has homologs in both *C. elegans* and vertebrates, all of which appear to be involved in regulating spindle orientation vis-à-vis the cell cortex.

Previous work had shown that a complex of cortical proteins involving a pair of cortical factors named Gai and Pins work in the regulation of spindle orientation in *Drosophila* neuroblasts, but the underlying mechanisms remained unclear. Using immunoprecipitation to identify molecules that in-



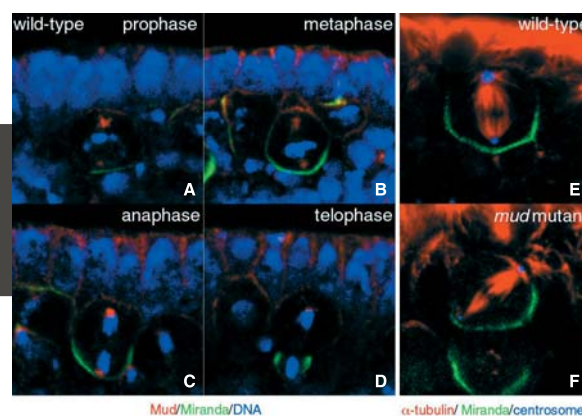


Izumi Y, Ohta N, Hisata K, Raabe T and Matsuzaki F. *Drosophila* Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. *Nat Cell Biol* 8:586-93 (2006).

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teract with the Pins-G α i complex, Izumi et al. found that Mud bound the complex in vitro. Looking at Mud's intracellular distribution, they found that it localized to the apical side of neuroblasts throughout the cell cycle as well as their centrosomes (the central hub from which spindle microtubules radiate) during the mitotic phase, in contrast to its binding partner Pins, which showed up only at the apical cortex. Similarly, in symmetrically dividing epithelial cells, both Mud and Pins were found at the lateral cortex, while Mud alone appeared at the centrosomes.

Mud localizes at apical cortex and centrosomal region in *Drosophila* neuroblasts (A–D). In *mud* mutant neuroblasts (F), the mitotic spindle fails to couple with the cortical polarity.



In both *pins* and *G α i* loss-of-function mutants, Mud failed to be recruited to the apical cortex of mitotic neuroblasts, but interestingly, during interphase when the cells are not dividing, Mud localization was unaffected in *pins* mutant cells. Contrastingly, centrosomal Mud accumulation was found to rely on microtubules, rather than the apical complex, suggesting that Mud distribution is governed by a pair of independent systems: recruitment to the apical cortex by the Pins-G α i complex, and to the centrosome via microtubules. Importantly, the Mud-Pins interaction works independent of the mechanism determining the localization of cell fate determinants, such as Prospero and Miranda, that function to ensure the asymmetry of neuroblast division; epithelial cells, which divide symmetrically and perpendicular to the apical-basal axis, also rely on this association to orient their division axes toward cortically localized Pins.

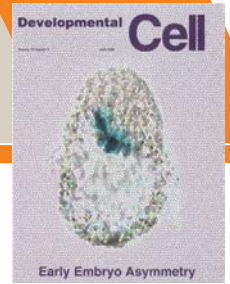
The question then was, how does Mud function in aligning the mitotic spindle along the apical-basal axis and thus enable the asymmetric parceling of cell fate determinants so crucial to fly neurodifferentiation? Mud mutant neuroblasts retain their cortical polarity, but the orientation of their mitotic spindles is severely compromised. This failure of "spindle coupling" is similar to a defect noted in Pins mutants, but with two significant differences: first, in Pins mutants, both the cortical factors and the spindle misorient, while only the spindle is askew in Mud-deficient flies; and second, spindle coupling recovers during telophase in *pins*, but not *mud*, mutants. These differences in phenotype point to an incomplete overlap of function between Mud and the Pins-G α i complex, resulting in distinct and independent roles at different mitotic stages.

In addition to their unequal inheritance of cell fate determinants, size matters in asymmetric daughters as well. Watching *mud* mutant neuroblasts divide, Izumi and colleagues observed that the angle of spindle orientation appeared to be linked to daughter cell size asymmetry, with highly skewed spindles tending to give rise to daughter cells closer to each other in size (which is abnormal in neuroblast division). In the most extreme cases, two daughter cells of equal size were produced. Additional centrosomes also frequently formed in these mutants, indicating the Mud may work in the establishment and/or maintenance of these structures. The exact manner in which Mud interacts with the cytoskeleton during mitosis and its function in centrosome organization await further study; for now, this work by the Matsuzaki group sheds new light on the extraordinary precision and coordination of processes that tell cells how and where to cleave apart.

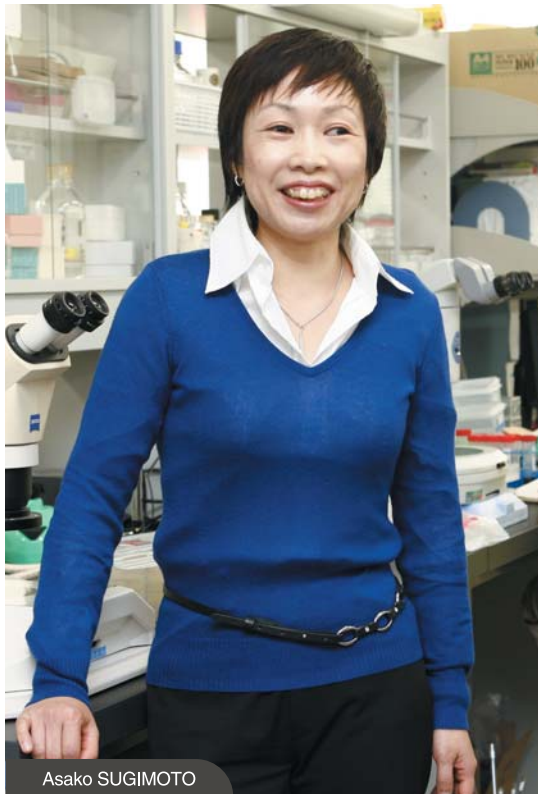
Astral tweaks

Motegi F, Velarde N V, Piano F and Sugimoto A. Two phases of astral microtubule activity during cytokinesis in *C. elegans* embryos. *Dev Cell* 10:509-20 (2006).

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Refining the picture of microtubule activity in cytokinesis



Asako SUGIMOTO

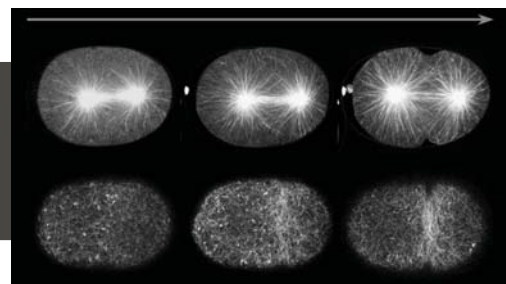
Cytoskeletal components known as microtubules are central to mitotic division, in which they provide the guidance and support backbone used to segregate replicated chromosomes. Spindle microtubules have also been implicated in fixing the position of the cleavage furrow, which splits the cell along an axis set up by the actin-based contractile ring during cytokinesis, and the question of how microtubules might determine the site of the nascent contractile ring has remained an open one for some years.

In an article published in *Developmental Cell*, Fumio Motegi and colleagues in the CDB Laboratory for Developmental Genomics (Asako Sugimoto; Team Leader) and New York University (USA) reported findings that point to a new model for astral microtubule activity during cytokinesis. Working with the nematode *C. elegans*, Motegi found that astral microtubules, which localize on opposing sides of the mitotic nucleus and help draw the chromosomes away from each other, in fact exhibit two separately mediated modes of action—one that localizes the contractile ring to the cell's equator, and a second that suppresses furrowing at all other regions of the cortex.

Early in the study, the team concentrated on the activity of astral microtubules at the cell cortex and, using GFP labeling to visualize the cells, they observed that these microtubules tended to behave differently at different stages of mitosis. They also found that microtubules were most densely concentrated in the part of the cell where the contractile ring arises, and that the early anaphase concentration of microtubules at the spindle equator appeared to promote the accumulation of RHO-1 (necessary for contractile ring formation). A focused gene screen and subsequent RNAi interference revealed that in the absence of γ -tubulin the cleavage furrow frequently failed to form, whereas the knockdown of *air-1* resulted in the contractile ring forming correctly at the central axis.

"Our analysis of these findings led us to a new model for astral microtubule activity during cytokinesis, in which early anaphase activity by γ -tubulin causes the contractile ring to assemble where it should, in the center of the dividing cell," says Sugimoto, "followed by later activity mediated by AIR-1, which prevents additional furrows from forming elsewhere by stabilizing the polar cortex."

Initiation of cytokinesis in the one-cell embryo of *C. elegans*. A mitotic spindle visualized by GFP-tubulin, is shown at top and filamentous actin visualized by GFP-moesin at bottom. A contractile ring forms at the spindle equator where the density of astral microtubules is high.

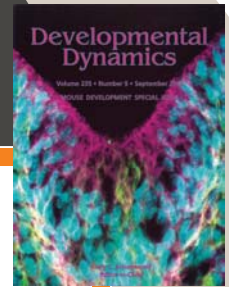


For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.

Binding to build the brain

Uemura M and Takeichi M. α N-catenin deficiency causes defects in axon migration and nuclear organization in restricted regions of the mouse brain. *Dev Dyn* 235:2559-66 (2006).

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Multicellular bodies such as ours are held together by strong bonds between cells, a function that is mediated by a variety of adhesion molecules, including members of the cadherin superfamily. The classic cadherins are transmembrane proteins, anchored in the cell membrane and protruding into the extracellular space to link with other cadherins tethered to neighboring cells. On the cytoplasmic side of the membrane, the cadherin tail serves as binding partner to a complex of catenin molecules. The catenin family itself is diverse; for example, the α -catenins comprise three subtypes, α N-, α E- and α T-catenins, which facilitate the process of cell-cell adhesion by binding to cadherin. α E-catenin is widely expressed in many different types of cells, and its loss of function proves lethal to developing embryos. α N-catenin, in contrast, is expressed primarily in cells of the nervous system.

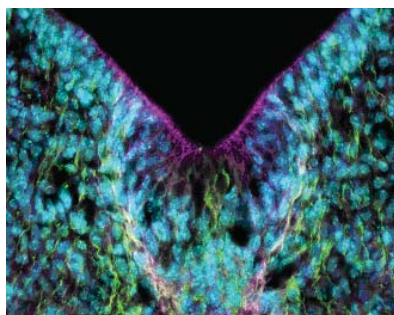


Masato UEMURA

In a study of catenin function in neurodevelopment, Masato Uemura of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) described the effects of loss of α N-catenin function on the embryonic nervous system of the mouse. The report, published in *Developmental Dynamics*, showed that in the brains of α N-catenin-deficient mice certain neuronal populations were missing, ventricular structures malformed, and axons of the anterior commissure failed to cross the brain midline, pointing to a diverse range of

functions for this molecule in neurodevelopment. Slices of neonatal brain obtained from these mutants showed no global defects on initial inspection, but a closer analysis showed a number of subtler local effects, including a loss of curvature and reduction of the ventricular wall in the striatum, resulting in abnormal enlargement of the ventricular cavity and a slew of structural defects in other regions.

Higher resolution analysis of the paths traced by migrating axons turned up defects in the midline crossing as well. In the α N-catenin knockouts, axons extending from the anterior olfactory nucleus migrated normally along their routes for a distance but became disoriented en route, and failed to cross the midline of the brain as they normally would. This set of defects strongly indicates the central role of α N-catenin in many different areas of the developing brain, but the molecular details of its function in these contexts remains to be studied.



α N-catenin expression (magenta) at the ventricular surface in the hindbrain of an E14. mouse. α N-catenin is widely expressed throughout the nervous system, but concentrates particularly strongly at the ventricular surface. It stains brightly in a number of radial glia (green) extending from the bottom of the image as well.

Clinically safe culture of human ES cells

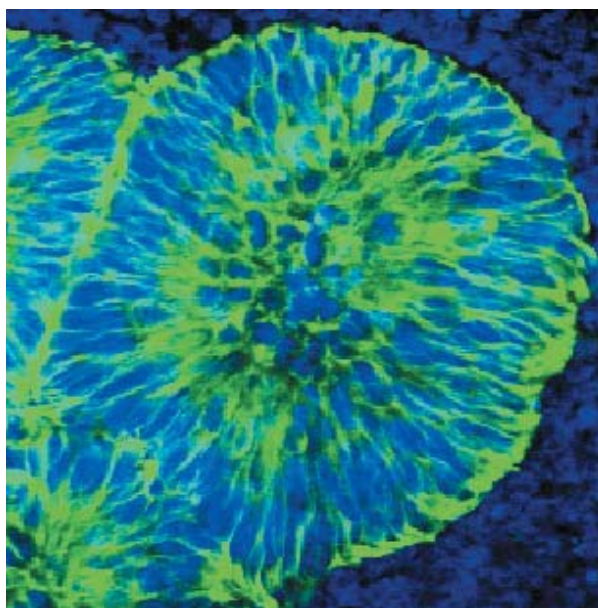
Much has been said about the great potential of embryonic stem (ES) cell-derived cells as a material resource for regenerative medical therapies. If such cells can be grown in culture in a safe and controlled fashion, and induced to differentiate into populations of specified human cell types, it would represent a significant step toward realizing the dream of generating replacement cells for bodies damaged by injury, disease or age. But, as with any clinically-oriented work, precautionary principles must be observed prior to the translation of findings from basic research into applications in human patients. For human ES cells, one of the primary concerns has been that most extant culture methods entail the use of non-human biological material to support growth and induce differentiation.

Morio Ueno of the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) and colleagues reported in an article published in the *Proceedings of the National Academy of Sciences USA* a new clinically-safe method for cultivating and steering the differentiation of human ES cells in vitro. This new method, which the authors of the study named AMED (for amniotic membrane matrix-based ES cell differentiation), uses only biological materials of human origin and demonstrates efficiencies similar to those of previously published methods in inducing ES cell differentiation into a range of neuronal cell types. The method was developed in collaboration with other CDB researchers, as well as labs in Kyoto Prefectural University of Medicine and Kyoto University, and was funded in part by the MEXT Leading Project in Regenerative Medicine.

Several methods for driving ES cells to differentiate into specialized neurons characteristic of the central nervous system have been published in the past, including a technique first reported by the Sasai lab in 2002 that relies on stromal cell-derived inducing activity (SDIA) exerted by mouse feeder cells, resulting in highly efficient differentiation into midbrain dopaminergic neurons. The clinical potential of these SDIA-induced neurons was evident from experimental trials in which these cells were transplanted into a non-human primate model of Parkinson's disease (a condition affecting dopaminergic

neurons in humans), resulting in dramatic recovery of motor function and neurotransmitter uptake. But in spite of this great promise, cells derived using such methods cannot be transplanted into humans due to the risk of contamination or infection via contact with xenogeneic feeder cells or uncharacterized culture media.

In the present report, Ueno et al found that both mouse and human ES cells can be cultured on a substrate derived from the matrix layers of the human amniotic membrane (hAM; which encases the baby within the mother's uterus, and which ruptures and is sloughed at birth). This naturally occurring material is already widely used in surgical practice, including various grafting procedures, making it much more acceptable for use in the culture of cells that may one day be transplanted into human patients. The



Neural precursors derived from AMED-treated ES cells



Ueno M, Matsumura M, Watanabe K, Nakamura T, Osakada F, Takahashi M, Kawasaki H, Kinoshita S and Sasai Y. Neural conversion of ES cells by an inductive activity on human amniotic membrane matrix. *Proc Natl Acad Sci U S A* 103:9554-9 (2006).

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amniotic membrane used in this study were obtained following informed consent from patients undergoing cesarean sections at local hospitals; human ES cells were provided by Norio Nakatsuji's lab in Kyoto University, which established the line using surplus blastocysts from in vitro fertilization clinics, in accordance with Japanese national regulations.

To prepare the amniotic membrane for use, the group carefully removed the matrix from its overlying epithelium, then transferred it to cell culture plates. Human ES cells grown for two weeks using this AMED method differentiated into Nestin⁺ neural precursors at an efficiency of approximately 90%, comparable to the purities achieved by the mouse feeder cell-based SDIA. After four weeks, a full 40% of these had differentiated into mature neurons, about a third of which exhibited a dopaminergic phenotype. And importantly, just as had been shown for SDIA, AMED-treated ES cells could be induced to differentiate into a spectrum of central and peripheral nervous system neuronal types, including motor and sensory neurons, and aggregates of retinal pigmented epithelium and the lens cells of the eye, by the addition of growth factors at developmentally appropriate time points.

The Ueno publication stands as a new milestone in the rapid advance of Japanese ES cell research. Building on a method developed for culturing corneal cells on human amniotic membrane by Kyoto Prefectural University of Medicine researchers in 2000, this report represents the culmination of just one and a half years of intensive experimentation, but one whose implications extend well into the future of regenerative medicine. The source of the amniotic membrane matrix's inductive activity, however, remains obscure. "In a sense, it's almost satisfying that the molecular mechanism at work here is still a mystery," remarks Sasai, "but it's a mystery we'll be working hard to solve."



New player in RNAi-mediated gene silencing

Eri1 downregulates heterochromatin assembly



Heterochromatin, a configuration of DNA and proteins coiled upon itself in highly condensed bundles, packs so tightly that it frequently serves to keep gene-encoding regions out of the reach of the nuclear transcription apparatus, strongly preventing the genes so packaged from being expressed. The structure and role of such heterochromatic regions has been widely studied in organisms from yeast to human, and it is now known to have important functions in epigenetic regulation and the control of gene expression during cell differentiation as well. RNA interference (or RNAi) represents a second means by which the expression of genes can be blocked, by causing the degradation of short stretches of RNA prior to their translation into proteins. Interestingly, recent studies have shown that the RNAi machinery is involved in heterochromatin assembly, but the details of this mechanism remain tantalizingly obscure.

Work by Tetsushi Iida of the Laboratory for Chromatin Dynamics (Jun-Ichi Nakayama; Team Leader), showed that, in the fission yeast *Schizosaccharomyces pombe*, the ribonuclease Eri1 breaks down small interference RNAs derived from heterochromatic regions, thereby controlling heterochromatin assembly. These findings are of particular interest, as the molecular players involved are widely conserved across the biological spectrum, including in man. The results of the Nakayama team's study were published in *Current Biology*.

Eri1 was first discovered in the nematode, *C. elegans*, and this ribonuclease has since been found to have orthologs in a broad range of



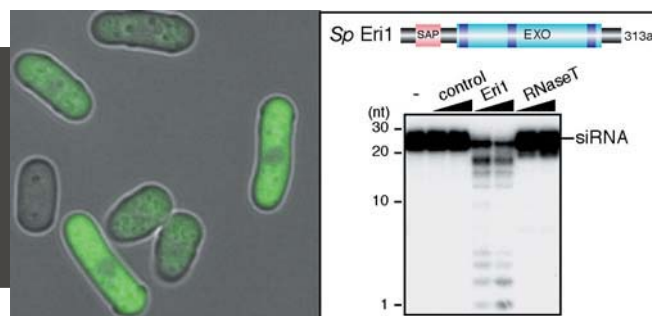
Iida T, Kawaguchi R and Nakayama J. Conserved ribonuclease, *eri1*, negatively regulates heterochromatin assembly in fission yeast. *Curr Biol* 16:1459-64 (2006).

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genomes. It is known to play a regulatory role in the RNAi machinery, in which short interfering double stranded RNA pairs up with complementary strands of messenger RNA, marking them for degradation. This RNAi mechanism is also believed to contribute to heterochromatin assembly. In this model, a bloc of proteins called the RITS (for RNA induced transcriptional silencing) complex ushers siRNAs to complementary chromosomal regions, and thus initiates heterochromatinization.

Iida et al began by identifying the *S. pombe* ortholog of Eri1, and verifying that it too had ribonuclease activity against siRNAs in vitro. Zeroing in on a possible role in heterochromatin formation, they engineered mutant yeast cells that lacked *eri1*, and found that centromeric and other heterochromatic regional gene expression was repressed, apparently due to the absence of Eri1 enzymatic activity. They next showed that Eri1's function is linked to the RNAi machinery, which was already known to control the heterochromaticity of these chromosomal precincts. From this evidence, they surmised that Eri1 has a negative regulatory effect on heterochromatic gene silencing, by targeting and breaking down specific siRNAs.

Eri1 expressed in fission yeast cells (Left). Schematic diagram of Eri1 protein (Right top). Eri1 degraded double-stranded siRNA to the level of short oligonucleotides (Right bottom).



In *S. pombe*, heterochromatin is defined by the highly specific protein modifications (the methylation of histone H3 at lysine 9), which, thus marked, becomes a target for the factor Swi6, leading to the tight winding for the affected sequence into a gene-silencing heterochromatic coil. In *eri1* mutants, histone methylation and Swi6 were enriched, and heterochromatin assembly at the centromeres upregulated. These same mutants also saw an increase in the abundance of siRNAs generated by the RNAi machinery, as evidenced by their increased association with the RITS complex, which suggests that *eri1* normally functions to downregulate the buildup of siRNAs and their binding to RITS complexes.

Eri1 negatively regulates heterochromatin formation by degrading siRNA molecules; with the RITS complex activated by association with siRNAs, the chromosome region having sequence homology to the siRNAs subsequently undergoes heterochromatinization. Eri1 degrades siRNA and is thought to regulate heterochromatin formation at an appropriate level.

The findings from these experiments led the Nakayama team to develop a model of heterochromatin assembly in fission yeast in which Eri1 enzymatically attacks heterochromatic region-derived siRNAs which would otherwise activate the RITS complex to drive further heterochromatin assembly, creating a perfectly titrated set of molecular checks and balances that keeps the cell's gene expression in tune. "It looks like Eri1 pulls off the trick of ensuring that heterochromatin assembles only where it should by maintaining siRNAs at just the right level," says Nakayama. "We've already begun to see from work in other labs how Eri1 can affect siRNA accumulation in *C. elegans* under certain conditions, so it will be exciting to find out more about its range of functions in RNAi."

Two nos make a yes

Tsuda L, Kaido M, Lim Y M, Kato K, Aigaki T and Hayashi S. An NRSF/REST-like repressor downstream of Ebi/SMRTER/Su(H) regulates eye development in *Drosophila*. *Embo J* 25:3191-202 (2006).

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Double-negative regulation in *Drosophila* eye development

The existence of genetic homologies underlies much of the recent interest in the study of model organisms as it becomes clearer that a surprisingly large amount of genetic turf is shared by nearly all living things. Researchers frequently note these similarities as part of the rationale for studying non-human organisms, but the converse logic also holds: human genes and sets of genetic interactions can also shed light on fly biology.

A study published by Leo Tsuda (now at the National Institute for Longevity Sciences in Obu, Japan) of the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) and colleagues at the CDB and Tokyo Metropolitan University, discovered that the fly gene, *charlatan* (*chn*), occupies a key position in a signaling cascade in *Drosophila* eye development. The results appeared in the online edition of *The EMBO Journal*.

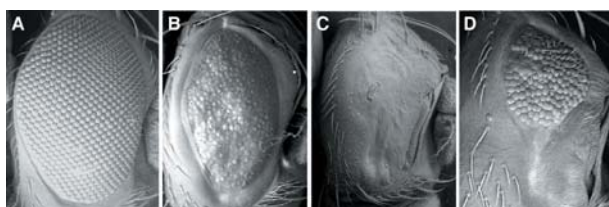
Previous work by Tsuda and colleagues had revealed that a protein complex that includes Ebi, SMRTER, Su(H) (Suppressor of Hairless) and strawberry notch, serves to upregulate the expression of the intercellular signaling factor, Delta (DI), and that a gene named *charlatan* was downregulated by the interaction between Su(H) and Ebi. Confirming that both Ebi and its co-repressor SMRTER associate with the *charlatan* promoter region in vivo, they next turned to the third element of the signaling complex, Su(H). Analysis showed that the activator, NICD, and the Ebi-SMRTER corepressors are targeted to the same binding site on the *charlatan* promoter, PR(*chn*), such that binding by one prevents binding by the other.

Intrigued by this shared binding site, the Hayashi group identified a range of similarities between *Drosophila* *chn* and the human gene *NRSF/REST*, involved in human neural regulation. Tsuda et al. then found that a consensus binding sequence for *chn* (which they named CBE, for Charlatan-binding element) was present in a number of *Drosophila* neural genes. This led to the finding that Delta (a Notch ligand) includes two CBEs and that ectopic expression of *chn*

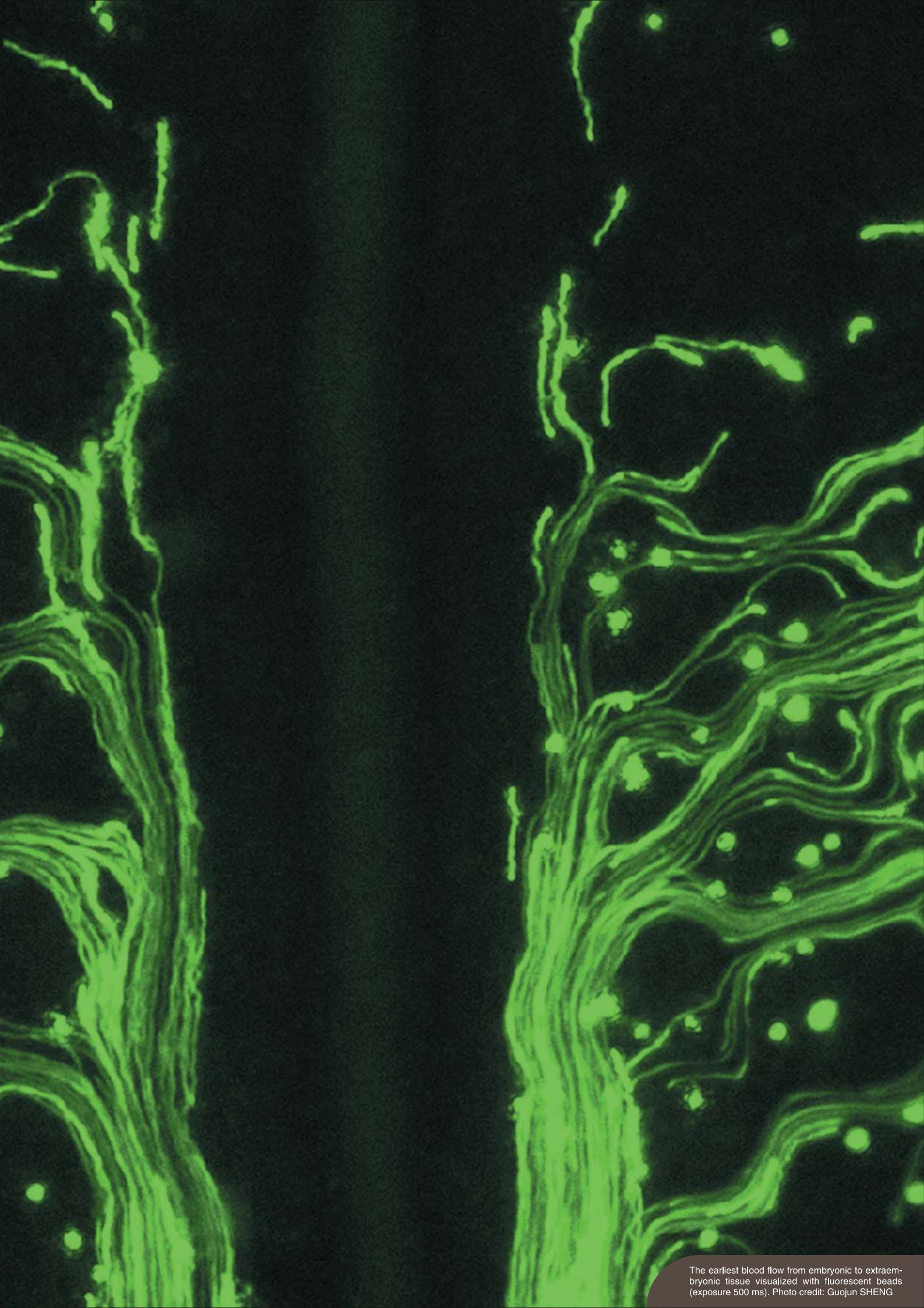
in committed photoreceptor cells reduced Delta levels, blocking cone cell differentiation without affecting their basic neuronal status. The sum of these findings points to a "double-negative" model involving *ebi* repressing *chn* to permit expression of Delta, which then induces cone cell differentiation. Commenting on the work, Hayashi states, "we're hopeful that these findings will lead to a clearer understanding of the crosstalk between EGFR and Notch signaling in neural differentiation."



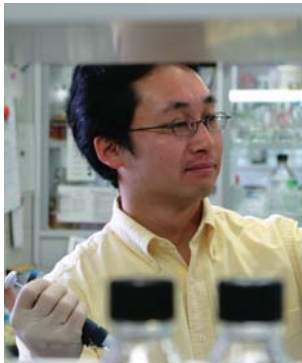
Leo TSUDA



Charlatan (Chn) is required for proper eye morphogenesis in *Drosophila*. (A-D) Scanning electron micrographs of adult eyes. (A) Wild type (WT). (B) Overexpression of Chn caused abnormal eye morphologies. (C) Reduction of Ebi activity markedly enhanced the abnormal eye phenotype caused by Chn overexpression. (D) *chn* mutation strongly inhibited eye formation.



The earliest blood flow from embryonic to extraembryonic tissue visualized with fluorescent beads (exposure 500 ms). Photo credit: Guojun SHENG



Chromatin Dynamics

Jun-ichi NAKAYAMA Ph. D.

Jun-ichi Nakayama received his bachelor's, master's and Ph. D. degrees in bioscience from the Tokyo Institute of Technology, the last in 1999 for his work on the cloning and characterization of mammalian telomerase components. He spent the period from 1999 to 2001 as a postdoctoral researcher at Cold Spring Harbor Laboratory in Shiv Grewal's lab, before returning to Japan in December 2001 as a PRESTO researcher in the Japan Science and Technology Corporation (JST). He was appointed team leader of the Laboratory for Chromatin Dynamics in 2002.

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Iida T, et al. Conserved ribonuclease, Eri1, negatively regulates heterochromatin assembly in fission yeast. *Curr Biol* 16:1459-64 (2006).

Sadaie M, et al. A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast. *EMBO J* 23:3825-35 (2004).

Nakayama J, et al. Alp13, an MRG family protein, is a component of fission yeast Ctr6 histone deacetylase required for genomic integrity. *EMBO J* 22:2776-87 (2003).

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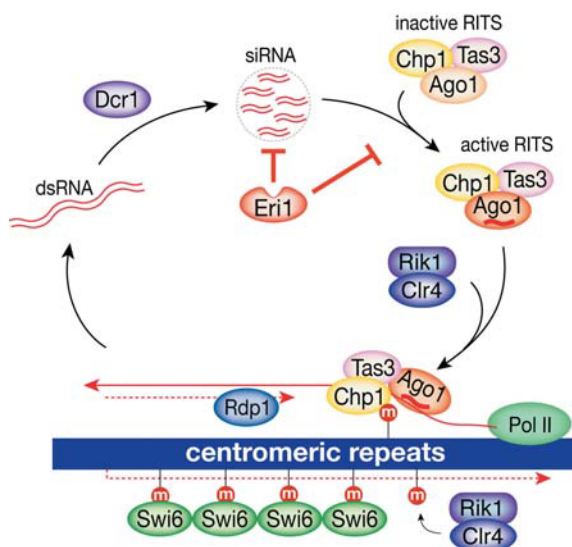
Nakayama J, et al. A chromodomain protein, Swi6, performs imprinting functions in fission yeast during mitosis and meiosis. *Cell* 101:307-17 (2000).

Multicellular organisms are made up of diverse populations of many different types of cells, each of which contains an identical set of genetic information coded in its DNA. Cell differentiation and the process of development itself depend on the ability of individual cells to maintain the expression of different genes, and for their progeny to do so through multiple cycles of cell division. In recent years, we have begun to understand that the maintenance of specific patterns of gene expression does not rely on direct modifications to the DNA sequence encoding the organism's genome, but rather takes place in a heritable, epigenetic manner. DNA methylation, chromatin modifications and post-transcriptional gene silencing by double-stranded RNA molecules are some of the best-known epigenetic phenomena. Recent studies have begun to show that these different mechanisms are closely interrelated, but a detailed understanding of these systems has yet to be developed.

Our team investigates how modifications to the structure and configuration of chromatin (complexes of nuclear DNA and proteins that provide

the structural basis of chromosomes) contribute to epigenetic gene regulation and how such modifications are transmitted over generations of cellular division by studying events at the molecular scale in the model organism, fission yeast (*Schizosaccharomyces pombe*), and in cultured mammalian cells.

Histones are a set of DNA packing proteins present in nucleosomes, the fundamental building blocks of chromatin. In our current studies, we are particularly interested in determining the specific histone modifications and the molecular recognition processes that enable modified histones to work together to construct and maintain higher-order chromatin structures. We also seek to clarify the picture of how dynamic rearrangements of chromatin structure are triggered by examining the structure and function of protein complexes that bind to and modify histones. In the future, we plan to perform more detailed analyses of the molecular mechanisms that underlie epigenetic function, as well as studies in higher organisms and epigenetic gene expression in developmental processes.



Model of RNAi-mediated heterochromatin formation

Stem Cell Biology

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

Shin-Ichi Nishikawa received his M. D. from the Kyoto University School of Medicine in 1973. He performed his internship and residency in the Department of Internal Medicine at the Kyoto University Chest Disease Research Institute, before taking an assistant professorship in the same department in 1979. He spent the period from 1980-82 at the University of Cologne Institute for Genetics before returning to the Chest Disease Research Institute, where he was appointed associate professor in the Department of Microbiology in 1983. He moved to the Kumamoto University Medical School in 1987 to take a professorship in the Department of Immunology, and returned to Kyoto in 1993, as professor in the Department of Molecular Genetics at the Kyoto Graduate School of Medicine. He was appointed CDB group director in 2000.



The stem cell system can be thought of as a means by which organisms maintain cell renewal. All forms of life require the endless recycling of both materials and energy, making it impossible for either organisms or individual cells to live independent of their environments. In bodies made up of many cells working in cooperation, such as our own, the need to replace and renew is not limited to simple materials and energy supply; the constant generation of new cells is also essential to the maintenance of the individual, a process that has developed in the evolution of multicellular life. In this sense, the process of cell renewal in maintaining the body's integrity and function provides a meaningful example of the relationship of the cell to the organism, the individual to the whole, which we hope to explore more fully using the biology of stem cells as our model.

We are especially interested in the question of how cells maintain the never-ending self-renewal that sustains the organism as a whole. This will require, at the very least, solving the riddles of two essential processes. The first of these is the need for old cells destined for replacement to be able to disengage or otherwise be dislocated from their environmental milieu. The second is the requirement for preparing new cells in replacement. To investi-

gate the first of these mechanisms, our group uses a system for labeling cells with special dyes, which allows us to monitor their location and behavior. We have also developed a system for differentiating embryonic stem (ES) cells in culture to study the second question of new cell production.

In addition to these two central themes, other members of the laboratory are studying angiogenesis, the formation of blood vessels, as blood supply is an absolute requirement to the establishment and maintenance of any deep or extensive biological tissue. These three ongoing research projects within our lab allow us to explore the problem of cell renewal in self-maintenance from multiple angles, an approach which we hope will provide new insights into this fundamental process.



■ LacZ- labeled cells in developing mouse embryo

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Publications

Uemura A, Kusuhara S, Wiegand S, Yu R, Nishikawa SI. Tlx acts as a proangiogenic switch by regulating extracellular assembly of fibronectin matrices in retinal astrocytes. *The Journal of Clinical Investigation* 116:369-377 (2006).

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Cell Migration

Kiyoji NISHIWAKI Ph. D.

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

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Suzuki N, et al. Chondroitin acts in the guidance of gonadal distal tip cells in *C. elegans*. *Dev Biol*, 300:635-646(2006).

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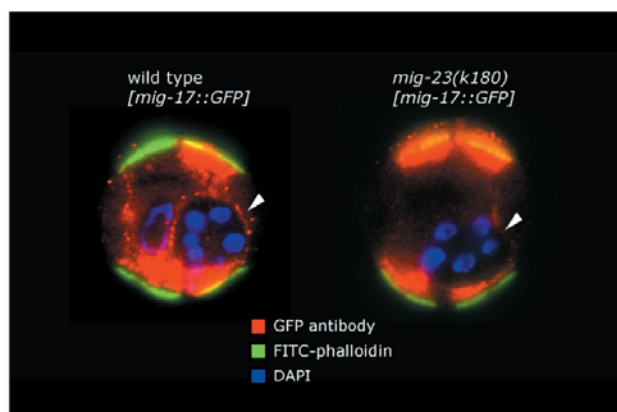
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In many forms of organogenesis, we can observe the coordinated movement of epithelial cells in sheets. For example, in the development of the lung, tubular epithelial sheets repeatedly extend and subdivide in branching patterns and thereby give rise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

We study the function of basement membranes in migration of epithelial sheets using a model organism called *C. elegans*, which is a species of tiny roundworm about 1 mm in length. In this worm, the gonads develop following a stereotyped pattern in which cells at the leading end of the migrating gonad (known as distal tip cells, or DTCs) travel along a U-shaped route in the larval body, thereby giving rise to an organ of that shape. The

proper migration of the developing gonad relies on surface interactions mediated by the basement membranes of the gonad and the body wall.

We study various mutant worms in which the direction of gonadal cell migration is abnormal to search for clues to the genetic and molecular bases of DTC guidance. One of the genes we have been focusing on encodes a metalloprotease named MIG-17, which localizes in the gonadal cell basement membrane and plays an important part in the determination of the DTC's migratory route by breaking down or modifying other membrane proteins. We have also discovered that a member of the fibulin family of secreted proteins is localized to the basement membrane in response to MIG-17 activity and also plays a role in directing cell migration. It is our hope that research such as this will provide insights into basic biological mechanisms that may one day make it possible to treat a range of health conditions in which cell migration is aberrant.

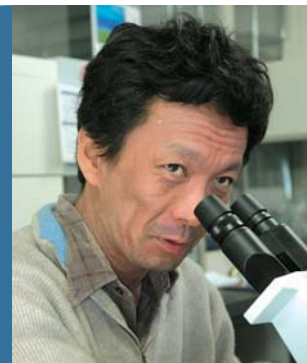


■ Localization of MIG-17 protein in wild type (left) and *mig-23* mutant (right)

Pluripotent Cell Studies

Hitoshi NIWA M. D., Ph. D.

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



Pluripotency is a term used to describe the ability of certain types of stem cells that are capable of giving rise to at least one type of cell from all three germ layers—endoderm, mesoderm and ectoderm. Stem cells are also characterized by their ability to produce copies of themselves with similar differentiative potential, as well as more specialized cells with various properties through differentiation. Embryonic stem (ES) cells are the best-known example of a type of stem cell possessing these properties of pluripotency and self-renewal. In our lab, we use ES cells as a model system for studying the molecular mechanisms that determine and enable the fascinating properties of pluripotent cells.

In previous work, we identified functions of various soluble factors, such as ACTH/SAF, Wnt and Activin/Nodal, to maintain the ability of ES cells to self-renew, and developed an ES culture medium using fully characterized components. These developments were made in parallel with studies aimed at resolving the functions of genes involved in the maintenance of stem cells in an undifferentiated state and the induction of differentiation. We have also identified a transcriptional factor that directs differentiation into placenta and yolk sac. Given their ability to generate all of the body's cell

types, ES cells have come to stand as a symbol for the emerging fields of cell replacement therapy and regenerative medicine, but they also represent an ideal system for the study of many of the processes of early mammalian embryonic development. The study of the basic biology of stem cells may one day bear fruit to shed light on the principles that control 'differentiation' events in general, as the sum of coordinated regulation of transcription, cell-cycle, cell morphology, cell adhesion and attachment by genetic and epigenetic mechanisms under the control of the environment.

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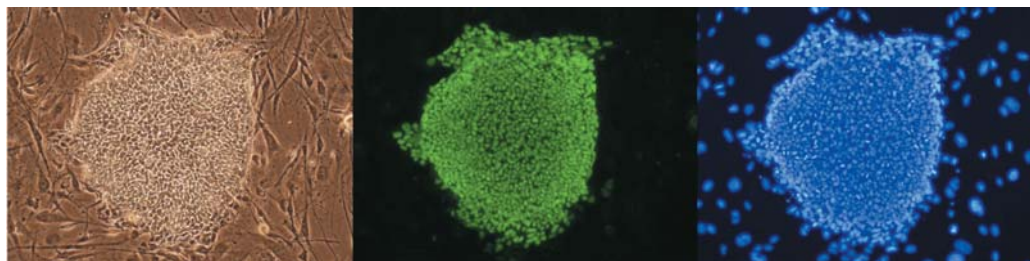
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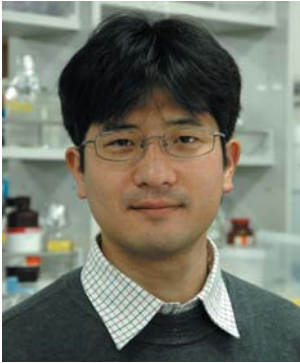
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■ Monkey ES cells phase contrast image (left). Immunostaining for OCT3/4 (center). Nuclear staining by Hoechst 33458. (right)



Mammalian Epigenetic Studies

Masaki OKANO Ph. D.

Masaki Okano received his baccalaureate and master's degrees from Kyoto University, and his doctorate in biochemistry from the Research Division in Agriculture at the same institution in 1994. He spent the period from 1994 to 1995 as a research fellow in cell biology at the Nagoya University Bio Science Center before moving to Massachusetts, where he worked as a research fellow in medicine at Harvard Medical School and Massachusetts General Hospital from 1995 to 2002, serving as an instructor in medicine at Harvard for the last two years of that period. He was appointed team leader at the RIKEN Center for Developmental Biology in 2001, and returned to work in Japan full-time in 2002.

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Publications

Oda, M., Yamagiwa, A., Yamamoto, S., Nakayama, T., Tsumura, A., Sasaki, H., Nakao, K., Li, E., and Okano, M. DNA methylation regulates long-range gene silencing of an X-linked homeobox gene cluster in a lineage-specific manner. *Genes & Development* 20, 3382-3394 (2006).

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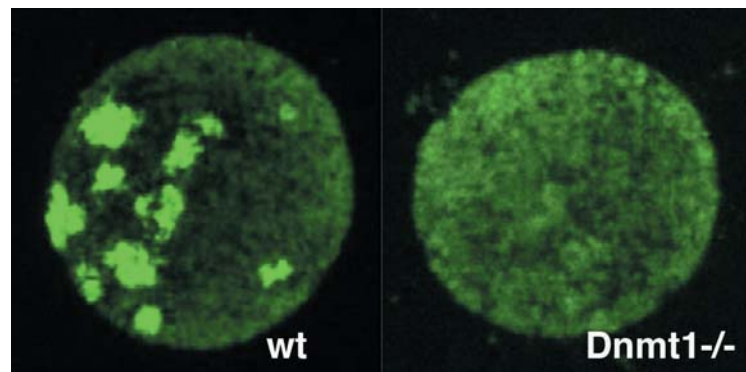
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The development and physiological function of multicellular organisms relies on the ability of their component individual cells, which as a rule contain identical genetic information, to express subsets of genes in a selective manner. In order to maintain lineages of cells of different types, the gene expression programs established in ancestral precursor cells must be transmitted stably to many generations of progeny. The fundamental mechanisms by which patterns of gene expression are preserved across cycles of cell division without a change to the underlying genome sequence are commonly referred to as "epigenetic" processes. These processes produce chemical modifications and structural remodeling of chromatin, nuclear structures that store the cell's DNA, allowing individual cells to regulate the switching on and shutting off of the expression of specific genes with great precision and flexibility.

Our team studies epigenetics by focusing on a particular set of molecules that act in the methylation of DNA, with the goal of identifying their roles in the regulation of chromatin function in embryonic development and the processes of fate deter-

mination and plasticity in cell differentiation. We conduct phenotype analyses of mice in which various genes involved in DNA methylation have been knocked out, as well as biochemical and cell biological studies of embryonic stem (ES) cells to explore how dynamic changes in DNA methylation function as regulatory factors in mammalian embryogenesis and cell differentiation, and the means by which DNA methylation works to maintain cell proliferation and chromosome function. We anticipate that a deeper understanding of these questions will lead to new insights in the context-specificity of the DNA methylation process as well as the roles of methylation in development, health and regeneration.



■ Loss of DNA methylation in pericentromeric heterochromatin in *Dnmt1*^{-/-} embryonic cells at E9.5 (Immunofluorescence staining with anti-methylcytosine antibody)

Mammalian Molecular Embryology

Tony PERRY Ph. D.

Tony Perry received his B.Sc. in Microbiology from the Department of Bacteriology at the University of Bristol, England and his doctorate from the University of Liverpool four years later. In 1989 he became a postdoctoral fellow working on epididymal sperm maturation at the University of Bristol and in 1996 won a European Molecular Biology Travel Fellowship to work on the mechanism of oocyte activation, which remains one of his research interests. He then moved first to the Rockefeller University and subsequently to Advanced Cell Technology, working primarily on novel methods of genome manipulation. In 2002 he took his present position as Team Leader of the Laboratory of Mammalian Molecular Embryology at the RIKEN CDB, where he works on mechanisms in mammalian preimplantation embryos.



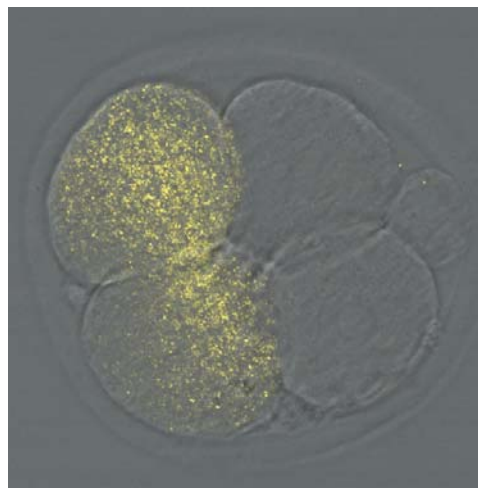
There can be few, if any, cells as specialized as sperm and egg; they appear unique (you can even see a mammalian oocyte with the naked eye) and don't typically divide by themselves. Yet when mammalian sperm and egg combine at fertilization, the single cell they generate is transformed within hours into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

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

During meiotic resumption, sperm chromatin undergoes almost complete remodeling; its nucleoproteins, which are mostly protamines, are removed and supplanted by maternal histones which are subsequently modified. We study and manipulate this overall process and have found that chromatin remodeling varies depending on the provenance of the nucleus; the fate of somatic cell chromatin is different from chromatin associated with a sperm-derived genome, even when

both are in the same oocyte. Moreover, chromatin remodeling is not necessarily of critical importance; gross hyper-acetylation during fertilization does have only a limited effect, if any, on development.

We are interested in additional interactions between sperm head components and the oocyte cytoplasm, in a bid to discover what happens during fertilization and the earliest moments of the new embryo. It would be useful to attribute molecular identities to the proteins involved in these interactions and characterize them functionally. This task is a daunting one, as the sperm contributes >1000 distinct nuclear and cytoplasmic protein species at fertilization, and yet detecting them in newly fertilized oocytes and embryos requires exquisitely sensitive methods. With a greater understanding of any sperm contribution to development, our lab hopes to gain insights into the processes by which embryonic stem cells are formed and carcinogenesis is initiated.



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

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Mammalian Germ Cell Biology

Mitinori SAITOU M. D. , Ph. D.

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

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Kurimoto K, et al. Global single-cell cDNA amplification to provide a template for representative high-density oligonucleotide microarray analysis. *Nature Protocols*, in the press (2007).

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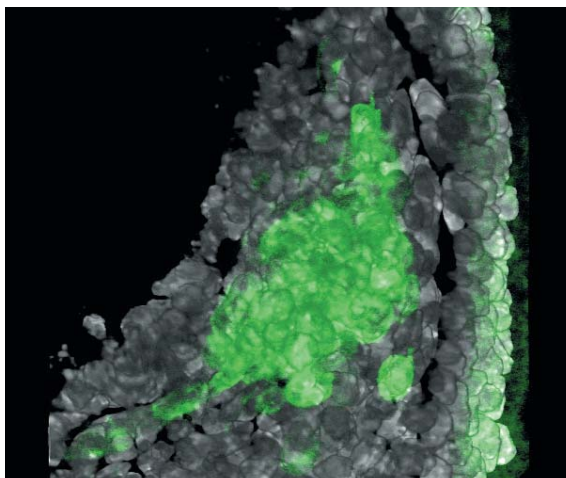
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All of the diverse cell types in the body can be broadly classed as either somatic or germline cells. In contrast to somatic cells, which work to maintain the constant, stable form and function of an individual organism's body, germ cells provide the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

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

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



Projected 3D image of *Blimp1*-positive PGCs (green, center) at E7.25 in mouse development.

Organogenesis and Neurogenesis

Yoshiki SASAI M. D., Ph. D.

Yoshiki Sasai received his M. D. from the Kyoto University School of Medicine in 1986, subsequently performing internships in general practice and emergency medicine. He completed the Ph. D. course at the same institution in 1992, for work on neural specific transcriptional regulators. In 1993, he took a postdoctoral fellowship in the De Robertis lab at the UCLA School of Medicine, remaining there until 1996 when he was appointed associate professor at the Kyoto University School of Medicine. He assumed a professorship at the Kyoto University Institute for Frontier Medical Sciences in 1998, and was appointed group director at the CDB in 2000. He serves on the editorial boards of *Neuron*, *Genesis* and *Developmental Dynamics*.

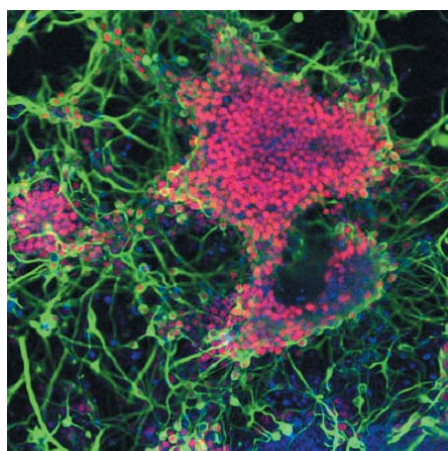


The complexity of the fully formed brain defies description, yet this organ arises from a nondescript clump of cells in the embryo. The specification of the dorsal-ventral axis is significant in neural development in that the central nervous system forms on the dorsal side of the body in all vertebrate orders. This process is dictated by the effects of a number of signaling factors that diffuse from organizing centers and direct dorsal ectoderm to maintain a neural fate. These molecules, which include Noggin, Chordin, Follistatin and their homologs, participate in elaborate signaling networks in which factors collaborate and compete to initiate the embryonic nervous system.

Using the African clawed frog, *Xenopus laevis*, as a model in molecular embryological studies, our group is engaged in clarifying the structure and extent of the signaling networks involved in setting up the dorsal-ventral axis and determining neural fate in the ectoderm. These studies focus on molecules that operate at early stages in embryonic development to lay down a complex of permissive, instructive and inhibitory patterns that determines the growth and differentiation of the brain in its earliest stages of development.

Our group is now also actively developing effective methods of inducing neuralization in mammals, work which it is hoped will contribute to basic research by providing in vitro experimental systems for use in the analysis of mammalian neurogenesis. In addition, this approach has potential for application in the treatment of neurodegenerative disorders, such as Parkinson disease. Using a system developed in our lab, we have succeeded in inducing mouse and primate embryonic stem (ES) cells to differentiate into a range of specialized neuronal types. The application of similar techniques to human ES cells represents a field of study that, although still at an early stage, shows immense clinical promise.

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



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

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Ikeya, M., Kawada, M., Kiyonari, H., Sasai, N., Nakao, K., Furuta, Y. and Sasai, Y. Essential Pro-BMP Roles of Cross-veinless 2 in Mouse Organogenesis. *Development* 133, 4463-4473 (2006).

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Embryonic Induction

Hiroshi SASAKI Ph. D.

Hiroshi Sasaki received his B. Sc. in zoology from the University of Tokyo, and his master's and Ph. D. in developmental biology from the same institution. He worked as a research associate in Atsushi Kuroiwa's group at Tohoku University from 1990 to 1992, and in Brigid Hogan's lab at Vanderbilt University from 1992 to 1994. He returned to Japan to take a position as assistant professor at Osaka University beginning in 1995, where he remained until his appointment as team leader at the RIKEN CDB.

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Sekimizu K, et al, The zebrafish iguana locus encodes Dzip1, a novel zinc-finger protein required for proper regulation of Hedgehog signaling. *Development* 131:2521-32 (2004).

Nishizaki Y, et al, Identification of essential sequence motifs in the node/notochord enhancer of Foxa2 (Hnf3beta) gene that are conserved across vertebrate species. *Mech Dev* 102:57-66 (2001).

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

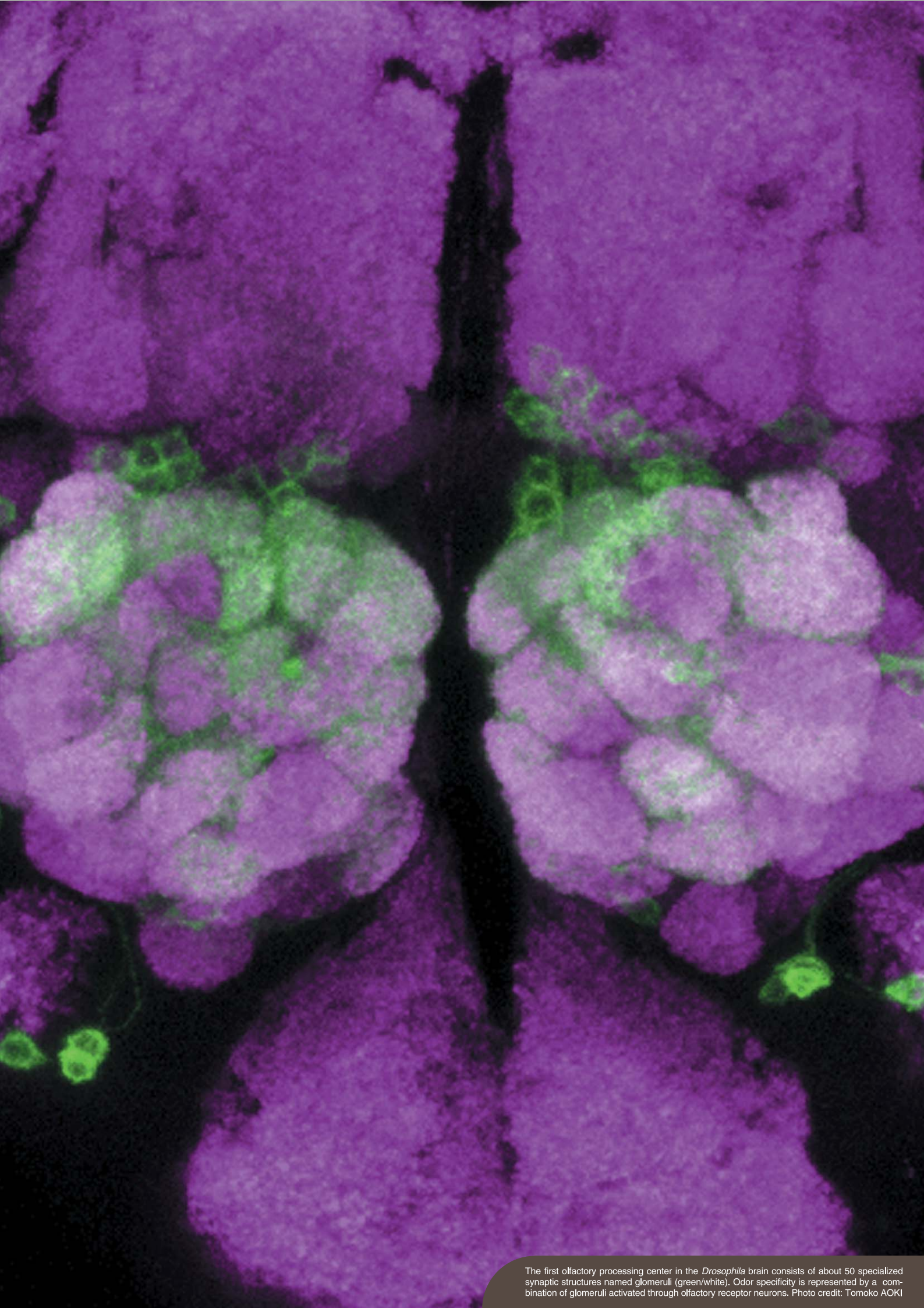
Research in our laboratory focuses on these signaling centers, especially those of the mouse, as a model for studying the regulation of embryogenetic processes. We focus particularly on the node and notochord, which are of central importance in the formation of the early embryo. Focus-

ing on analyses of the roles played by the transcription factor Foxa2/HNF3 β , and defects in head development that result in a loss of function mutant allele named *headshrinker*, we seek to determine the mechanisms that establish and maintain signaling centers during development.

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



■ Prechordal plate (a signaling center regulating head development; stained purple on left) is absent in *Ssdp1/headshrinker* mutants (right)



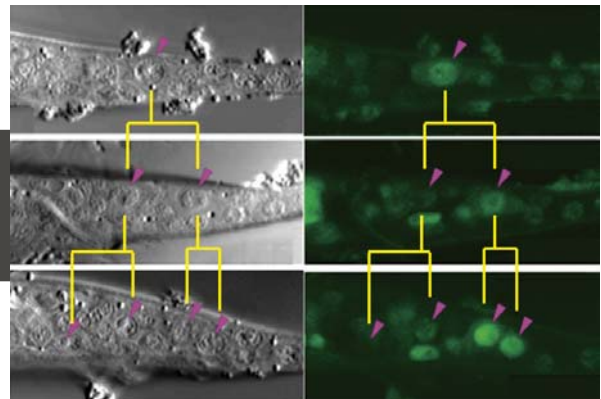
The first olfactory processing center in the *Drosophila* brain consists of about 50 specialized synaptic structures named glomeruli (green/white). Odor specificity is represented by a combination of glomeruli activated through olfactory receptor neurons. Photo credit: Tomoko AOKI

Wnt signaling and Hox regulate PSA-3 to determine cell fate

A diversity of cell fates is generated during animal development through processes including asymmetric cell division, in which cells' identities may be fixed even before they are born by the unequal allocation of fate-determining proteins to opposite sides of a cell prior to mitosis. This form of predestination is at work in the roundworm *C. elegans* just as it is in larger, more complex organisms. Surprisingly, in *C. elegans*, most cell asymmetry is the outcome of divisions regulated by the Wnt-MAPK signaling pathway, as mediated by POP-1. This general mechanism, however, cannot fully explain the spatio-temporally specific cell fates generated through asymmetric division.

Yukinobu Arata and colleagues in the Laboratory for Cell Fate Decision (Hitoshi Sawa; Team Leader) reported that cell specialization arises as the net regulatory effect of Wnt signaling and a Hox protein on a novel factor, PSA-3. "This is a nice demonstration of how cooperation between a general mechanism for asymmetric cell division and position-specific factors can lead to discrete outcomes during development," says Arata.

Asymmetric expression of *psa-3* in the tail region of *C. elegans* as visualized by differential interference contrast (DIC) microscopy (left) and GFP labeling (right)



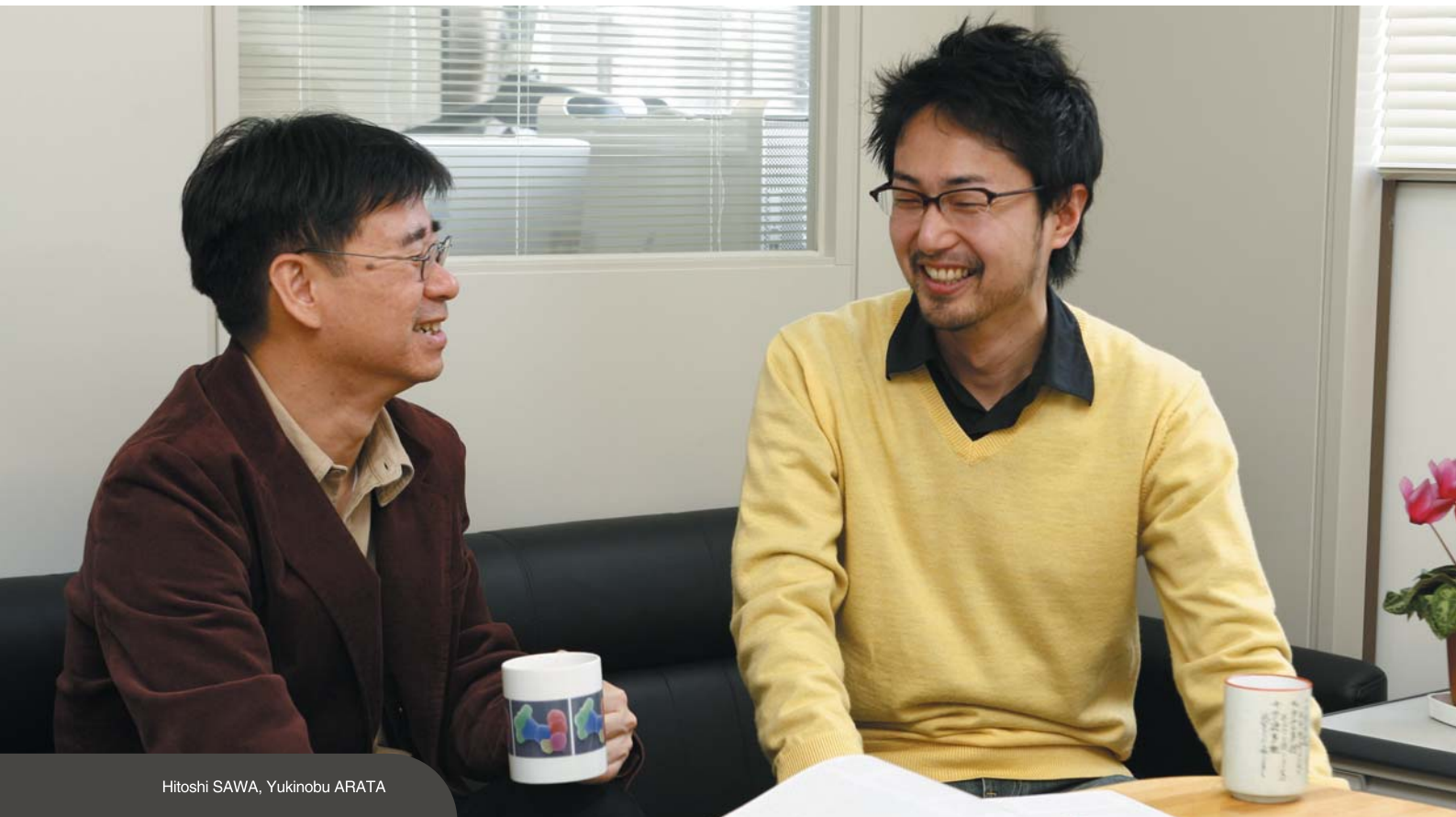
Recent works had shown the role a Wnt-related factor, POP-1, plays in asymmetric cell division of both embryonic and postembryonic cells in *C. elegans*. But the mechanism appeared to be general to a wide variety of asymmetrically dividing cells, suggesting that additional factors must help guide such Wnt-polarized cells to their ultimate fates. Arata et al. focused on at a single cell type, the asymmetrically dividing T cell in the roundworm tail, which ordinarily divides to give rise to a hypodermal anterior and a neural posterior daughter. Screening for mutations that disrupted this pattern, they identified a trio of mutants for the genes *psa-3*, *ceh-20* and *nob-1*, all of which yielded posterior daughters lacking structures known as phasmid sockets, which characterize cells in the neural lineage. Of these, *nob-1* and *ceh-20* had previously been identified as roundworm homologs of the positional specification genes *Hox* and *Pbx*, respectively. *psa-3*, however, showed up only as a presumptive entry in databases of *C. elegans* genes.

Analysis of its sequence revealed that *psa-3* shared similarities with genes in the Meis family of transcription factors, and even more intriguingly, contained a site in the promoter region that appeared to be a POP-1 binding sequence. The team created a GFP *psa-3* construct to allow them to follow its activity in T cells, and found that *psa-3* was gradually upregulated in the posterior descendants, following the initial T cell division. In worms engineered with a nucleotide substitution in the putative POP-1 binding sequence, this effect was lost, indicating to the team that *psa-3* expression in posterior T cell daughters is regulated by its interaction with the *C. elegans* Wnt signaling pathway.



Arata Y, Kouike H, Zhang Y, Herman M A, Okano H and Sawa H. Wnt Signaling and a Hox Protein Cooperatively Regulate PSA-3/Meis to Determine Daughter Cell Fate after Asymmetric Cell Division in *C. elegans*. *Dev Cell* 11:105-15 (2006).

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Hitoshi SAWA, Yukinobu ARATA

It remained to be seen, however, how *psa-3*'s effect could be kept specific to posterior descendants of T cells, when POP-1 was known to operate in the asymmetric divisions of all seam cells, of which T cells are only a subset. Roundworms with mutations in the *Hox* equivalent *nob-1* and the *Pbx* homolog *ceh-20* showed decreases in *psa-3* in the posterior lineage. Searching for the regulatory elements responsible, they found that the fourth *psa-3* intron contained a binding sequence for NOB-1, and that this interaction appeared to be facilitated by CEH-20, indicating that the tail Hox factor, NOB-1 allows *psa-3* to function selectively in posterior descendants of T cells. And interestingly, the Sawa team found that *psa-3* itself functioned in localizing CEH-20 to the nucleus in posterior T cells, suggesting a regulatory circuit in which *psa-3* and *ceh-20* cooperate to maintain each other's functions. These findings were borne out in vivo, where the results of heat shock rescue experiments indicated that PSA-3, NOB-1 and CEH-20 work as co-factors in regulate cell fate in the posterior lineage after asymmetric T cell division.

"It appears that similar mechanisms for asymmetric division are used repeatedly during the development, not only of *C. elegans*, but many other organisms as well," notes Sawa. "It will be interesting to find out whether Hox proteins cooperate with a common mechanism for asymmetry to specify diverse cell fates in other species as well."

This study, published in the July issue of *Developmental Cell*, was made in collaboration with scientists from the Keio and Osaka University (Japan) and Kansas State University (USA), and with funding support from the Japan Science and Technology CREST and PRESTO programs.

New role for Vasa in *Ciona* germline



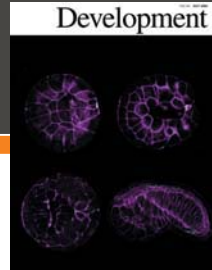
All differentiated animal cells can be categorized into either somatic or germ lineages. While somatic cells build and maintain the body itself, the cells of the germline differentiate into eggs and sperm, transmit genetic information to the next generation and retain the ability to differentiate into each and every cell type in the body. In many animals, including the research models *Drosophila* and *C. elegans*, the development of the germline founder population of primordial germ cells (PGCs) is characterized by the inheritance of germ plasm, a cytologically distinct assembly of electron-dense granules. These germinal granules later form perinuclear granules, or nuage—a distinguishing feature of the germ cell lineage in these species. Despite their centrality to germline development, however, both the germ plasm and nuage remain poorly understood.

Maki Shirae-Kurabayashi (Laboratory for Germline Development; Akira Nakamura, Team Leader) and colleagues shed new light on the mechanisms underlying germline formation in the ascidian *Ciona intestinalis* in an article published in *Development*. This remarkable organism has a unique form of cytoplasm known as the postplasm in its developing germline, which is believed to serve as the ascidian germ plasm. Previous studies have shown *Ciona* postplasm to contain a high concentration of maternal RNAs and proteins, as well as electron-dense masses highly similar to the germinal granules found in *Drosophila*; such masses give rise to the centrosome-attracting body (CAB), a specialized cytoplasmic structure.

Ascidian postplasm also contains a high concentration of RNAs and the protein product of the *CivH* gene (a homolog of the *Drosophila* germline-specific gene *vasa*, which is essential for germ cell development and highly conserved in vertebrates and invertebrates). These *CivH*-positive cells are subsequently incorporated into juvenile *Ciona* gonads and form the animal's germ cells. Given these similarities with pri-

Shirae-Kurabayashi M, Nishikata T, Takamura K, Tanaka K J, Nakamoto C and Nakamura A. Dynamic redistribution of vasa homolog and exclusion of somatic cell determinants during germ cell specification in *Ciona intestinalis*. *Development* 133:2683-93 (2006).

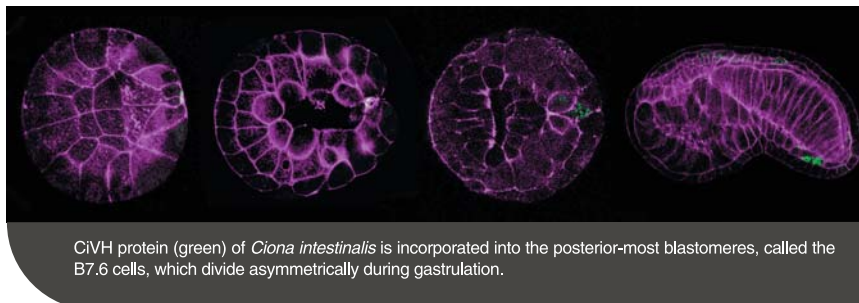
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mordial germ cells in other species, many researchers now believe that postplasm serves as the ascidian germ plasm, in which the progeny of a highly specific subset of blastomeres, termed B7.6 cells, develop into PGCs.

A number of postplasmic components, including the centrosome-attracting body itself, are also known to have a role in promoting asymmetric cell division and the regulation of somatic cell differentiation during early embryogenesis. One of these factors, *posterior end mark* (PEM) RNA, appears to control the positioning of cleavage planes, thereby promoting asymmetric division. Intriguingly, the detection of these postplasmic factors in presumptive B7.6 cells opened up the possibility that, in addition to their known function in somatic cell differentiation, they might also play roles in germ cell formation as well.

Previous studies were unclear, however, as to the stage at which B7.6 cells directly merge into the gonads, and whether the postplasmic components involved in somatic cell development do in fact contribute to germ cell formation. Shirae-Kurabayashi et al. first established that *Ciona* B7.6 cells undergo an asymmetric cell division to produce two daughter cells, B8.11 (the CAB-containing anterior cells) and B8.12 (CAB-negative posterior cells). As most of the postplasmic components are associated with the CAB, these too are partitioned solely into the B8.11 daughters, subsequently losing *CiVH* protein expression and associating with the animal's gut wall. The B8.11 cells, however, did retain the remnants of the CAB and other postplasmic components, which were not seen in B8.12 cells, suggesting that B8.11 cells do not function in the specification of germ cells.



The team did find, however, that maternal *CiVH* RNA and proteins entered the cytoplasm from the CAB immediately prior to B7.6 division, and were then inherited by the B8.12 progeny. Subsequent upregulation of *CiVH* protein production in B8.12 cells resulted in the formation of perinuclear *CiVH* granules, which they tentatively identified as the nuage characteristic of germ cells. Using Dil labeling and *CiVH* immunostaining, the Nakamura team found that the descendants of the B8.12 cells are incorporated into *Ciona* primitive gonads and go on to form the germ cells. This suggests that the *Ciona* B8.12 cells are indeed early germline progenitors, and that formation of a nuage-like structure occurs immediately after the B7.6 cell division, much earlier than was previously thought.

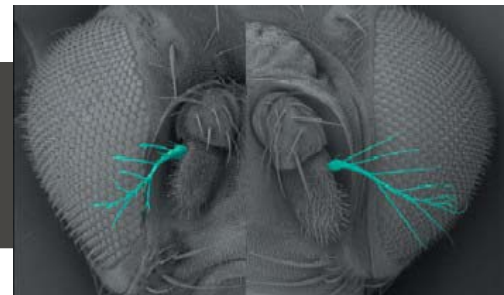
Shirae-Kurabayashi proposes that the centrosome-attracting body and most postplasmic components involved in somatic differentiation are segregated from the PGCs through a single asymmetric cell division, and that germline development in ascidian embryogenesis requires the diffusion of specific postplasmic components into the cytoplasm prior to the B7.6 division. Studies of other ascidian species showing the localization of postplasmic/PEM RNAs into two distinct embryonic regions indicate that the asymmetric distribution of postplasm components at the B7.6 cell division may be a conserved feature of germ cell specification in ascidians. "In this work, we showed that a postplasm factor participates in the formation of the *Ciona* germline," says Shirae-Kurabayashi, "but we also know that the germline will regenerate itself if it is ablated from a larval ascidian, which suggests that there are two separate mechanisms—embryonic and postembryonic—for constructing the germ lineage in these animals, so of course we're very curious to find out how each of those works."

Novel factor keeps cells in shape

The study of morphogenesis commonly looks at the ways in which organized groups of cells extend, compress, fold inward, or roll up to form three-dimensional shapes. But on an even minuter scale, the shapes of individual cells are also important to their ability to move, make connections, and otherwise interact with other cells and their microenvironments. Cells change and maintain their shapes by closely regulating the activity of their cytoskeletons, the molecular support structures that play a fundamental role in controlling cell shape, polarity and locomotion. The cytoskeletal component F-actin is a filamentous polymer whose localization and activity in specific regions of epithelial cells is critical to enabling cell motility, but the mechanisms that regulate such activity are incompletely understood.

In a study published in the journal *Current Biology*, Kenzi Oshima and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi; Group Director) identified a new regulator of F-actin in cellular morphogenesis. This study, which was conducted in collaboration with scientists at the University of Tokyo, National Institute of Genetics and Tokyo Metropolitan University, showed that the protein kinase IKK ϵ negatively regulates F-actin assembly at the peripheral edges of *Drosophila* cells in culture and during morphogenetic processes.

Scanning electron micrographs of *Drosophila* adult heads. Expression of a dominant negative form of IKK ϵ in antenna arista (left; pseudocolored) caused excess branching of lateral (compare with normal adult head; right), demonstrating a critical role for IKK ϵ in actin-dependent cellular morphogenesis.



Oshima first noted IKK ϵ in a screen of genes involved in epithelial morphogenesis in the fly trachea, finding that the overexpression of this protein led to epithelial disruption, the loss of apical-basal cell polarity, and the abnormal accumulation of F-actin in the apical region of cells. Looking at its expression pattern in wild type cells, the group found that IKK ϵ tended to accumulate in thicker, cytoplasmic regions of cells, rather than at the thinner peripheries, where F-actin localizes. Sites where the expression of these two proteins intersected, however, frequently developed the “ruffling membrane” structures characteristic of cell motility.

Examining the effects of downregulation of IKK ϵ in vitro, they found that while cells survived even when the gene's activity was interfered with, the loss or lessening of IKK ϵ function resulted in dramatic changes in cell morphology, with such cells exhibiting a ruffled, serrate or spiky, stellate morphology at much higher frequency than the smooth phenotype typical of control cells. Overexpression had the opposite effect, causing more of the cells to develop smooth edges and, interestingly, to die by spontaneously entering the process of programmed cell death, apoptosis. Analysis of the rates of F-actin turnover in IKK ϵ up- and downregulated cells indicated that retrograde flow of F-actin from the peripheral edges of the ruffling membrane back toward the cytoplasmic interior was reduced in IKK ϵ -deficient cells.

Cell motility and migration is fundamental to numerous morphogenetic processes during *Drosophila* development, so Oshima and colleagues next looked at the role of IKK ϵ in living embryos, focusing on the formation of the trachea, a branching network of epithelial lumina. In the loss-of-function mutant, the tra-



Oshima K, Takeda M, Kuranaga E, Ueda R, Aigaki T, Miura M and Hayashi S. IKK epsilon regulates F actin assembly and interacts with *Drosophila* IAP1 in cellular morphogenesis. *Curr Biol* 16:1531-7 (2006).

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chea developed normally at first, but in later stage embryos the terminal tracheal branches went haywire, exhibiting defects including misorientation, splitting and duplication at a frequency of about 30%. The authors surmised that these phenotypes were the result of ectopic accumulation of F actin, leading to abnormalities in the morphology and movement of the terminal branches. Similar defects were observed in the development of other extended structures in which F-actin assembly is involved, including sensory bristles and the terminal segment of the antennae, known as arista.

Returning to the possible link with apoptotic cell death factors suggested by the overexpression phenotype, the Hayashi group looked for a potential interaction between IKK ϵ and an apoptosis inhibitor, DIAP1, which had previously been shown to negatively regulate IKK ϵ expression. Increased levels of DIAP1 intensified the IKK ϵ loss-of-function dysmorphology phenotype, while overriding the effects of IKK ϵ overexpression. Loss of IKK ϵ function, meanwhile, had a positive effect on DIAP1 expression, resulting in a threefold increase. This set of findings led Oshima et al. to propose that IKK ϵ and DIAP1 play opposing roles in regulating F-actin-driven cellular morphogenetic events at the periphery of epithelial cells. Interestingly, however, the role of DIAP1, which is primarily known as a regulator of programmed cell death, appears to be independent of its anti-apoptotic function.

"The IKK family of protein kinases is known to include regulators of immune response, so we were very surprised to find that IKK ϵ has a completely different role in F-actin-dependent cellular morphogenesis," admits Hayashi. "We were even more surprised to find that IKK ϵ functions as a negative regulator of the non-apoptotic functions of DIAP1 and caspase. Since the IKK ϵ -IAP pathway seems to be present in vertebrates, this finding might provide a clue to investigate evolutionarily conserved roles of non-apoptotic functions of caspases."



Novel pathway establishes embryonic cell polarity in *C. elegans*

Embryos of the roundworm *C. elegans* develop distinct polarity even at the one-cell stage. Apparently triggered by cues linked to the sperm entry point at fertilization, this initial polarization helps set up the highly stereotyped and well-characterized sequence of cell divisions and differentiative events that ultimately results in a viable worm larva. The roots of this anterior-posterior cellular polarity have been traced to the asymmetric distribution of PAR-family proteins at the cortical periphery of the cell, in which PAR-3 and -6 localize to the anterior cortex along with their co-factor PKC-3, while PAR-2 accumulates at the cell's posterior margin. But the means by which these proteins are conveyed to their appropriate destinations following a presumed signal from the sperm-contributed centrosome has never been worked out, although it is thought to involve the conveyance of the anterior PAR complex by the actomyosin cytoskeleton.

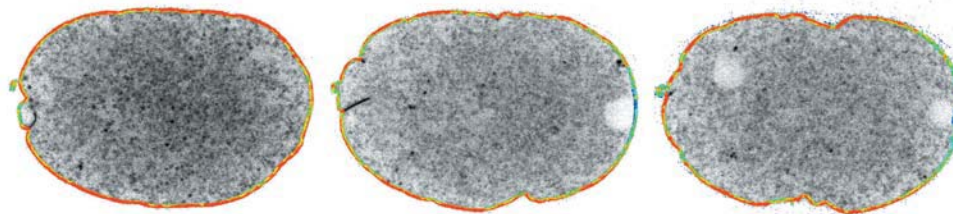
In August 2006, Fumio Motegi and Asako Sugimoto of the Laboratory for Developmental Genomics (Asako Sugimoto; Team Leader) followed the trail several steps farther upstream, elucidating a molecular pathway that goes a long way toward bridging the gap between the sperm entry site cue and the asymmetric allocation of PAR factors. In a report published in *Nature Cell Biology*, Motegi and Sugimoto showed that two small GTPases, RHO-1 and CDC-42, and their potential regulator, ECT-2/RhoGEF, act in series to establish polarity in the one-cell roundworm embryo.





Motegi F and Sugimoto A. Sequential functioning of the ECT-2 RhoGEF, RHO-1 and CDC-42 establishes cell polarity in *Caenorhabditis elegans* embryos. *Nat Cell Biol* 8:978-85 (2006).
Cover image copyright © Nature Publishing Group 2006

The Rho family of GTPases is known to participate in the regulation of the actin cytoskeleton, and as cytoskeletal activity had been implicated as a possible mode of transport for PAR proteins during cell polarization in *C. elegans*, the team examined the consequences of the loss of function of two Rho-family genes, *cdc-42* and *rho-1*, and the gene for a putative RHO-1 activator, *ect-2*. RNAi suppression of the activity of these genes revealed an interesting disparity in the phenotypes. For all three factors, RNAi led to differing degrees of compromise of the posterior migration of the posterior mitotic spindle pole. *cdc-42* loss-of-function cells showed normal initial polarization at the cell cortex, with ruffling at the anterior edge and smoothening at the posterior, but in both *ect-2* and *rho-1* RNAi cells, this effect was lost.



Local exclusion of ECT-2/RhoGEF from the posterior cell cortex leads to the establishment of anterior-posterior polarity in *C. elegans* embryos. Cortical ECT-2 visualized with GFP is pseudocolored. Higher and lower amounts of ECT-2 are represented by red and blue, respectively. Three sequential images are shown (posterior to the right).

This preliminary finding led them next to look for possible relationships between these three factors and PAR localization. Developing a method for labeling and tracking the movement of PAR and cytoskeletal proteins using fluorescent tags, they determined that the polarization process is a two-stage affair. Phase I is characterized by the flow of cytoskeletal elements to the anterior cortex, whereas in phase II the newly established anterior cortical domain appears to undergo structural reorganization. Watching the effects of the loss of function of the three proteins, Motegi and Sugimoto found that when *ect-2* or *rho-1* function was interfered with, PAR-6 failed to localize to the anterior, while in *cdc-42(RNAi)* embryos, PAR-6 localized correctly in phase I, only to disappear in phase II. Similarly disparate effects were observed in the behavior of the actomyosin cytoskeleton in the RNAi embryos, which led Motegi and Sugimoto to conclude that RHO-1 and its putative GEF, ECT-2, work in phase I to establish the anterior-posterior polarity of the cell, while CDC-42 functions in phase II to consolidate and maintain that polarization.

Experiments using fluorescent-tagged transgenes for *ect-2*, *rho-1* and *cdc-42* under the control of a germline-specific promoter enabled the direct monitoring of these factors. Analysis of the three proteins yielded observations that tended to confirm the predictions of the team's working model, and revealed the dynamics and timing of their localization at new levels of detail. Prior to polarization, ECT-2 is uniformly distributed to the entire cortical region, but, perhaps in response to a molecular signal from the centrosome, ECT-2 gradually becomes depleted from the posterior cortex. It appears that this anterior concentration of ECT-2 then leads to the anterior accumulation of RHO-1. This prompts the anterior flow of actin cytoskeletal components, which is thought to be involved in conducting CDC-42 to localize in the same region enabling it to interact with both actomyosin and the anterior PAR complex.

"This study helps to explain some of the mechanisms underlying the earliest events in the A-P patterning of the roundworm embryo," says Sugimoto. "It will be interesting to find out whether the establishment of polarity by the sequential action of Rho and Cdc42 has been conserved in other organisms as well."

Neural crest gene *ARID3B* linked to childhood cancer malignancy

Neuroblastomas are the most common form of pediatric tumor, growing in the sympathetic nervous system, which originates from the neural crest. In roughly half of all patients diagnosed with neuroblastoma, the tumor spontaneously regresses without metastasizing, but in the other half the cancer spreads and the prognosis is poor. Expression of the oncogene *MYCN* has previously been shown to be associated with the development of neuroblastoma, but the reasons behind this form of tumor's variable clinical course continue to perplex physicians.

In September, Kenichiro Kobayashi and colleagues in the Laboratory for Stem Cell Biology (Shin-Ichi Nishikawa; Group Director) identified a second factor, *ARID3B*, involved in the development of malignant neuroblastoma. Their work, conducted in collaboration with the Kobe University Graduate School of Medicine and published in the journal *Cancer Research*, indicates that this gene may play a critical role in determining whether or not these tumors follow the path to malignancy, making it a potentially attractive target for cancer therapeutics.

Tumorigenicity of *MYCN*+*ARID3B* mouse embryonic fibroblasts. Mice receiving transplants of MEFs expressing either *ARID3B* (left) or *MYCN* (middle) alone do not develop tumors (7 days post-transplant).



The Nishikawa group had previously revealed that the development of the neural crest was hampered in mice with null mutations for *ARID3B*, pointing to a potential role for the gene as a survival factor in the proliferating neural crest. Other work had also shown that the closely related gene, *ARID3A*, induces the malignant transformation of mouse embryonic fibroblasts when transfected together with *ras* oncogenes. Following these evidence trails, Kobayashi et al. decided to test the hypothesis that *ARID3B* also influences the tumorigenesis of neuroblastomas.

They began by looking at five human neuroblastoma cell lines, which can be grown indefinitely in culture, and measuring the expression levels of *MYCN*, *ARID3A* and *ARID3B* using RT-PCR, a method of amplifying mRNA transcripts. All of the cell lines expressed *MYCN*, as expected, as well as *ARID3B*, which stood in contrast to cells of other cancer types, where *ARID3B* was detected in only two of eight lines. Next they used microarray data from clinical samples obtained from neuroblastoma patients and found that nearly half (9 of 21) of the cells expressed *ARID3B*, and, importantly, 80% were from stage IV neuroblastomas, aggressive tumors which have typically spread to distant sites in the body and are difficult to treat. Statistically speaking, the case for possible linkage between *ARID3B* and malignancy was worth pursuing.



Kobayashi K, Era T, Takebe A, Jakt L M and Nishikawa SI.
ARID3B Induces Malignant Transformation of Mouse Embryonic
 Fibroblasts and Is Strongly Associated with Malignant
 Neuroblastoma. *Cancer Res* 66:8331-6 (2006).

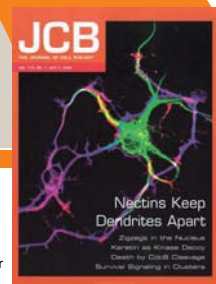
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 American Association for Cancer Research 2006

To test the gene's function, the group conducted gene knockdowns by short interfering RNA and antisense technology to block *ARID3B* in neuroblastoma cell lines, and found that in four of the five lines, this resulted in the suppression of cell proliferation, suggesting the potential of this molecule as a therapeutic target. The transformative potential of the gene was then demonstrated in experiments using mouse embryonic fibroblasts (MEFs). Normally, these cells gradually lose their proliferative ability, but those transfected with *ARID3B* became immortalized, a characteristic of some stem cell and cancer cell lines in which the cells acquire the ability to survive and grow indefinitely in culture. When co-expressed with *MYCN*, *ARID3B* conferred tumorigenicity and anchorage-independent growth, two hallmarks of cancer malignancy, on the MEFs.

The physiological role of *ARID3B* during embryogenesis is thought to involve protecting cells of the neural crest lineage from apoptosis, a function that appears to be hijacked in neuroblastoma tumors. "We first discovered *ARID3B* from its role in abnormal crest development, but online data from cancer samples has now enabled us to link this basic research to a clinically relevant finding," notes Nishikawa. "We have been also able to take advantage of published data sets to identify a possibly related role in some other types of cancers, such as pancreatic cancer, which provides a nice demonstration of what can be achieved relatively simply by harnessing information mining technology with the huge amount of data now available to researchers in the life sciences."



Neurite sorting by nectins



Togashi H, Miyoshi J, Honda T, Sakisaka T, Takai Y and Takeichi M. Interneurite affinity is regulated by heterophilic nectin interactions in concert with the cadherin machinery. *J Cell Biol* 174:141-51 (2006).

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Hideru TOGASHI

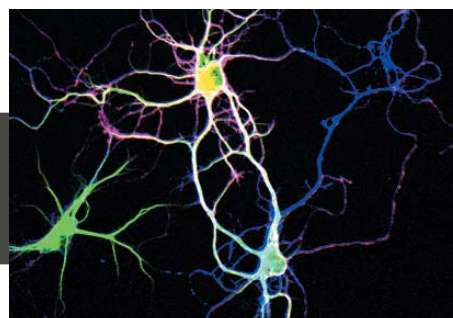
Synapses typically arise when the dendrite of one neuron develops a stable bridge to the axon of another, and indeed in some classes of neuron, such axodendritic junctions are the only type of synapse to form. The question of how axons and dendrites, collectively known as neurites, are able to recognize each other and thus set the stage for this preferential synaptogenesis has been something of a challenge for neuroscientists. In a report featured on the cover of *The Journal of Cell Biology*, Hideru Togashi and colleagues from the Laboratory of Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) outlined a novel molecular mechanism for regulating the affinity between neurites through the preferential binding of adhesion molecules.

The Togashi study started by examining the behavior of two members of the nectin family of adhesion molecules. Previous reports had shown that nectin-1 (N1) localizes mainly to the presynaptic neuronal membrane, while nectin-3 (N3) prefers the postsynaptic side of the junction. Togashi used immunostaining to show that, in hippocampal neurons, N3 is distributed about equally in both axons and dendrites, while N1 is far more abundant in axons. When neurites of different type encountered each other and began to form synapses, both N1 and N3 became concentrated at the point of contact, but interestingly, when a pair of dendrites happened to cross paths, no such concentration was evident. Tests in which these genes were overexpressed resulted in abnormalities in neurite morphology and behavior, which was pinpointed to interactions between different regions of the N1 and N3 molecules.

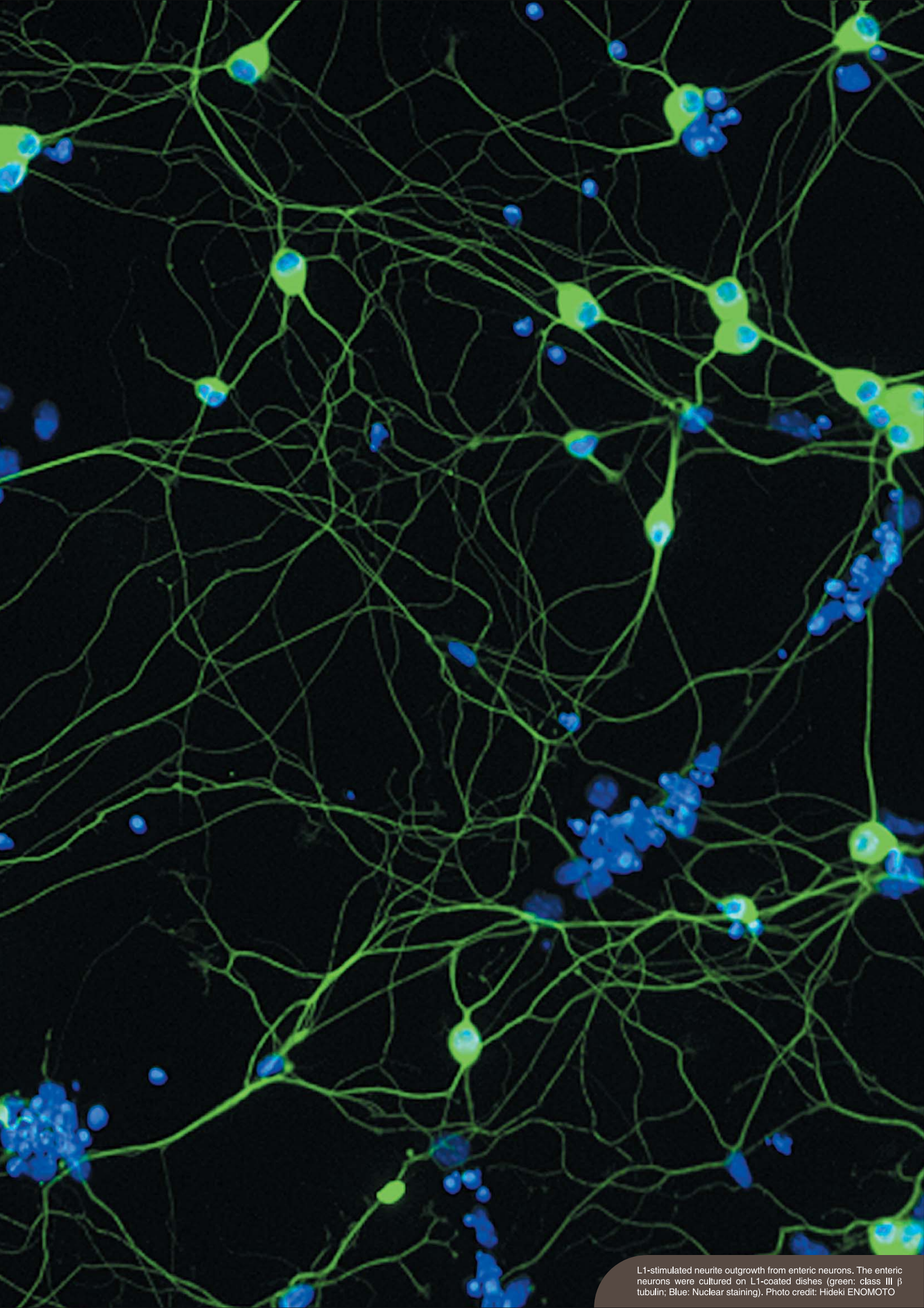
Further immunostaining showed that a component of the cadherin cell-cell adhesion machinery, β -catenin was also present at nectin binding sites, and that its concentration increased proportionally to the intensity of nectin accumulation, indicating possible linkage between nectin and cadherin activity in determining inter-neurite affinities.

This study led Togashi et al. to propose a new model of the nectins' roles in neurite interaction. In this model, N1 molecules on the axon preferentially bind their N3 counterparts on dendrites, which has the secondary effect of promoting homophilic cadherin binding. Without N1, cadherin-based adhesion occurs much less intensely, resulting in weaker connections between cells, while in N1-transfected cells, opportunities for binding increase, causing dendrites (which are normally N1-free) to associate with other dendrites, and even normal axodendritic contacts to overstabilize.

A mixed culture of hippocampal neurons overexpressing nectin-1 (red) and nectin-3 (green). MAP2 is also stained to visualize dendrites (blue). Neurites of these neurons are tightly intertwined due to the heterophilic interactions between the two nectins.



For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.



L1-stimulated neurite outgrowth from enteric neurons. The enteric neurons were cultured on L1-coated dishes (green: class III β tubulin; Blue: Nuclear staining). Photo credit: Hideki ENOMOTO

Cell Fate Decision

Hitoshi SAWA Ph. D.

Hitoshi Sawa obtained his baccalaureate, master's and doctoral degrees from Kyoto University in the period from 1982 to 1991. He worked as a postdoctoral fellow at the California Institute of Technology on a Human Frontier Science Program grant during the period from 1991 to 1994, then at the Massachusetts Institute of Technology from 1994 to 1997 under support from the Howard Hughes Medical Institute. He returned to Japan in 1997 to continue his work at Osaka University, first as a postdoc, then as researcher funded by the Japan Science and Technology Corporation PRESTO program. He took his current position at the RIKEN CDB in 2001.

Staff

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Publications

Arata Y, et al. Wnt signaling and a Hox protein cooperatively regulate *psa-3/Meis* to determine daughter cell fate after asymmetric cell division in *C. elegans*. *Dev Cell* 11, 105-115 (2006).

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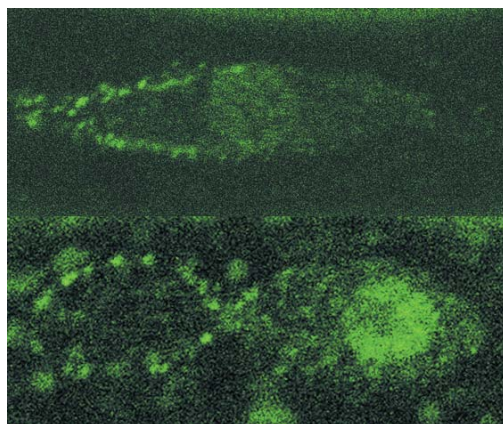
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The development of multicellular organisms involves a single cell (a fertilized egg) that gives rise to a diverse progeny through successive cell divisions. The process of cell division in which the resulting daughter cells are of different characters or "fates" is known as asymmetric cell division. One example of this form of cell division is seen in stem cells, which can produce one self-copy and one more specialized (differentiated) cell in a single division. Asymmetric cell division is central to the generation of cellular diversity, but because in higher organisms a given cell's "family relations" (known as cell lineage) are frequently unclear, it can be difficult to study the process in detail.

In the nematode, *C. elegans*, however, thanks to the transparency of the worm's body it is possible to track the division and other activity of individual cells in the living animal using only a low-magnification light microscope. In our laboratory, we take advantage of this ability to monitor developmental processes in real time, a method that we use in combination with genetic studies to investigate the mechanisms that underlie asymmetric division. We have determined that nearly every asymmetric cell division in *C. elegans* is mediated by β -catenins acting in the Wnt signaling pathway. This pathway works to polarize cells fated to undergo asymmet-

ric division; we seek to gain a better understanding of how it achieves this by studying the intracellular localization of the Wnt pathway's molecular components.

We have also discovered numerous mutant *C. elegans* phenotypes in which the asymmetric division of specific cells is disturbed, allowing us to identify more than 50 genes involved in this process. By investigating the function of these genes, we hope to clarify the biological mechanisms of asymmetric cell division even further.

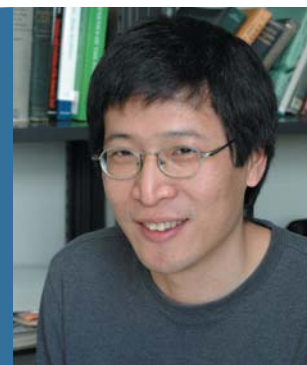


■ Asymmetric localization of WRM-1/ β -catenin before (top) and after (bottom) division

Early Embryogenesis

Guojun SHENG Ph. D.

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



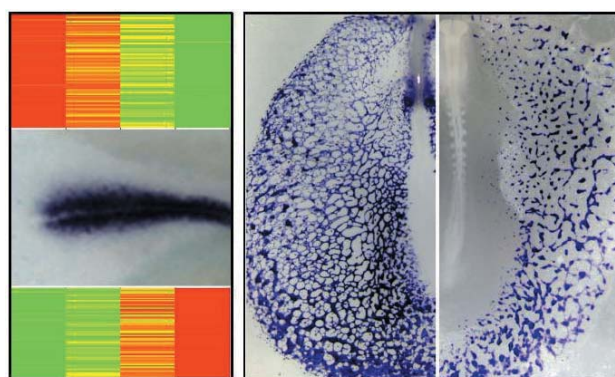
We study the molecular and cellular mechanisms of gastrulation, an early developmental process conserved throughout the animal kingdom. During gastrulation, three germ layers (ectoderm, mesoderm and endoderm) are formed from a uniform layer of epiblast cells. Each of these germ layers undergoes further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

In the chicken embryo, signals from the organizer (Hensen's node) and the primitive streak induce molecular markers specific to either neural ectoderm or mesoderm cells, and control their distinct migratory behaviors. This induction is a multistep

process involving gradual commitment, ultimately leading to the assignment of a terminal fate. We are studying the signaling processes involved in the induction of cell fate-specific gene expression and behavioral changes in these systems.

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

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



■ Left panel: Genechip analysis reveals several hundred dorsal or ventral specific genes for streak mesoderm precursors (red: high; green: low).
Right panel: Lmo2 is expressed in both primitive blood and endothelial cells (left). Inhibition of FGFR signaling abolishes endothelial specific expression while promoting blood specific expression (right).

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Nakazawa F, Nagai H, Shin M and Sheng G. Negative regulation of primitive hematopoiesis by the FGF signaling pathway. *Blood* 108: 3335-43 (2006).

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Developmental Genomics

Asako SUGIMOTO Ph. D.

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

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Motegi F and Sugimoto A. Sequential functioning of the ECT-2 RhoGEF, RHO-1 and CDC-42 establishes cell polarity in *Caenorhabditis elegans* embryos. *Nat Cell Biol* 8:978-85 (2006).

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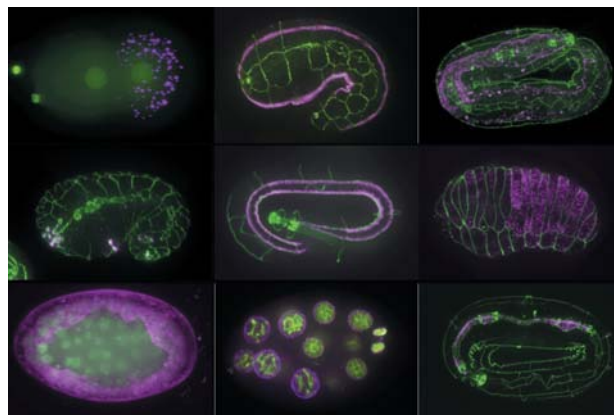
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The cooperation between groups of genes encoded in an organism's genome is essential to the development of complex bodily structures from a single cell, the fertilized egg. Our laboratory has adopted the nematode *Caenorhabditis elegans* as an experimental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

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

We are now beginning to perform live imaging and other cell biological studies of discrete sets of genes identified in our phenomics analyses. One focal topic is the regulation of cell division patterns in the context of development. In the development of multicellular organisms, the timing of the cell cycle needs to be properly regulated in conjunction with processes of cell fate determination and differentiation. An important developmental concern in regard to cell division is that orientation of the cell cleavage plane must be coordinated with the cell's polarity so that cell fate determinants can be properly segregated in the daughter cells. We are interested in how cell division and differentiation are regulated interactively in the context of embryonic ontogeny. We use 4-dimensional imaging and quantitative evaluation of the image data thus obtained to analyze these dynamic events. And, in a concurrent project, we are developing new techniques and tools (e.g., cell-type- or sub-cellular-structure-specific monoclonal antibodies and GFP markers) to improve the temporal and spatial resolution of phenome analysis. By studies such as these, we hope to provide a more detailed picture of the regulation of dynamic processes by networks of genes.



Immunostaining with new series of monoclonal antibodies recognizing specific cell types or subcellular structures.

Retinal Regeneration

Masayo Takahashi M.D., Ph.D.

Masayo Takahashi received her M. D. from Kyoto University in 1986, and her Ph. D. from the same institution in 1992. After serving as assistant professor in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1996, where she discovered the potential of stem cells as a tool for retinal therapy. She came back to Kyoto University Hospital in 1998, and since 2001 served as an associate professor at the Translational Research Center in the Kyoto University Hospital. She joined in the CDB as a team leader of the retinal regeneration research team in 2006. Her clinical specialty is retinal disease, macular diseases and retinal hereditary diseases in particular. Her aim is to understand these diseases at a fundamental level and develop retinal regeneration therapies.



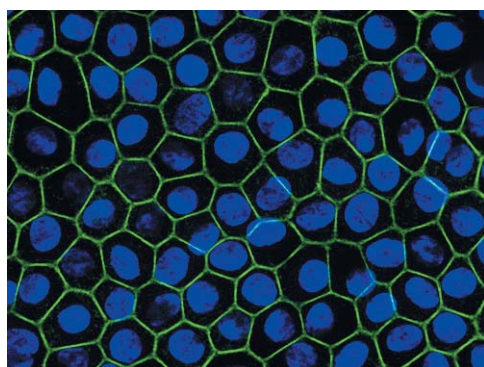
The retina has been called the "approachable part of the brain," owing to its relatively simple structure and its location near the body surface, and for these reasons it serves as a useful and experimentally amenable model of the central nervous system. Until very recently, it was thought that in adult mammals the retina was entirely incapable of regenerating, but we now know that at least new retinal neurons can be generated after being damaged. This has opened up new hope that the ability to regenerate neurons and even to reconstitute the neural network may be retained in the adult retina. We are now exploring the exciting prospect that, by transplanting cells from outside of the retina or by regeneration from intrinsic progenitor cells, it may one day be possible to restore lost function to damaged retinas.

Our research into retinal regeneration seeks to achieve clinical applications by developing methods for inducing stem cells to differentiate into retinal neurons and retinal pigment epithelial cells in sufficient quantities for use in the treatment of patients suffering from conditions in which such cells have been damaged or lost, and to ensure

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

Therapeutic applications cannot be developed from basic research alone; the clinical approach—a thorough understanding of the medical condition to be treated—is equally important. For conditions such as retinitis pigmentosa, even the successful transplantation of cells in animal models may not necessarily be translatable to a human clinical therapy without an understanding of the underlying genetics and possible immunological involvement.

Our goal is to study retinal regeneration based on both a strong foundation in basic research and solid clinical evidence.



■ Hexagonal shapes of retinal pigment epithelial cells derived from monkey ES cells.

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Suzuki T, Mandai M, Akimoto M, Yoshimura N, Takahashi M. The simultaneous treatment of MMP-2 stimulants in retinal transplantation enhances grafted cell migration into the host retina. *Stem Cells*. 24: 2406-2411, (2006).

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Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph. D.

Masatoshi Takeichi is director of the RIKEN Center for Developmental Biology as well as director of the Cell Adhesion and Tissue Patterning research group. He completed the B. Sc. and M. S. programs in biology at Nagoya University before receiving a doctorate in biophysics from Kyoto University in 1973. After attaining his Ph. D., he took a research fellowship at the Carnegie Institution Department of Embryology under Richard Pagano. He then returned to Kyoto University, attaining a full professorship in the Department of Biophysics (1986-1999), before becoming professor in the Department of Cell and Developmental Biology in the Graduate School of Biostudies at the same university. He assumed the directorship of the CDB in 2000.

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Publications

Kametani Y and Takeichi M. Basal-to-apical cadherin flow at cell junctions. *Nature Cell Biol* 9:92-98 (2007).

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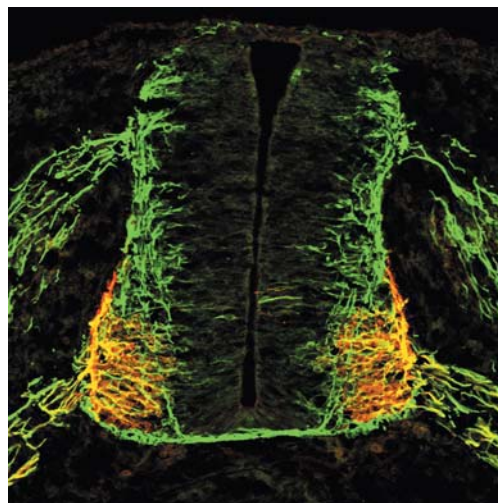
Tanoue T and Takeichi M. Mammalian Fat1 cadherin regulates actin dynamics and cell-cell contact. *J. Cell Biol.* 165, 517-528. (2004)

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

Cell-cell adhesion is dynamic and reversible, which is important for regulating many types of morphogenetic cell behavior. Cadherin activity is modulated by interactions with cytoskeletal or signaling factors mediated by members of the catenin family of cadherin-associated proteins. We are studying the mechanisms underlying the crosstalk between cadherins and cytoskeletal or signaling systems, with the goal of uncovering novel regulatory mechanisms specific to cell-cell adhesion and tissue construction. The outcomes of these studies are expected to contribute to our understanding of the molecular basis of not only normal morphogenesis but also cancer invasion, in which cell-cell adhesion is assumed to be destabilized.

Another area of interest to our lab is the mechanisms by which neural tissues are organized through the processes of dynamic cell rearrangement, such as cell migration and relocation. Using cerebral cortices and cerebellum as model systems, our team is attempting to determine how cell migration and positioning are controlled by cadherins and catenins during the formation of laminar structures in these tissues.

We are also interested in the mechanisms underlying the formation of neural networks, particularly interneuronal recognition during synapse formation. The cadherin/catenin complex is localized in synaptic contacts, and different cadherin subtypes are expressed by different neurons of the brain. We are investigating ways to determine how synaptic contact formation is regulated by this adhesion system. We are also interested in the roles of other cell surface molecules, such as OL-protocadherin, Fat cadherins and nectins, in neural morphogenesis, particularly in interneuronal recognition processes.



Double-immunostaining for OL-protocadherin (red), a member of the cadherin superfamily, and neurofilament (green) in the spinal cord of chicken embryo.

Systems Biology

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

Hiroki R. Ueda received his M. D. from the Faculty of Medicine of the University of Tokyo in 2000, and received his Ph. D. from the University of Tokyo Graduate School of Medicine in March 2004. While an undergraduate, he worked as a research assistant on a biological simulation system project at Sony Computer Science Laboratories. As a graduate student he next went on to work, first as a researcher (2000) and then as a group leader (2002), at Yamanouchi Pharmaceutical, on a project studying biological clock mechanisms in fly and mouse. He was appointed team leader of Laboratory for Systems Biology in 2003, and Leader of the Functional Genomics Subunit at the CDB in 2004. He was also appointed as Professor (Invited) of Osaka University in 2006.

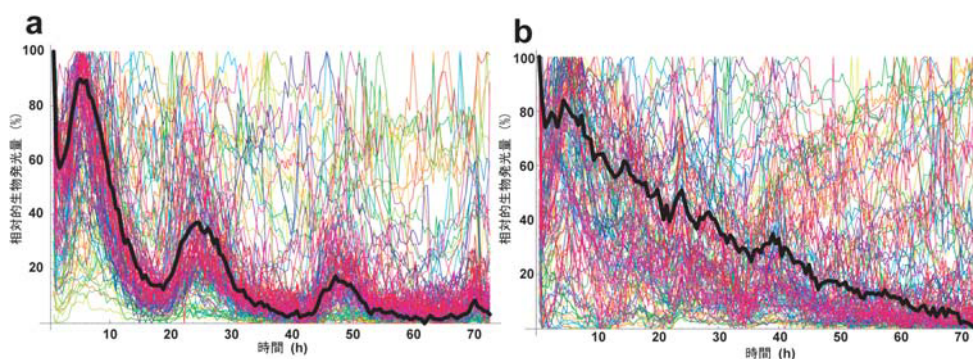


Recent large-scale efforts in genome-sequencing and expression analysis have produced an embarrassment of riches for life science researchers—biological data can now be accessed in quantities that are orders of magnitude greater than were available even a few years ago. Now, the growing need for integration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

Our lab takes the mammalian circadian clock as a relatively simple and self-contained initial model for the study of a biological system. In addition to its advantages as a basic research model, the function of the circadian clock is intimately involved in the control of metabolic and hormonal cycles, and its dysregulation is implicated in the etiology of numerous human diseases, including sleep disorders. An improved understanding at

the system level promises to provide biomedical and clinical investigators with a powerful new arsenal to attack these conditions.

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



■ Feedback repression is required for mammalian circadian clock function

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Sato T K, et al. Feedback repression is required for mammalian circadian clock function. *Nat Genet* 38:312-9 (2006).

Ueda H R, et al. System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat Genet* 37:187-92 (2005).

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Genomic Reprogramming

Teruhiko WAKAYAMA Ph. D.

Teruhiko Wakayama received his B. Sc. and M. Sc. from Ibaraki University, and was awarded a Ph. D. in reproductive biology from the University of Tokyo Department of Veterinary Anatomy in 1996. He received a postdoctoral fellowship from the Japanese Society for the Promotion of Science in 1996 and spent the next two years at the Yanagimachi lab in the University of Hawaii Medical School, where he succeeded in creating the world's first cloned mouse. He was appointed to an assistant professorship at the same institution in 1998, and moved to the Rockefeller University as a research assistant professor in 1999. He spent a year as a researcher at Advanced Cell Technology before returning to Japan to take his current position at the RIKEN CDB.

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Wakayama S, et al. Establishment of mouse embryonic stem cell lines from somatic cell nuclei by nuclear transfer into aged, fertilization-failure mouse oocytes. *Curr Biol* 17(4):R120-1 (2007).

Hikichi et al. Differentiation potential of parthenogenetic embryonic stem cells is improved by nuclear transfer. *Stem Cells* 25:46-53 (2007).

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A theoretically limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual cells and their nuclei, that fiction has become reality. At only a few percentage of all attempts, the efficiency of the cloning procedure (measured as the ratio of live offspring to cloning attempts), however, remains quite low, and even in those attempts that do lead to live births, the cloned animals consistently exhibit a range of severe developmental abnormalities and pathologies.

The cause of these defects is thought to be due to imperfections in the nuclear transfer technique used to remove the nucleus from a host cell and replace it with another from a donor, imperfections that presumably lead to total or partial failure of reprogramming, the process in which a cell's developmental potential is reset to a totipotent state (such as is exhibited by a naturally fertilized egg). However, the specific details of the technical

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

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



■ Transfer of a somatic nucleus into an enucleated egg

Cellular Morphogenesis

Shigenobu YONEMURA Ph. D.

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

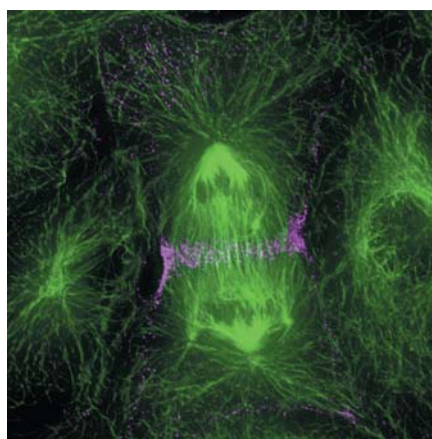


The body's cells exhibit a variety of shapes, a diversity that reflects the truism that form is at least a partial determinant of function. Cells' interiors contain networks of protein polymers called cytoskeletons. Although these structures are thought to play a major role in cellular morphogenesis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

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

determined the signaling pathway connecting microtubules and Rho by knocking down a number of microtubule-associated proteins. Regulation by Rho appears also to be deeply involved in the determination of the cell division plane.

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



■ Distribution of microtubules (green) and Rho (magenta) in a dividing cell.

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Publications

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Animal Resources and Genetic Engineering

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

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

provides a number of other services, such as cloning by nuclear transfer and cryopreservation of mouse zygotes and sperm. The lab also performs a number of maintenance and logistical functions, such as the specific pathogen free (SPF) housing, cleaning, processing and distribution of animals.

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



Chimera mice.

Leading Project Research Units

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

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

Cell Plasticity

Mitsuko KOSAKA Ph. D.

Recent extensive studies have reported that mammalian stem cells residing in one tissue have the capacity to produce differentiated cell types for other tissue and organs. However, despite a number of promising studies describing the plasticity of adult stem cells, many questions remain to be answered.

The regeneration of lens tissue from the iris of newts has become a classical model of developmental plasticity, although little has been known about the corresponding plasticity of the mammalian iris. We have recently demonstrated the retinal stem/progenitor properties of postnatal and adult mammalian iris pigment epithelium (IPE) cells, and suggested that discrete cell populations with heterogeneous developmental potencies exist within the IPE. Using the IPE system, we are trying to unravel the cellular and molecular basis of multipotent stem cells in adult ocular tissue. We hope this project will open new avenues in the field of tissue stem cell biology and therapeutic approaches.



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Organ Regeneration

Hideki TANIGUCHI M. D. , Ph. D.

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

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



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The Genome Resource and Analysis Subunit aims to support a wide range of gene analysis research, ranging from DNA sequencing and gene expression analysis to storage and distribution of gene resources. Our facility can perform gene expression analysis and high-throughput gene screening by using a DNA sequencing system which can analyze over 100,000 genes a year and can also custom make DNA microarray systems. Gene resources derived from a variety of species can also be stored and distributed to researchers according to their requests. By building upon existing technology with the challenge of creating new techniques and ideas, as well as providing solid support, we aim to respond flexibly to the needs of each research request.



Functional Genomics

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The Functional Genomics Subunit provides genome-wide expression analysis using DNA chip service for all laboratories in the Center for Developmental Biology (CDB), and develops advanced DNA chip technologies such as single-cellular genome-wide expression analysis for future DNA chip service. In addition to these services, the Functional Genomics Subunit will develop advanced high-throughput assay technologies such as high-throughput transfection assays, using tens of thousands of RNAi vectors and full-length cDNA clones.



Proteomics Support Unit

Shigeo HAYASHI Ph. D.

Mass Spectrometry Analysis

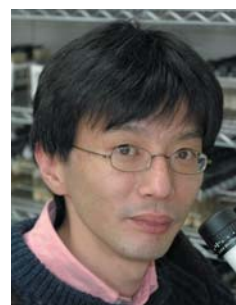
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Akira NAKAMURA Ph. D.

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



Animal Facility



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

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

Research Aquarium



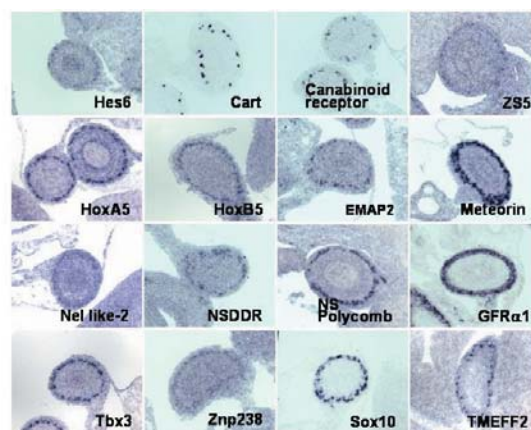
The CDB research aquarium serves as home to a number of water-dwelling species used in research into development and regeneration, including the zebrafish and the African clawed frog (*Xenopus laevis*). These, and other aquatic species, provide useful models for studies ranging from developmental genomics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

The aquarium includes 7 temperature and humidity-controlled rooms geared to providing optimal climates for the handling and breeding of freshwater and marine species, and utilizes reverse osmotic technology to maintain consistent tank-water purity. In addition to the commonly used zebrafish and *Xenopus* frog, the facility also houses specimens from more novel models used in evolutionary development research, such as the lamprey, *Lampetra japonica*, and the hagfish, *Eptatretus burgeri*.

Researchers SNARE novel role for protein family

The complex system of nerves that surround the esophagus, stomach, and intestines consists of millions of sensory and motor neurons, information processing circuits and glial cells that are involved in transmitting and processing messages throughout this region. Known as the enteric nervous system (ENS), this network comprises neurons and supporting glia that line the intestinal wall and are derived from neural crest cells. These proliferating neural crest cells move through the developing intestine and migrate through the intestinal wall to subsequently differentiate into various neurons and supporter glial cells. The ensuing adult ENS plays a number of crucial roles within the enteric environment, including regulating intestinal motility and secretion, and controlling intestinal blood flow. Although a small number of genes have been identified as being involved in ENS development, they are insufficient to explain the complex processes involved in forming the ENS. Molecular mechanisms underlying neurite extension and ENS precursor migration, in particular, remain poorly understood.

Expression patterns of genes identified by microarray analysis of enteric nervous system of E13.5 mouse embryo



In an article published in *Developmental Biology*, Hideki Enomoto (Team Leader; Laboratory for Neuronal Differentiation and Regeneration) and colleagues from the Washington University School of Medicine identified a number of genes that are highly expressed in the mouse ENS. In attempting to investigate these genes in more detail, Enomoto and colleagues found that members of the SNARE protein family play an important role in neurite extension and ENS precursor migration. Although SNARE proteins were known to function in mature synapses, this is the first report to show these proteins are involved in such an early stage of development as the formation of the ENS.

In an attempt to shed light on the genes involved in ENS development, Enomoto and colleagues first compared gene expression in wild-type and aganglionic bowels from embryonic and newborn mice. This involved using a combination of DNA microarray analysis and quantitative real-time polymerase chain reaction (qRT-PCR). The results of this analysis revealed 83 genes that were expressed at two-fold higher levels in the wild-type bowel than in the aganglionic bowel. The authors then selected 42 of these 83 genes for in-situ hybridization, of which 39 were found to be expressed in the ENS. This analysis also revealed a further 9 genes that had higher expression levels in the aganglionic bowel compared to wild-type mice, suggesting to the authors that intestinal innervation may influence gene expression in adjacent cells, such as the intestinal epithelium.

The majority of genes identified in this study all play an important role in synaptic function, such as *Synaptotagmin 1*, involved in fast neurotransmitter release, and *Reticulon 1*, which has a role in regulating exocytosis. Of particular interest to the authors, however, was the fact that these genes were expressed

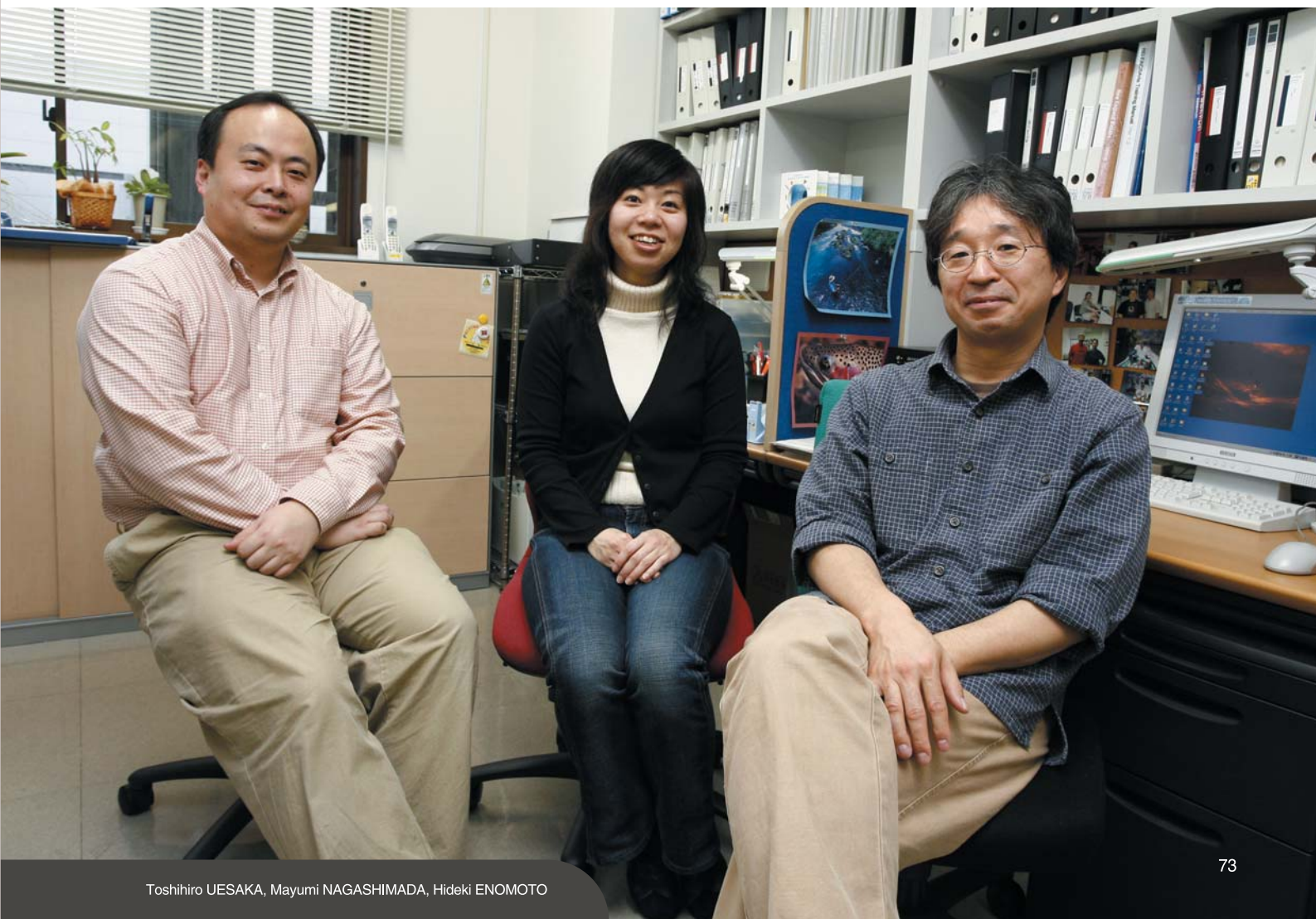


Vohra B P, Tsuji K, Nagashimada M, Uesaka T, Wind D, Fu M, Armon J, Enomoto H and Heuckeroth R O. Differential gene expression and functional analysis implicate novel mechanisms in enteric nervous system precursor migration and neuritogenesis. *Dev Biol* 298:259-71 (2006).

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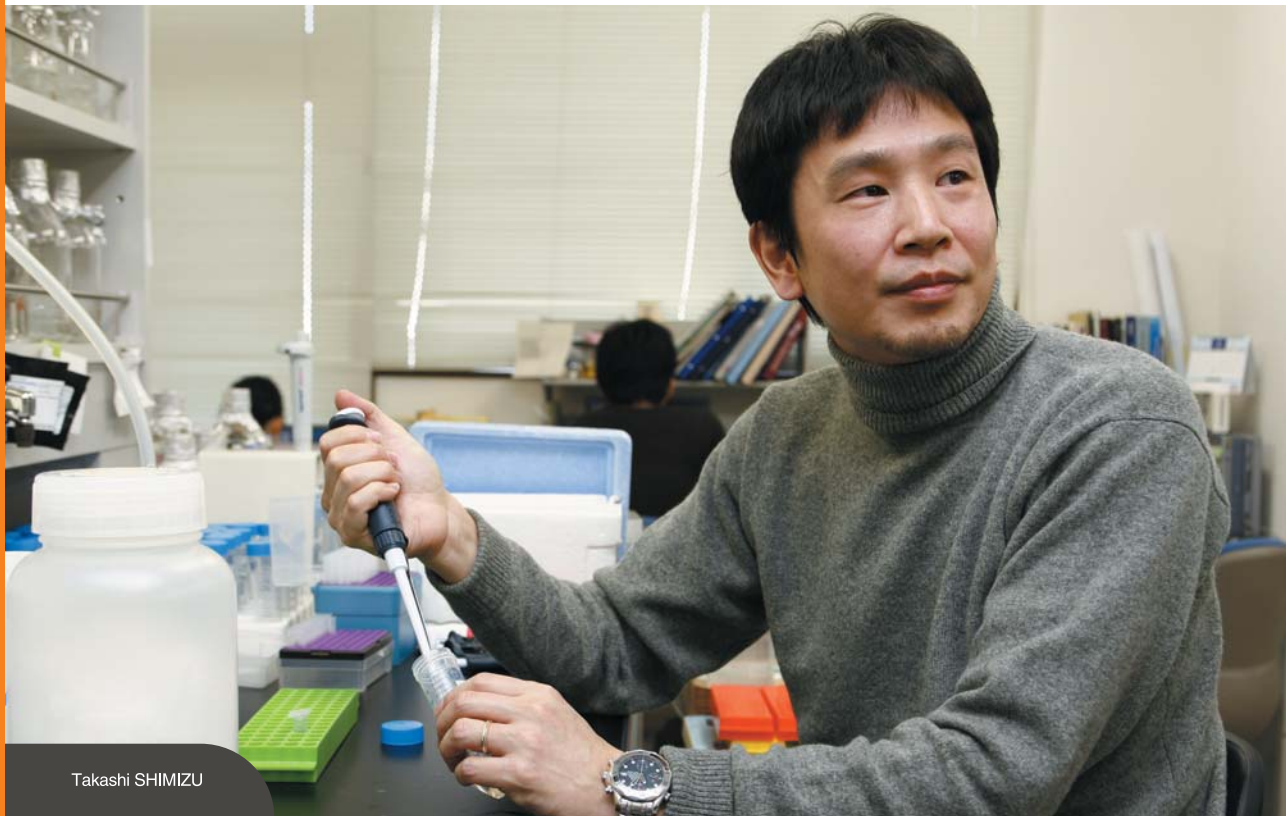
at E.14—a stage of development in which the ENS is not yet required for survival. This prompted Enomoto and colleagues to put forward a scenario in which SNARE proteins are involved in the developing ENS. Armed with the knowledge that the SNARE family of proteins acts in membrane fusing of synaptic vesicles, the team set out to examine the possibility that SNARE-mediated vesicle fusion is essential for both neurite extension and ENS precursor migration. In attempting to examine this issue in more detail, the authors subjected cultured embryonic bowel to a chemical inhibitor that blocked certain SNARE proteins, resulting in delayed neural-crest-derived cell migration and reduced neurite growth. Based on these findings, Enomoto et al. concluded that the SNARE family of proteins is not only involved in synaptic function, but also plays an important role in ENS development.

In addition to identifying a number of genes expressed in the enteric nervous system, this is the first study to show that SNARE proteins have an important role in ENS development. The authors hope that their current findings, together with their ongoing analysis of genes of interest, will provide a fresh perspective on irregularities in the ENS developmental process. Enomoto commented that, “developmental anomalies in the enteric nervous system are a direct cause of impaired bowel function in the form of Hirschsprung’s disease. We hope that the results of this study provide a basis for further research findings that could help in curing such diseases.”



A question of competence

Neural tissue responsiveness to FGF and RA controlled by Cdx



Takashi SHIMIZU

Fibroblast growth factors (FGFs) and retinoic acid (RA) represent two important molecular signals in the formation and patterning of the vertebrate posterior hindbrain, where the spinal cord meets the tail end of the brain. They function in a broad range of other developmental processes as well, indicating that the ability to respond to these signals must be closely linked to the site of activity, and regulated accordingly. The brain itself is highly ordered along anterior-posterior (A-P) axis—regionalized into forebrain, midbrain, hindbrain and spinal cord compartments in a head-to-tail direction. It has been suggested that FGF and RA are involved in the formation of these neural structures, but it remains unclear how they can form these structurally distinct regions, and the factors controlling tissue responsiveness to FGFs and RA remain unknown.

In an article published in the journal *Development*, Takashi Shimizu and colleagues in the Laboratory for Vertebrate Axis Formation (Masahiko Hibi; Team Leader) showed that a pair of Cdx-family proteins, Cdx1a and Cdx4, controls the neural tissue's responsiveness to FGF and RA signaling. Studying the effects of the inhibition of these two factors in the embryonic zebrafish, they found that loss of Cdx1a/4 function induces the ectopic expression of markers of posterior hindbrain and anterior spinal cord, dependent on RA and FGF.

Shimizu et al. used antisense technology (a gene knockdown technique in which small synthetic molecules called morpholinos are used to bind to complementary sequences of RNA, blocking transcription), and found that Cdx1a and Cdx4 are required for posterior spinal cord development, and that their loss of function results in the ectopic formation of posterior hindbrain and anterior spinal cord, suggesting that they normally suppress this fate. Interestingly, these aberrant regions develop as a kind



Shimizu T, Bae Y K and Hibi M. Cdx-Hox code controls competence for responding to Fgfs and retinoic acid in zebrafish neural tissue. *Development* 133:4709-19 (2006).

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of mirror image of the normal tissues. The ectopic sites include neuronal populations typical of the hindbrain, and exhibit reversed patterns of expression of a number of hindbrain markers. Tests in which Cdx1a and -4 were inhibited singly indicated that Cdx4 works at least partially redundantly in suppressing hindbrain development, but that the phenotypes in the *cdx1a/4* and *cdx4* solo morphants were different, nonetheless.

The team turned next to the FGFs and retinoic acid, which are also known to impact on hindbrain development, and whose opposing patterns of expression overlap at the approximate intersection of hindbrain and spinal cord. In the *cdx* morphants, these countervailing gradients also manifested in the caudal end, but were flipped along the A-P axis, with subtly different peaks of activity in *cdx4* and *cdx1a/4* embryos, with the region of maximal FGF and RA activity being more posterior in the double morphant.

Suspecting that the region of overlapping FGF and RA signaling represented an ectopic environment that mimics the normal developmental field for the posterior hindbrain, Shimizu used specific inhibitors to interfere with FGF (*fgf3* and *fgf8*) and retinoic acid signaling in the *cdx* morphants. While the perturbation of either of these signaling pathways had no discernible effect on the other, their loss of function did affect the expression of other hindbrain markers in the ectopic neural tissue, with FGFs responsible for markers for rhombomeres 4 to 6, and retinoic acid necessary for the ectopic expression of genes specific to the anterior spinal cord and the caudal-most region of the hindbrain. In *cdx1a/4* morphant embryos in which both FGF and RA signaling was blocked, the mirror-image ectopic region failed to form.

The Cdx-defective embryo has an extra hindbrain. Ectopic expression of *krox20* is detected in the posterior neural tissue of a *cdx1a/4* (italics) morphant embryos.



Of the genes whose expression was lost in the *cdx1a/4* morphants, several were posterior-expressed members of the Hox family of patterning genes, suggesting that Hox signaling functions downstream of Cdx. Hox genes are known to work both as activators and repressors, prompting the team to try to untangle their role in this context. On constructing and expressing sequences in which either a repressor or an activator domain of was fused to a posterior Hox gene, the Hibi team found that while the repressor construct had no effect on ectopic gene expression in the *cdx1a/4* morphant, the activator fusion protein inhibited both normal and ectopic expression of its target gene (as wild-type did), suggesting that the posterior Hox proteins function as transcriptional activators that indirectly suppress posterior hindbrain development.

Shimizu's findings highlight a surprisingly complex web of signaling at work in the formation of the posterior hindbrain and anterior spinal cord, in which Cdx activity in setting up regional competence to respond to FGF and retinoic acid signaling appears to be mediated by posterior members of the Hox family. In normal development, the expression of Cdx1a and -4 appear to control the responsiveness of the neural tissue to FGF and RA signals and suppress more anterior fates, such as hindbrain and anterior spinal cord; in the absence of inhibitory input from these Cdx factors, anterior tissues form ectopically.

"Cdx genes were thought to control posterior body formation," notes Hibi on the surprising findings, "but our study reveals that Cdx genes also function to repress anterior fate by causing a switch from tissue responsiveness to inductive signals."

Asymmetric chromatin remodeling in mammalian oocytes



Yoshida N, Brahmajosyula M, Shoji S, Amanai M and Perry A C. Epigenetic discrimination by mouse metaphase II oocytes mediates asymmetric chromatin remodeling independently of meiotic exit. *Dev Biol* (2006).

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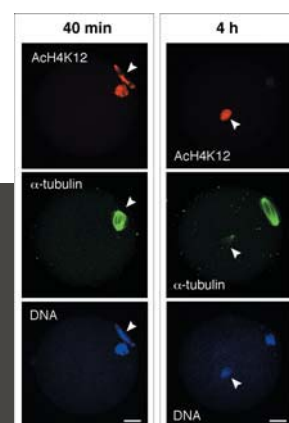
Naoko YOSHIDA, Manjula BRAHMAJOSYULA, Anthony C. F. PERRY, Manami AMANAI, Shisako SHOJI

In an article published in the journal *Developmental Biology*, Naoko Yoshida and colleagues from the Laboratory of Mammalian Molecular Embryology (Tony Perry; Team Leader) addressed the question of whether chromatin remodeling is accomplished by proteins synthesized after fertilization, or by maternally-supplied factors. During fertilization, the entry of a sperm induces cell-cycle (meiotic) progression, causing the oocyte to start behaving like an embryo. To measure the remodeling effects of oocyte (maternal, as opposed to embryonic) cytoplasm on sperm chromatin, the team eliminated the ability of sperm to induce cell cycle resumption (which normally occurs at fertilization), and analyzed the changes undergone by inactivated sperm chromatin within mII-arrested oocytes. Immunofluorescence microscopy showed that the sperm chromatin acquired maternally-derived histones independently of meiotic progression. However, the maternal cytoplasm exerted differential effects on the genomes supplied by each parent; maternal histone H3 methylation was high, while methylation of paternal H3 on lysines 4 and 9 (K4 and K9) remained low.

In mitotic cells, the level of histone acetylation usually up-regulates gene expression, so it could play an important role in very early embryos. In the mII oocyte, paternal, but not maternal, chromatin acquired maternally-derived K12-acetylated H4 (AcH4-K12) independently of microtubule assembly and regardless of whether or not maternal chromatin was present. In contrast, somatic cell nuclei underwent rapid H4 deacetylation; sperm and somatic chromatin even exhibited unequal AcH4-K12 dynamics when injected together into the same mII oocyte. This implies an extraordinary ability of the mII oocyte to discriminate between two types of exogenous chromatin and modify them in different ways.

But how and why do mII oocytes achieve this discrimination? The inhibition of somatic histone deacetylation showed that histone acetyl transferase (HAT) activity was present in the oocytes together with histone deacetylase (HDAC). From a series of experiments, Yoshida and colleagues inferred that mammalian oocytes are able to specify the histone acetylation status of given nuclei by differentially targeting HAT and HDAC activities. However, the team also found that asymmetric H4 acetylation of the two parental chromatin sets during and immediately after fertilization was dispensable for development when both were hyperacetylated, so the function of this differential targeting remains enigmatic. These studies delineate non-zygotic chromatin remodeling and suggest a powerful standardized model with which to study de novo genomic reprogramming.

All chromatin remodeling events are equal, but some are more equal than others. Immunofluorescence microscopy of AcH4-K12 (red) in enucleated mII oocytes coinjected with an inactivated sperm head plus a cumulus cell nucleus. Spindles are indicated by α -tubulin staining (green, second row) and genomic DNA is stained blue (third row). Each column represents the same injected oocyte, with times post-injection indicated at the top. Arrowheads show the sperm-derived genome. AcH4-K12 is high in both the sperm and cumulus nucleus after 40 min but markedly declines only in the cumulus nucleus. Scale bars = 20 μ m.



For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.

Cv2 boosts BMP signal

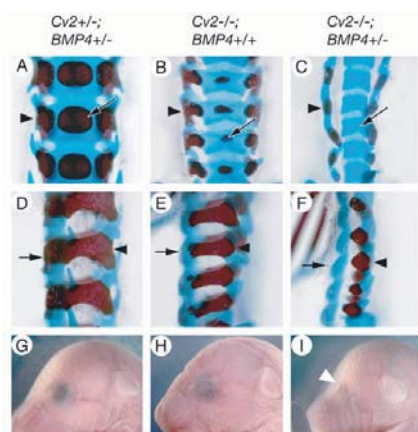


Ikeya M, Kawada M, Kiyonari H, Sasai N, Nakao K, Furuta Y and Sasai Y. Essential pro-Bmp roles of crossveinless 2 in mouse organogenesis. *Development* 133:4463-73 (2006).

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Potent signaling factors, such as the bone morphogenetic protein (BMP) family of molecules, need to be regulated by a system of checks and balances to ensure that they operate at precisely the right time, location and intensity during development. The BMPs are famously counteracted by a set of BMP antagonists in the dorsal side of the embryo. But positive regulation plays an important role as well, occurring when amplified signaling is called for at a particular site or stage.

In an article published in the journal *Development*, Makoto Ikeya and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) reported such a role for the *Cv2* gene, which works to amplify BMP signaling in the development of a number of tissue and organ systems in mouse development.



The deletion of one copy of *BMP4* strongly and cooperatively enhanced the vertebral defect of *mCv2*^{-/-} mutants, causing reduction of the vertebral body in size and of its ossification and suppression of vertebral arch development. Moreover, the reduction of the *BMP4* gene dosage synergistically increased the frequency of the microphthalmic phenotype.

Ikeya et al began the study by generating knockout mice carrying a null mutation for *Cv2* to study its role in murine embryogenesis. Although the appearance of these mutants was not dramatically different than that of wild type animals, they showed a range of embryonic phenotypes and defects and all died in the perinatal period. The pattern of these defects suggested that the loss of *Cv2* prevents the differentiation of precursor cells derived from the sclerotome, a subset of cells that migrates out from the somites to give rise to the vertebrae. But further analysis indicated that the effect was not due to a simple, generalized reduction in BMP gene expression, but rather pointed to a role for *Cv2* upstream of or at the level of the BMP molecular receptor. Ikeya next found that in mice carrying a null mutation for *Cv2*, the loss of a single copy of the gene *BMP4* significantly intensified the defects in both vertebral and eye development, suggesting a cooperative relationship between the genes.

As the BMPs are known to be required for renal development the group next looked at kidneys in *Cv2* mutants, and found that they were smaller and contained fewer renal glomeruli than normal. Subsequent testing revealed that the genes work together in the regulation of kidney development. This thoroughgoing tissue-by-tissue analysis of the effects of loss of *Cv2* function reveals that the gene is required in highly specified processes to enhance BMP. Whether this context-sensitive pro-BMP of *Cv2* is achieved by enhancing the association of BMP ligands and receptors or by some other mechanism, remains unknown.

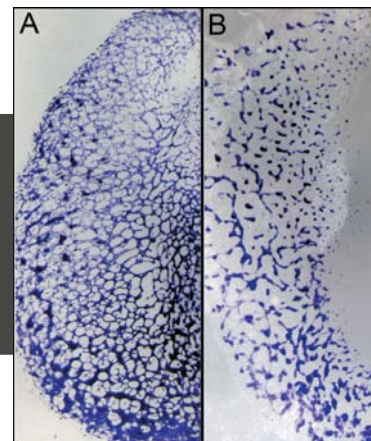


Makoto IKEYA, Masako KAWADA

FGF signaling keeps primitive blood in check

Hematopoiesis, the formation of blood, begins enigmatically, with embryonic development featuring two distinct and indeterminately related phases of this process—a late wave of definitive hematopoiesis and an earlier transient burst of primitive blood generation by clusters of cells known as blood islands scattered throughout the extraembryonic mesoderm. These clusters give rise to both blood and endothelial cells that form vascular lining. The decision-making process by which uncommitted precursors in the blood islands are induced to settle on a terminally differentiated state is known to involve regulation by a number of signaling factors, but the role of the fibroblast growth factor (FGF) family of molecules, which function pleiotropically in many aspects and stages of development, is only poorly understood.

Lmo2 marks both blood and endothelial cells in control (A), but is only expressed in blood cells when FGF signaling is blocked by SU5402 treatment (B).



Fumie Nakazawa and Hiroki Nagai in the Laboratory for Early Embryogenesis (Guojun Sheng, Team Leader), in article published in the journal *Blood*, showed an important function for the FGF receptor FGFR2 in regulating cellular fate during primitive hematopoiesis. Investigating patterns of gene expression and FGF gain and loss of function in the embryonic chicken, Nakazawa and Nagai demonstrated that FGF signals potently inhibit hemoglobin expression (a hallmark of hematopoiesis) and upregulate endothelial markers, while loss of FGF signaling results in ectopic blood formation.

The study began with the development of an alternative to conventional approaches to identifying blood during development by staining differentiated cells with chemical labels. Looking instead at expression patterns of mRNAs for a quartet of hemoglobin genes known to be expressed in primitive blood cells, the Sheng team found they were able to detect blood cells as they formed in situ in the chick embryo, and selected one of the genes, ρ , as their marker of choice for more detailed investigations of primitive hematopoiesis. First, the researchers examined the effects of embedding tiny beads soaked in various signaling molecules into regions within and outside the future ρ domain and found that FGF-soaked beads exerted a strongly inhibitory effect on ρ expression without, however, affecting the region's ability to express endothelial markers.

Nakazawa and Nagai next tried the converse experiment, using beads soaked in a molecular inhibitor of FGF signaling, and found that primitive blood cells began to form ectopically in both grafts and intact embryos, while the endothelial cells that give rise to the vasculature failed to form. The inhibitory agent used was a general inhibitor of all FGF receptors (FGFRs), of which there are four in the chick, so they next examined the patterns of gene expression for each of the FGFRs in situ and found FGFR2, which is expressed in endothelium and absent in blood cells, to be the likeliest candidate for a specific regulator of primitive hematopoiesis in the extraembryonic mesoderm.

Nakazawa F, Nagai H, Shin M and Sheng G. Negative regulation of primitive hematopoiesis by the FGF signaling pathway. *Blood* 108:3335-43 (2006).

Image courtesy Guojun SHENG



Zeroing in on this target, they used electroporation to introduce a constitutively active version of FGFR2 into early embryos and compared their subsequent cellular composition with that in control embryos. In the control sample, roughly one-third of the cells expressed ρ (indicating a blood fate), another third were of endothelial lineage, with the remainder being of other cell types. In the FGFR2 embryo, however, less than 3% of the cells were found to express ρ , and more than 80% differentiated into endothelium. When morpholinos were used to block the function of the constitutively active FGFR2, the opposite effect was seen.

Having demonstrated a strongly inhibitory effect for FGFR2 on primitive hematopoiesis, the Sheng lab sought to put the receptor in context within the many molecules signals known to impact on blood differentiation. Inhibition of VEGF signaling, another pathway implicated in blood and endothelial development, yielded no appreciable effect on the expression of either ρ or the endothelial marker, *Lmo2*. But beads soaked in the FGF ligand FGF4 had a negative effect on the expression of *Gata1*, a member of a second set of genes known to be expressed in the blood lineage immediately prior to the onset of globin expression. And in FGF-inhibited embryos, *Gata1* expression was found to be upregulated and expanded in patterns similar to those seen for ρ .

This work by Nakazawa, Nagai and colleagues points to a role for the receptor FGFR2 in inhibiting primitive blood differentiation, but it remains to be seen which FGF ligand is at work in this process. And while FGFR2 has now clearly been shown to play an important part, the intricate web of molecular pathways that establish and maintain the tight balance between blood and vascular differentiation in clusters of cells during early development continues to hold a few secrets.

"We know very little of how blood island cells are set aside initially and how the differentiation of smooth muscle cells (which are not derived from blood islands) is coupled with that of the endothelial cells," says Sheng. "And it is of tremendous importance, both for us and for people working in stem cell biology, to know how a very small percentage of blood island cells remains undifferentiated."

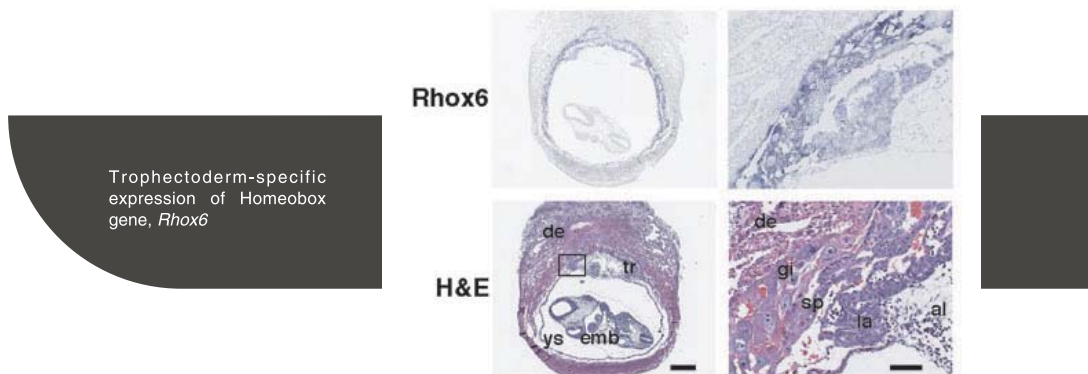


Dnmts discriminate

Lineage- and stage-specific *Rhox* regulation in the early embryo

Essentially every cell in the body contains a full set of the same genetic information, making it critically important for cells to ensure that not all genes are switched on at the same time. In mammals, DNA methylation, which may either block access to DNA binding sites by transcriptional regulators or by attracting repressors to methylated sites, is one of the most commonly used means of keeping genes under lock and key. This form of regulation is known as epigenetic, as it is both heritable across generations and independent of genomic sequence. DNA methylation involves the activity of a family of enzymes known as DNA methyltransferases (or Dnmts). Dnmt1 is responsible for maintaining methylation, while a pair of factors, Dnmt3a and -3b, work together in the process of establishing methylation *de novo*. The lethality of knockout phenotypes has shown that all of these factors are required for embryonic survival, but little is known of exactly how they operate in genetic regulation during development.

In a report published in *Genes and Development*, Masaaki Oda and colleagues from the Laboratory for Mammalian Epigenetic Studies (Masaki Okano; Team Leader) showed that DNA methylation regulates the transcription of a cluster of genes expressed in the extraembryonic trophectoderm lineage, but not in the mouse embryo proper. This demonstration of DNA methyltransferase activity in the lineage- and stage-specific silencing of a large genomic region was carried out in collaboration with scientists from the Novartis Institute for Biomedical Research (USA).

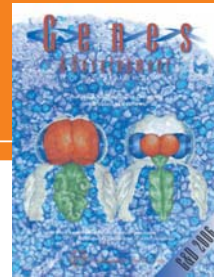


One of the first instances of cellular differentiation in mammalian development takes place in the blastocyst, a hollow ball of little more than 100 cells, which initially comprises primordial embryonic and extraembryonic lineages and subsequently fastens itself to the uterine lining at implantation. The *Rhox* (Reproductive Homeobox) family of genes is known to be repressed in the post-implantation embryonic lineage, which led the team to examine whether DNA methylation might have a role in this silencing process. Oda and colleagues found that two genes, *Rhox6* and *Rhox9*, were both highly methylated in the embryo proper after implantation, but hypomethylated in the trophectoderm (extraembryonic tissue that gives rise to placenta). This high level of DNA methylation was not seen, however, in pre-implantation embryos, indicating that the effect was dependent on both developmental stage and cell lineage.

They next looked at the effects of various combinations of gene deletions involving members of the Dnmt family, and found that while mutations of either *Dnmt3a* and *Dnmt3b* produced loss-of-function phenotypes in the form of reduced methylation of *Rhox* genes in the embryo proper, the result was much more dramatic in the *Dnmt3a/3b* double knockout, in which such methylation was lost altogether. Similar ef-

Oda M, Yamagiwa A, Yamamoto S, Nakayama T, Tsumura A, Sasaki H, Nakao K, Li E and Okano M. DNA methylation regulates long-range gene silencing of an X-linked homeobox gene cluster in a lineage-specific manner. *Genes Dev* 20:3382-94 (2006).

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fects were seen in the methylation of *Oct4* in the trophectoderm, indicating the widespread nature of the activity of these DNA methyltransferases. The loss of methylation of *Rhox6* and *-9* in the embryonic regions of the double knockouts, where their transcription is normally shut down, resulted in their de-repression, an effect that was observed for several other, but importantly, not all, trophectoderm-specific genes tested.

The *Rhox* genes occupy a stretch of the mouse genome that includes many genes that are expressed in trophectoderm but not the embryo proper. In the double knockout mice, a cluster of these *Rhox* and neighboring genes were found to be de-repressed, indicating that the regulatory effect of *Dnmt3a* and *-3b* extends over a substantial genomic region, an effect that manifests only after implantation. Using embryonic stem cells as a model of the inner cell mass/epiblast (the embryonic lineage that gives rise to embryo proper), the Okano team confirmed that, *in vitro* as well, the double *Dnmt3a/3b* knockout showed loss of methylation for *Rhox6*, *Rhox9*, and other genes in the vicinity of the *Rhox* cluster, which could be rescued by the expression of exogenous *Dnmt3a*.

The Oda study provides a solid demonstration of a specific regulatory role for DNA methylation in mammalian development. "The large-scale changes in chromatin structure that take place in the germline and early embryo are thought to bear a close relationship to the loss or acquisition of differentiative potency," says Okano. "The *Rhox* cluster provides us now with a nice model system for studying one of the molecular bases of chromatin regulation in early embryogenesis, which until now had been quite challenging to study."



Fez and Fezl share the burden in rostral brain regionalization



Hirata T, Nakazawa M, Muraoka O, Nakayama R, Suda Y and Hibi M. Zinc-finger genes *Fez* and *Fez-like* function in the establishment of diencephalon subdivisions. *Development* 133:3993-4004 (2006).

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The mammalian brain emerges progressively during development, unfolding as a linear series of sections from spinal cord to forebrain. The embryonic forebrain can be subdivided into rostral and the caudal regions, and the caudal forebrain (or diencephalon) itself comprises three main subdivisions: prethalamus, thalamus and pretectum. Brain development relies on tightly regulated and highly specific patterns of gene expression to ensure that each part integrates seamlessly with the whole. A number of genetic markers have been identified for each of the specified caudal forebrain regions, but the means by which the boundaries between these subdivisions are established earlier in development has remained elusive.

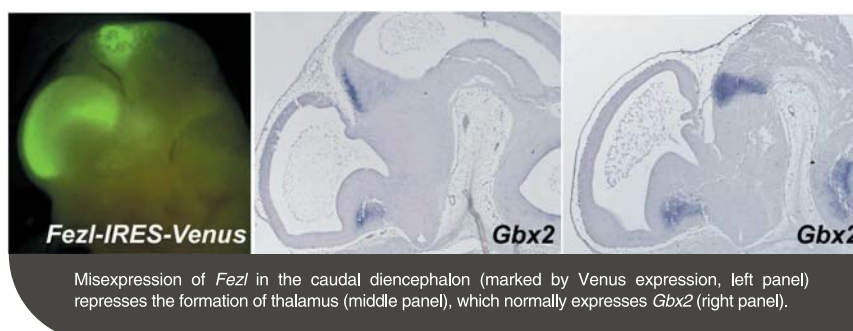
In a study published in the journal *Development*, Tsutomu Hirata and Masato Nakazawa and colleagues in the Laboratory for Vertebrate Axis Formation (Masahiko Hibi; Team Leader) identified a pair of genes that work together to set up the initial subdivisions of the mouse diencephalon. Hirata and Nakazawa, working with researchers from a number of other CDB labs, describe how the zinc-finger genes *Fez* and *Fezl* cooperate to delineate and maintain forebrain patterning by repressing caudal specification in the rostral forebrain.

Fez/Fezl double homozygous mice showed severe caudal forebrain defects, including the loss of the prethalamus and ZLI, and a significantly reduced thalamus (the pretectum, however, was intact). Analyses of genes normally expressed in diencephalic regions showed that many genes directly or indirectly involved in the regionalization patterning of the forebrain were affected by the double homozygous mutation, suggesting that *Fez* and *Fezl* work together to regulate rostro-caudal patterning.

Interestingly, in contrast to the deleterious effects on prethalamus and ZLI, the double homozygous phenotype showed a rostral expansion of the caudal regions of the diencephalon. Where the expression of genes normally associated with the more rostral prethalamus was lost, that of typically caudal markers was found to have shifted to the anterior, even from the earliest stages of neural patterning. Tests using misexpressed *Fez* and *Fezl* tended to support the interpretation that these two genes in combination work to repress caudal diencephalon fate. Although morphologically abnormal, the diencephalon is not significantly reduced in size in the *Fez/Fezl* mutants, which led Hirata and Nakazawa to propose that these two genes function early in neural patterning to repress caudal gene expression in the rostral diencephalon, while at the same time promoting the establishment of the prethalamus.



Masato NAKAZAWA



Misexpression of *Fezl* in the caudal diencephalon (marked by Venus expression, left panel) represses the formation of thalamus (middle panel), which normally expresses *Gbx2* (right panel).

For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.

Keeping an eye on cadherin function in retinal synaptogenesis



Tanabe K, Takahashi Y, Sato Y, Kawakami K, Takeichi M and Nakagawa S. Cadherin is required for dendritic morphogenesis and synaptic terminal organization of retinal horizontal cells. *Development* 133:4085-96 (2006).

Journal cover image reproduced with permission of the Company of Biologists.

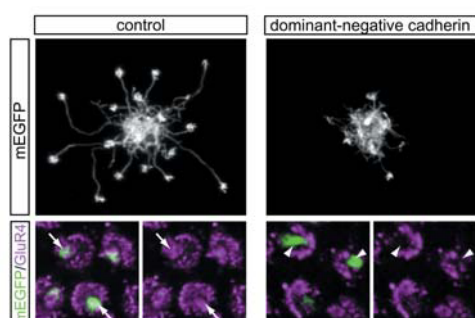
The role of cadherin cell-cell adhesion machinery in regulating the organization of the vertebrate synapse has been amply shown in cultured neurons, but the difficulty of performing and observing the effects of genetic manipulations in the intact vertebrate nervous system has prevented researchers from confirming what the in vitro data suggest: that cadherins play similar roles in vertebrate synapses. In an article published in the journal *Development*, Koji Tanabe and colleagues in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) reported the use of a new gene transfer system that enabled them to study in vivo the function of cadherins in horizontal cells in the embryonic retina in chicken. These cells, which receive input from photoreceptors and modulate intercellular feedback in the retinal network, form elaborate dendritic fields that enable them to make contacts with multiple surrounding neurons. Tanabe discovered that in neurons in which cadherin function had been lost, the size of these fields was smaller and that the individual dendrites frequently failed to connect properly with photoreceptor cells.



Koji TANABE

This success in observing cellular phenotypes in living tissue was made possible by their development of a novel transposon-based system of conditional transgenesis. The group first used this method to introduce a combination of plasmids whose net effect was to allow the group to observe a specific subset of the retinal cellular population. Looking at late-stage chick embryos, they discovered that they were able to group these cells into three classes by their distinct shapes. The group was able to observe the initially indistinguishable cells gradually transform, extending dendrites that went on to form synaptic connections in patterns specific to their morphological type.

After verifying that horizontal cells express the cadherin family member N-cadherin in vivo, blocked cadherin function and found that while the dendritic fields were smaller than normal, axon outgrowth was unaffected. Although dendrites appeared to home to their appropriate targets, the morphologies of the dendritic terminals were severely affected. Given that the cadherin-negative horizontal cells showed defects in both the global organization of the dendritic and the local ability to form functional synapses, Tanabe et al. suggest that cadherins may be involved in two distinct stages of dendritic morphogenesis; first as the dendrites elongate, then again later as they form synaptic contacts.



Overexpression of dominant negative cadherin inhibits dendrite growth and disrupts the morphologies of dendritic terminals (Upper panels, Type III horizontal cells are shown). Cadherin blockage also perturbs synapse formation. Although the synapse marker GluR4 accumulates strongly on dendritic terminals of control cells (arrows), the accumulation is perturbed in dominant-negative cadherin expressing cells (arrowheads).

For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.

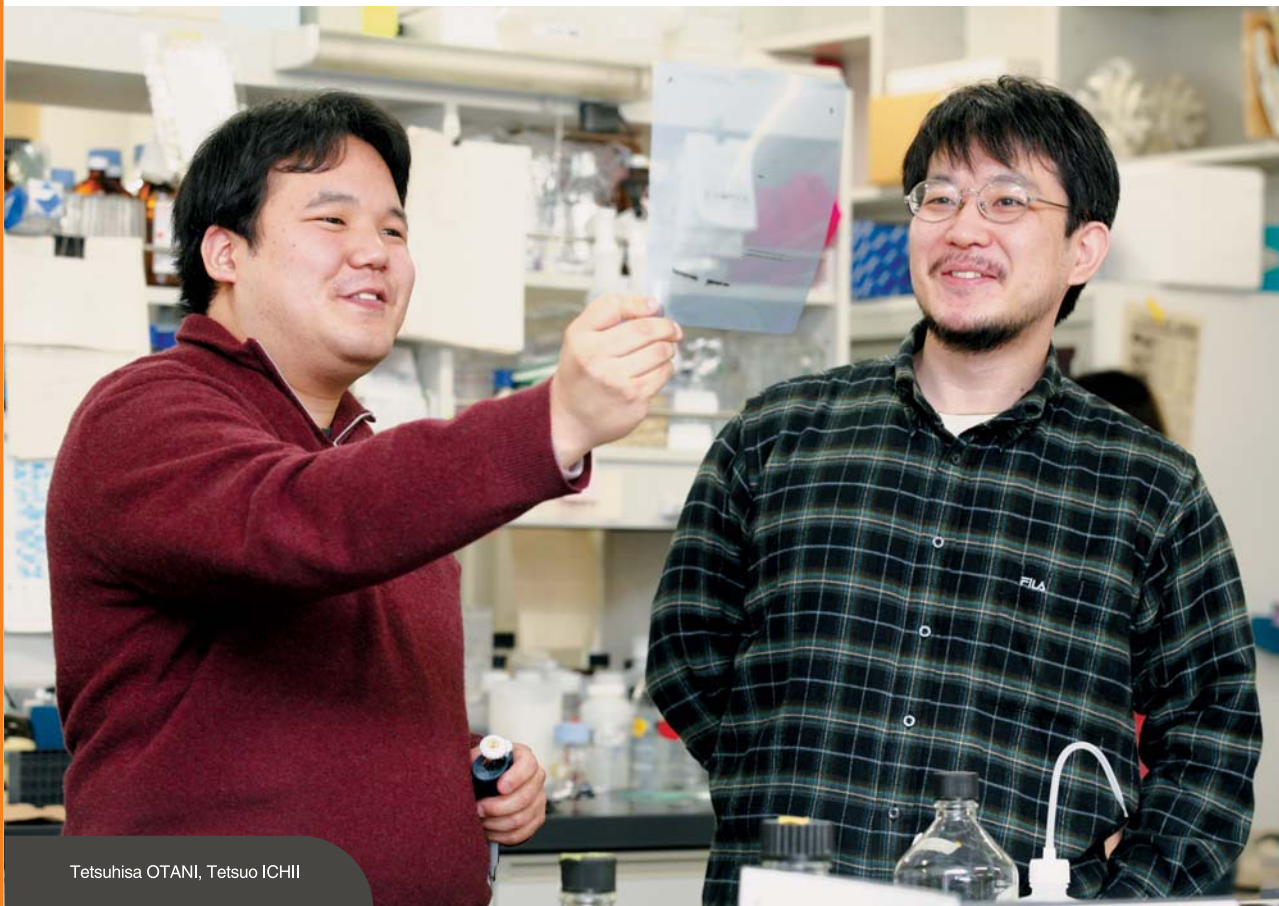
Tuba joins the band

A Cdc42-specific activator regulates epithelial cell-cell junctions

Epithelial cells can be easily identified by a honeycomb-like structure that minimizes cell-cell contact surface areas, which suggests some form of tension exists in maintaining this construction. One candidate molecule involved in this arrangement is the scaffolding protein Tuba, a Cdc42-specific guanine nucleotide-exchange factor (GEF). Scaffolding proteins, such as Tuba, help to coordinate cellular processes by bringing together interacting proteins. GEFs also activate Rho family small GTPases, essential regulators of the actin cytoskeleton and implicated in cadherin activities in epithelial cells. Although previous studies have identified a number of GEFs and their precise roles, the exact role of Cdc42 and its GEFs in epithelial cells remain poorly understood.

In a report published in *The Journal of Cell Biology*, Tetsuhisa Otani and colleagues in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) and Kyoto University identified Tuba as a protein concentrated at the apical-most regions of epithelial cell junctions through its interaction with ZO-1, a protein associated with the tight junction area of cell-cell junctions. In the absence of Tuba, cell junctions in a human intestinal cell line became morphologically abnormal, and took on a curved and distorted appearance.

Previous studies using *Drosophila* have shown the cadherin group of cell-adhesion molecules to be important in controlling the tensile properties of cell junctions. These events are likely to take place in the apical-most area of cell-cell junctions known as the adherens junction (AJ), which is found beneath the tight junction (TJ). The AJ is lined with actin fibers that contribute to the adhesive activity of this area and regulators of cadherin or actin, such as GEFs, are thought to be involved in the fine-tuning of cell-cell boundary shape. Using a series of gene knockdown experiments the authors were able to confirm this by demonstrating that RNAi-mediated Tuba depletion in human intestinal epithelial cells distorted the outline of the AJ-TJ complex and disrupted F-actin and E-cadherin distribution.



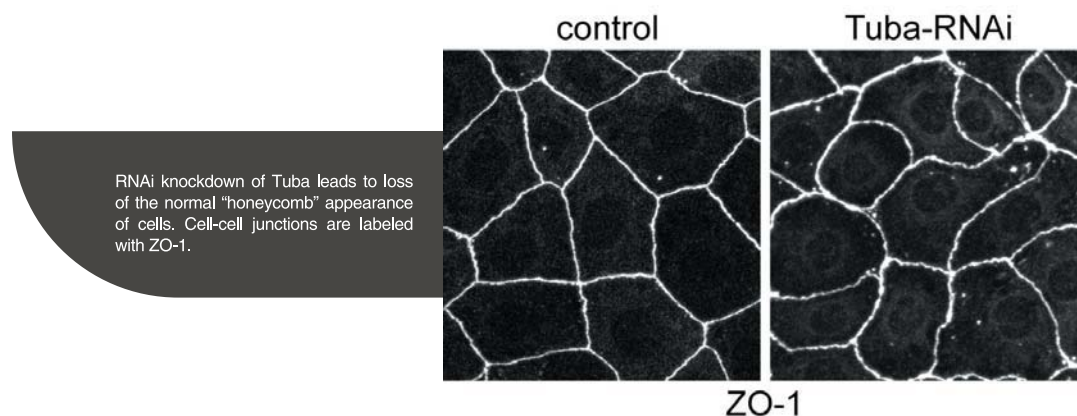


Otani T, Ichii T, Aono S and Takeichi M. Cdc42 GEF Tuba regulates the junctional configuration of simple epithelial cells. *J Cell Biol* 175:135–46 (2006).

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This is the first study to report on the apical-specific localization for Cdc42 and one of its GEFs, indicating that Tuba may play a unique, position-specific role in junctional organization. Otani and colleagues took their results to suggest that Tuba is required in regulating the configuration of apical junctions, and that it regulates actin polymerization—an important process in the development of the actin cytoskeleton—through Cdc42 and its effectors. The subsequent strands of colocalized F-actin and E-cadherin increase surface tension so that the surface area of cell junctions is minimized, and may be involved in stabilizing the linear morphology of cell junctions; this is all disrupted by the loss of Tuba.

In examining ZO-1, Otani et al. found that knockdown of this particular protein revealed its function as both a partner of Tuba and a regulator of myosin activity, which affects cell junction morphology. As a reduction in Tuba had no corresponding effect on myosin distribution, the authors concluded that the slackened phenotype of Tuba-depleted cell junctions is brought about by Tuba-mediated actin organization, rather than by actomyosin activity.



Using these findings to delve deeper into the sub-cellular layer, Otani and colleagues went on to reveal that Tuba inactivation modified the assembly pattern of junctional F-actin and E-cadherin, which act in holding neighboring cells together. They further found that while depletion of Cdc42 mimicked Tuba reduction, this factor's overexpression rescued the Tuba knockdown phenotypes, indicating that Tuba functions via Cdc42 and that Cdc42 acts downstream of Tuba signaling. Depleting N-WASP, another protein and an effector of Cdc42, resulted in distorted apical junctions similar to Tuba depletion, suggesting that N-WASP is necessary for the normal accumulation of E-cadherin and F-actin at cell junctions. This was of particular interest to the authors as it implied that N-WASP works downstream of the Tuba-Cdc42 pathway, prompting Otani to propose a scenario in which Tuba-Cdc42 activates N-WASP to enhance actin polymerization at the apical junction level. This results in the delivery of polymerized actin filaments to the lower portions of cell junctions. This area then serves as the dock to which the E-cadherin-catenin complex is anchored.

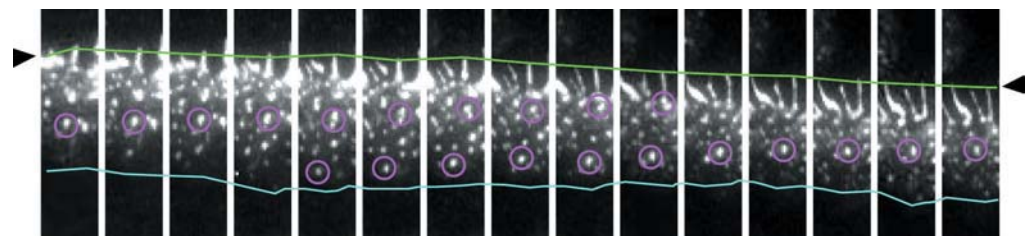
A number of studies had implicated other GEFs, in addition to Tuba, as contributing to the cell-cell adhesion process. Revealing Tuba's role in maintaining cellular morphology is an important step forward in understanding how different GEFs share cell assembly regulation roles—an intriguing topic and one that Otani and his colleagues look to tackle in the future. “We are now focusing on the role of Tuba in epithelial remodeling during morphogenesis,” says Otani. “This is in addition to adopting a cell biological approach to examine Tuba's role in the organization of lateral actin filaments and its relationship with apical junctions.”

Cadherins go with the flow

Adhesion between cells must be robust enough to provide a stable basis for the organization of tissues, but dynamic enough to provide the flexibility and mobility required in certain situations, such as development and wound healing. This cellular freedom of movement is essential to both the formation and maintenance of multicellular life, but the means by which it is regulated by molecular adhesion machinery remains one of the mysteries of biology.

In a study published in *Nature Cell Biology*, Yoshiko Kametani and Masatoshi Takeichi of the Laboratory for Cell Adhesion and Tissue Patterning described a new aspect to the behavior of cadherin proteins, showing that these cell-cell adhesion molecules, in certain cellular contexts, move in coordinated, unidirectional flows. They further show how such cadherin flow is linked to cytoskeletal movements, opening up the possibility that plays a significant role in the remodeling of epithelial tissues.

Cadherins are transmembrane molecules that extend long tails through the cell surface to form links with other cadherins of like type on adjacent cells. These proteins are anchored in the cellular interior by binding with members of the catenin family of proteins, which themselves are believed to associate with the actin cytoskeleton, a dynamic structural component of cells of fundamental importance to cell motility. Kametani used green fluorescent protein to label a specific type of cadherin known as VE-cadherin and monitor its behavior in epidermoid cancer cells in vitro. These epithelial cells tend to organize into sheets, but when she observed the junction zones between cells, she found that these often appeared to be tilted, sloping upward from the cells' base to their apical surface. And, interestingly, clusters of cadherin molecules appeared to form and gradually move upward on these junctional inclines, resulting in a clearly identifiable basal-to-apical flow.



Time-lapse images of cadherin movements. Cadherins flow in a unidirectional manner at the cell-cell junction.

Intrigued by this "cadherin flow," the group began to analyze the phenomenon in molecular-level detail, and found that the mobile cadherins maintain both their transdimer links with cadherins on adjacent cells, and their colocalization with associated catenin molecules. By introducing mutations into both cadherins and catenins, Kametani revealed that this flow relies on the connection between the adhesion machinery and filamentous actin mediated by catenin family members. Double immunostaining of VE-cadherin and F-actin showed that the cadherin clusters tended to associate with actin fibers and in some cases even jumped from fiber to fiber. Further analysis of actin dynamics near the cellular junction revealed that these cytoskeletal molecules as well were continuously reorganizing themselves and moving in a retrograde flow. Actin flow had been shown in previous studies to depend on the function of the motor protein myosin II, and when the group used a myosin inhibitor to block its function, they found that cadherin flow was stopped as well.



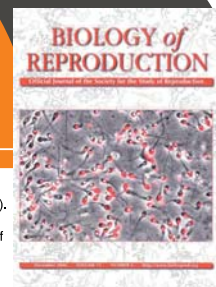
Kametani Y and Takeichi M. Basal-to-apical cadherin flow at cell junctions. *Nat Cell Biol* 9:92-8 (2007).
Cover image copyright © Nature Publishing Group 2006

Cadherins are found in a wide variety of cell types, so to determine whether the flow effect was lineage-specific, Kametani next tested for flow-like movements of cadherin clusters in other cell lines transfected with VE-cadherin. She found that some, but not all cell types showed similar cadherin flow—in MDCK cells for example, cadherin movements seemed random. But even in these cells, flow could be induced by scraping the culture to simulate a wound; when cell migration began at the wound edges, cadherins began to flow in the direction of the migration as well. The picture that develops from this set of findings suggests that cadherin flow is an actin-dependent phenomenon that enables cells to move between cells in a continuous layer.

This study shows a sophisticated balance between epithelial cellular adhesion and motility that underlies the ability of the multicellular body to organize and maintain itself. “It seems that cadherins may be working as a kind of clutch during cell migration that uses actin flow to provide the impetus,” says Kametani. “And as cellular adhesion and migration are important both to physiological development and pathological processes such as cancer invasion and metastasis, we hope that this work may one day have clinical implications as well.”



Insights into small RNA function in mammalian fertilization



Amanai M, Brahmajosyula M and Perry A C. A Restricted Role for Sperm-Borne MicroRNAs in Mammalian Fertilization. *Biol Reprod* 75:877-84 (2006).

Amanai M, Shoji S, Yoshida N, Brahmajosyula M and Perry A C. Injection of Mammalian Metaphase II Oocytes with Short Interfering RNAs to Dissect Meiotic and Early Mitotic Events. *Biol Reprod* 75:891-8 (2006).

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In a pair of reports published in the same issue of the journal *Biology of Reproduction*, Manami Amanai and colleagues in the Laboratory of Mammalian Molecular Embryology (Tony Perry; Team Leader) described new applications for and insights into siRNAs and miRNAs in mammalian fertilization and early embryonic development. The first describes the role of miRNAs in the earliest stages of mammalian development, while the second reports technical advances in the use of siRNAs to silence genes in the oocyte and during and after fertilization.

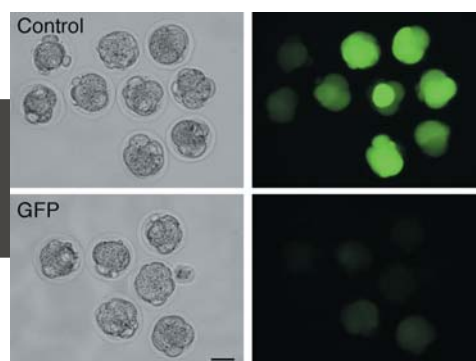


Shisako SHOJI, Naoko YOSHIDA, Anthony C. F. PERRY, Manjula BRAHMAJOSYULA, Manami AMANAI,

In the first study, the team tested whether oocyte mRNAs might be inhibited by miRNAs delivered by sperm. They first removed the sperm membrane coats, enabling them to isolate the portion of the sperm that actually enters the oocyte. Microarray analyses identified a spectrum of miRNAs whose presence was confirmed by quantitative PCR (qPCR). However, these sperm-borne miRNAs were not abundant, and did not significantly raise the level of corresponding oocyte miRNAs immediately after fertilization. Loss of miRNA function had no discernible effect on meiotic exit or early embryogenesis. In contrast, nuclear transfer resulted in a significant change in the level of one of the 10 miRNAs tested; this may represent some functional significance, as embryonic development following nuclear transfer is so poor.

In the second study, Amanai and colleagues described the effectiveness of gene silencing by siRNAs in mII oocytes. Although siRNAs have been widely employed to dissect gene function, they have not been characterized in mII oocytes. The team also sought to test whether injected siRNAs could similarly be applied to the inhibition of regulatory factors at the first mitotic cell division following fertilization. They injected siRNAs targeting cell cycle regulators expressed in the mII oocyte, and confirmed they brought about reductions in levels of mRNAs and corresponding proteins. They also showed that siRNA injected at the time of fertilization can knock down transcripts for both native genes and transgenes, with measurable effects up to the blastocyst stage, and that RNAi is effective in nuclear transfer as well. Since this regulation may be reversible, the approach promises one day to enhance reprogramming and the development of nuclear transfer clones by transiently eliminating detrimental proteins.

Paired images of modulation contrast (left) and fluorescence microscopy showing embryos on the fourth day of development following coinjection of enucleated mII oocytes with GFP-expressing cumulus cell nuclei plus buffer alone (Control, upper panels) or with an siRNA for GFP mRNA. Bar = 50 μ m



For the full story, see the Annual Report CD-ROM, or visit www.cdb.riken.jp.



Night view, Kobe O-hashi

RIKEN Kobe Institute

RIKEN Kobe Institute

Center for Developmental Biology

The Center for Developmental Biology is organized as the initial research center within the RIKEN Kobe Institute, which also comprises the Kobe Research Promotion Division, which provides administrative services and support, and the institutional Safety Center. The CDB is home to 29 laboratories in its Core Research (7 groups), Creative Research Promoting (20 teams) and Supporting Laboratories (2 teams) programs, as well as technical support units and ancillary offices. The CDB Director is assisted by two deputy directors and advised by the Center's Advisory Council and Institutional Review Board.

Director

Deputy Directors

Core Program

The Core Program aims to promote highly innovative developmental and regeneration studies in a strategic and multi-disciplinary manner. This program constitutes the core research framework to achieve the aims of the Millennium Project, and focuses on the main themes of the CDB: the mechanisms of development and regeneration, and the scientific bases of regenerative medicine.

Creative Research Promoting Program

The Creative Research Promoting Program provides solid support to encourage young researchers to carry out innovative and independent research plans. The teams are allowed a great deal of flexibility in regard to projects, budget, and lab size. The program also places great emphasis on cooperation and international participation.

Supporting Laboratories

Support Unit

Leading Project Research Unit

Research Promotion Division

Safety Center

Institutional Review Board

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

Advisory Council

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

In February 2006, the DBAC held a plenary meeting in Kobe, in which it praised, "the overall high quality of scientific research carried out in the CDB," and made recommendations for future directions for the Center's research and administration. The full text of the report can be viewed on the CDB website (www.cdb.riken.jp).

Vertebrate Body Plan

Shinichi AIZAWA Ph.D.

Morphogenetic Signaling

Shigeo HAYASHI Ph.D.

Evolutionary Morphology

Shigeru KURATANI Ph.D.

Cell Asymmetry

Fumio MATSUZAKI Ph.D.

Stem Cell Biology

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

Organogenesis and Neurogenesis

Yoshiki SASAI M.D., Ph.D.

Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph.D.

Stem Cell Translational Research

Takayuki ASAHARA M.D., Ph.D.

Neuronal Differentiation and Regeneration

Hideki ENOMOTO M.D., Ph.D.

Neural Network Development

Chihiro HAMA Ph.D.

Vertebrate Axis Formation

Masahiko HIBI M.D., Ph.D.

Cell Lineage Modulation

Toru KONDO Ph.D.

Sensory Development

Raj LADHER Ph.D.

Germline Development

Akira NAKAMURA Ph.D.

Chromatin Dynamics

Jun-ichi NAKAYAMA Ph.D.

Cell Migration

Kiyoji NISHIWAKI Ph.D.

Pluripotent Cell Studies

Hitoshi NIWA M.D., Ph.D.

Mammalian Epigenetic Studies

Masaki OKANO Ph.D.

Mammalian Molecular Embryology

Tony PERRY Ph.D.

Mammalian Germ Cell Biology

Mitunori SAITOU M.D., Ph.D.

Embryonic Induction

Hiroshi SASAKI Ph.D.

Cell Fate Decision

Hitoshi SAWA Ph.D.

Early Embryogenesis

Guojun SHENG Ph.D.

Developmental Genomics

Asako SUGIMOTO Ph.D.

Retinal Regeneration

Masayo TAKAHASHI M.D., Ph.D.

Systems Biology

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

Genomic Reprogramming

Teruhiko WAKAYAMA Ph.D.

Animal Resources and Genetic Engineering Shinichi AIZAWA Ph.D.

Cellular Morphogenesis Shigenobu YONEMURA Ph.D.

Genomics Support Unit Fumio MATSUZAKI Ph.D.

Genome Resource and Analysis Subunit Hiroshi TARUI Ph.D.

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

Proteomics Support Unit Shigeo HAYASHI Ph.D.

Mass Spectrometry Analysis Subunit Akira NAKAMURA Ph.D.

Research Unit for Cell Plasticity Mitsuko KOSAKA Ph.D.

Research Unit for Organ Regeneration Hideki TANIGUCHI M.D., Ph.D.

Margaret Buckingham

Institut Pasteur, France

Stephen Cohen*

EMBL, Germany

Hiroshi Hamada*

Osaka University, Japan

Haifan Lin*

Yale University, USA

Yo-ichi Nabeshima

Kyoto University, Japan

Austin Smith

University of Cambridge, UK

Toshio Suda

Keio University, Japan

Yoshimi Takai

Osaka University, Japan

Patrick Tam*

Children's Medical Research
Institute, Australia

Chris Wylie

Cincinnati Children's Hospital
Research Foundation, USA

*New appointment in 2006.

The following members of the
DBAC completed their terms fol-
lowing the 2006 Advisory Council
meeting:

William Chia

Temasek Life Sciences
Laboratory, Singapore

Elaine Fuchs

The Rockefeller University/
HHMI, USA

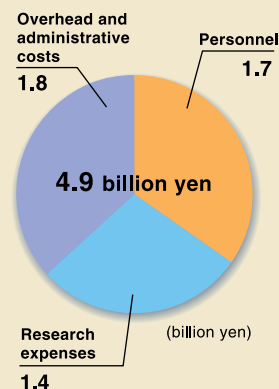
Hajime Fujisawa

Nagoya University, Japan

Yoshiki Hotta (2006 Chair)

Research Organization of
Information and Systems, Japan

2006 CDB Budget



In addition to these intramural funds, individual labs and investigators are encouraged to compete for external funding as well, from sources such as the Japan Society for the Promotion of Science (JSPS), other government agencies, private foundations and industry. These external funds form an important component of the CDB's overall funding mix.

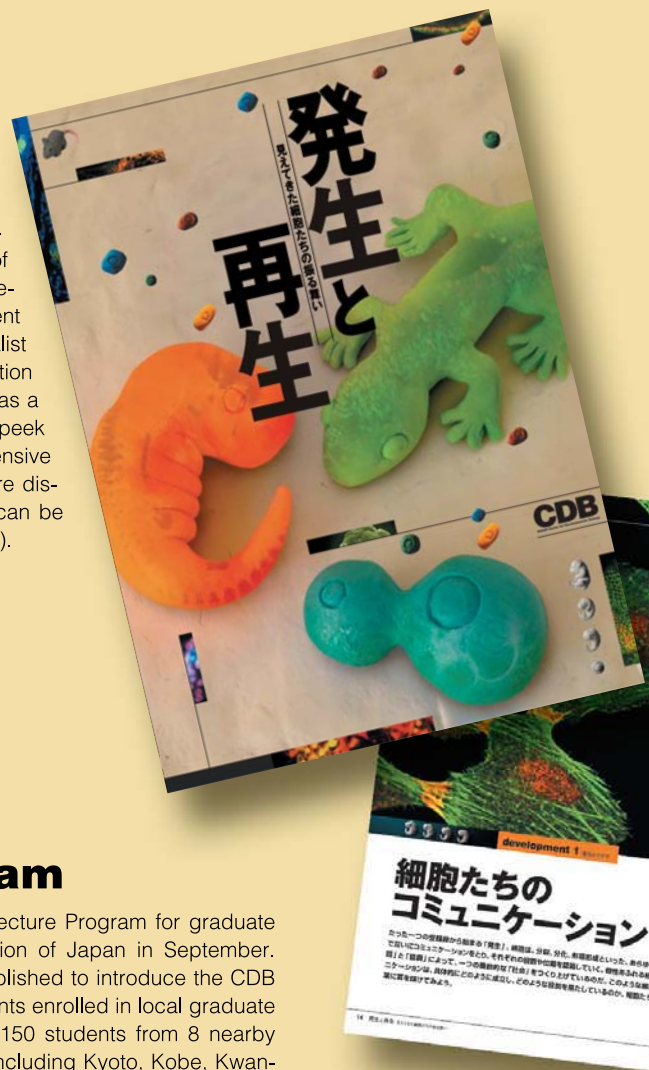
2006 CDB Staff

Laboratory heads	31
Research scientists	126
Research associates	8
Technical staff	124
Assistants	29
Visiting scientists	54
Student trainees	55
Part-time staff	33
Research Promotion Division	40
Other	55
Total	555

Academic and Outreach Activities

Millennium Project Commemorative Book

The Millennium Project research initiatives funded by the Japanese government, under the auspices of which the CDB was established, came to a close in March 2005. To commemorate the advances made under this support, and to highlight the questions and that remain to younger generations of developmental biologists, the CDB produced a lavishly illustrated Japanese-language book, *Hassei to saiesi: Mietekita saibotachi no furumai* (Development and Regeneration: The Lives of Cells Made Visible), targeting a non-specialist audience. The book introduced important topics in development, regeneration and regenerative medicine, and featured engaging sidelight sections such as a roundtable discussion between scientists on the future of their fields, and a peek into the daily life of a young CDB researcher. *Hassei to saisei* received extensive publicity in Japanese print and online media, and 2,500 printed copies were distributed free of charge via the CDB website. The full content of the book can be browsed on the Japanese-language website (www.cdb.riken.jp/jp/index.html).



Intensive Lecture Program

The CDB held its third Intensive Lecture Program for graduate school affiliates in the Kansai region of Japan in September. This yearly lecture series was established to introduce the CDB and its research programs to students enrolled in local graduate study programs. This year, about 150 students from 8 nearby universities and medical schools, including Kyoto, Kobe, Kwansei Gakuin and Osaka Universities and the Nara Advanced Institute of Science and Technology, visited the CDB to participate in lectures, demonstrations and lab visits. Talks on topics ranging from the epigenetics of mammalian development to cadherins in neural morphogenesis were given by eleven CDB research scientists and PIs over the course of the two-day program, which also featured practical demonstrations using embryonic stem cells, various model organisms and the micromanipulator equipment used in such procedures as somatic cell nuclear transfer and the generation of knockout mice.

Academic Affiliations and Programs



Kyoto University

Graduate School of Biostudies
Graduate School of Medicine



Kobe University,
Graduate School of
Science and Technology

Department of Life Science
Department of Developmental and
Regenerative Medicine



Osaka University,
Graduate School of Science



Nara Advanced Institute of Science
and Technology, Graduate School of
Biological Sciences



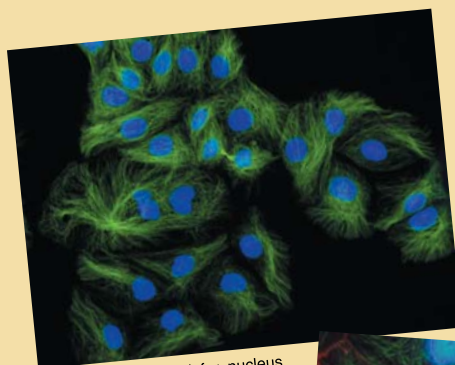
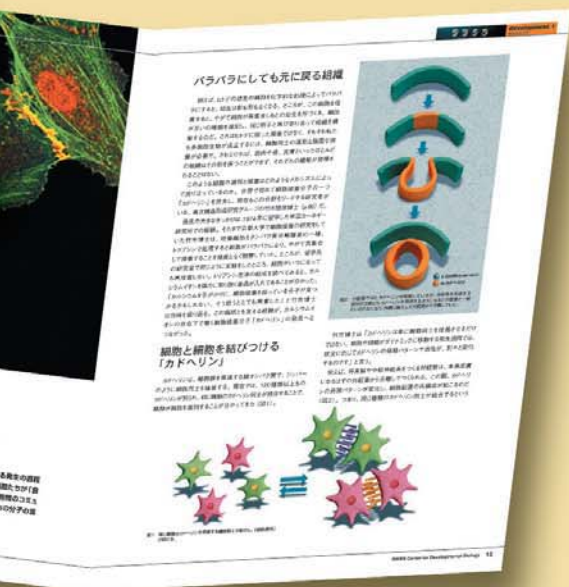
Kwansei Gakuin University,
Bioscience Department

As part of its commitment to promoting the fields of developmental biology and regenerative medicine in Japan, the CDB has cultivated strong relationships with a number of local graduate and medical school programs. While RIKEN is not an academic institution and does not grant degrees, many of the CDB's scientists serve as visiting professors at affiliated graduate schools and host students enrolled in these programs to do the bench work for their theses in CDB labs. The CDB has established formal affiliations with the following programs:

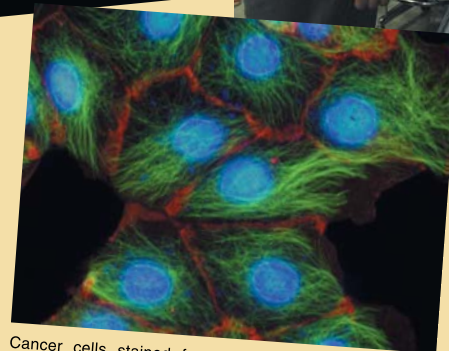
The Center for Developmental Biology devotes significant effort and expense to activities intended to deepen public understanding of developmental biology and related issues such as somatic cell nuclear transfer, embryonic stem cells, evolution and regenerative medicine, through a range of onsite, online and in-print media and activities.

High School Class Visits

In early August, a group of ten students from the Super Science High School in Kokura, Fukuoka Prefecture, visited the CDB to participate in a two-day summer school program organized by members of the Laboratory for Cell Adhesion and Tissue Patterning and the Office for Science Communications and International Affairs. The students enjoyed a tour of the facilities and visits to a number of labs before rolling up their sleeves and getting some hands-on lessons in biological research. Using chemical and antibody stains, after only one day in the CDB's demonstration laboratory, the students were able to visualize sub-cellular structures including DNA, the Golgi complex and microtubules (see images below).



Cancer cells stained for nucleus (blue) and microtubules (green)
Photo by: Risa Yamamoto

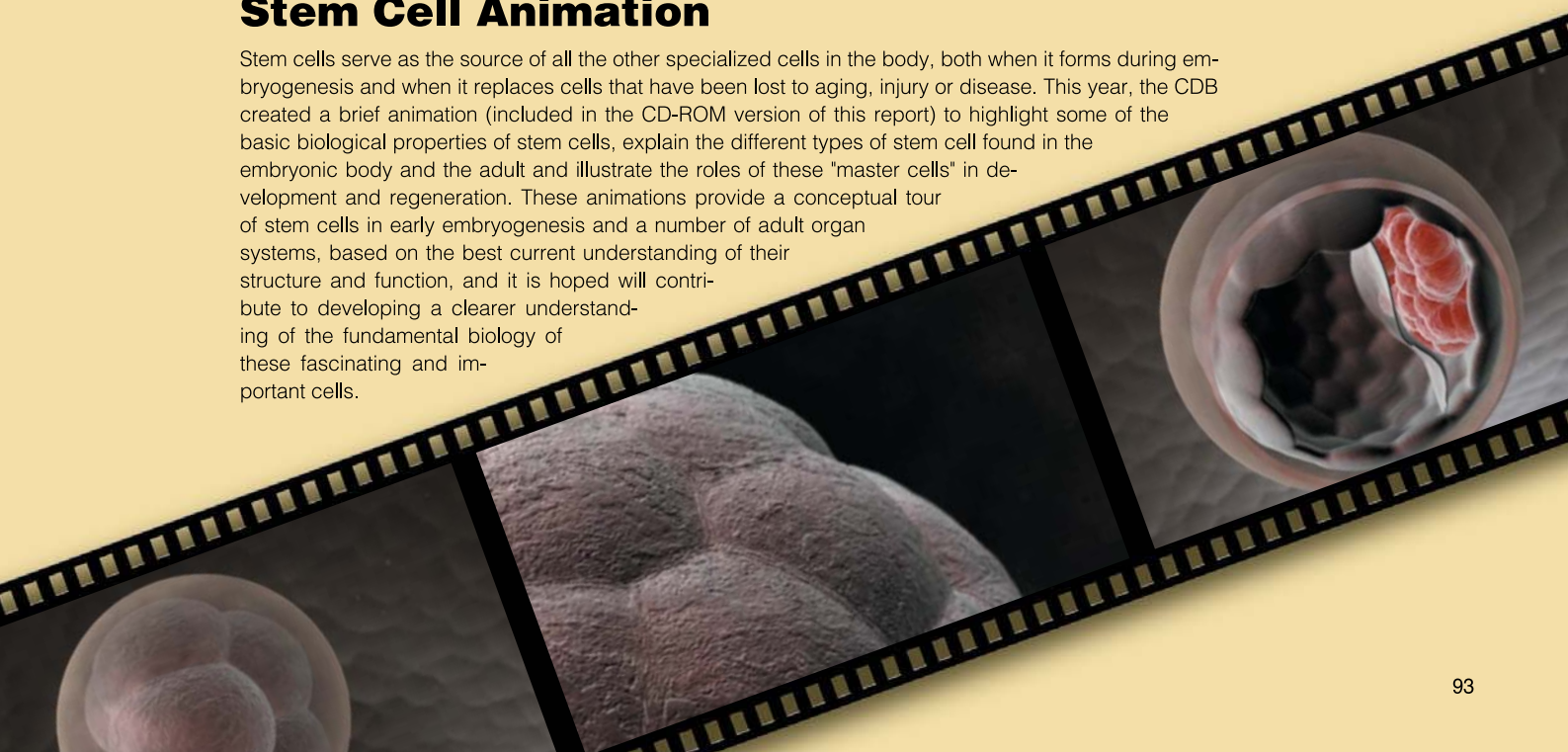


Cancer cells stained for nucleus (blue), microtubules (green) and β -catenin (red)
Photo by: Keiichi Nakao



Stem Cell Animation

Stem cells serve as the source of all the other specialized cells in the body, both when it forms during embryogenesis and when it replaces cells that have been lost to aging, injury or disease. This year, the CDB created a brief animation (included in the CD-ROM version of this report) to highlight some of the basic biological properties of stem cells, explain the different types of stem cell found in the embryonic body and the adult and illustrate the roles of these "master cells" in development and regeneration. These animations provide a conceptual tour of stem cells in early embryogenesis and a number of adult organ systems, based on the best current understanding of their structure and function, and it is hoped will contribute to developing a clearer understanding of the fundamental biology of these fascinating and important cells.



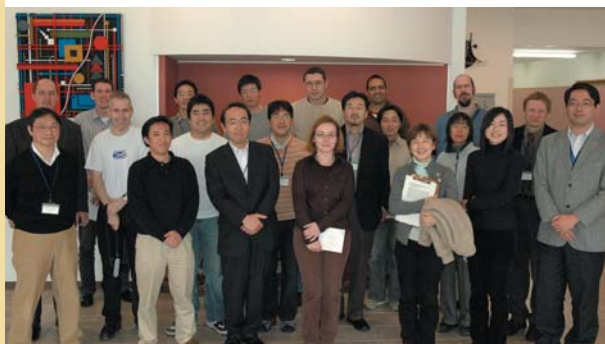
International Meetings and Activities

In 2006, the CDB hosted or organized a number of academic gatherings, continuing its commitment to promoting the interests of the developmental biology and regenerative medical communities, with a particular focus on the emerging Asia-Pacific region. In addition to its meeting series, the CDB also continued to strengthen its interactions with overseas affiliate institutions in Europe, North America and Asia through research visitation, collaborative studies and the holding of joint meetings. The year also saw the CDB take expanded roles in a number of global and regional organizations dedication to the promotion of research into development and related fields.

Morphogenetic Signaling: The View from the Frog (February 21)

Organizer: Raj Ladher

The African clawed frog, *Xenopus laevis*, has long been used in the study of early development and morphogenesis. Participants in this meeting presented and discussed recent advances in the understanding of signaling and tissue patterning in this important model organism.



Morphogenetic Signaling: The View from the Frog

GSF-CDB Joint Meeting (April 13 – 14)

Organizers: Timm Schroeder, Shin-ichi Nishikawa, Shinichi Aizawa

This meeting was the inaugural event for the CDB's most recent institutional affiliation, with the GSF National Research Center for Environment and Health (Germany). Researchers from both institutions gathered in Kobe for two days following the 2006 CDB Symposium to introduce their latest findings and exchange views.



GSF-CDB Joint Meeting

EPC Biology Conference (March 23)

Organizer: Takayuki Asahara

Scientists working with endothelial progenitor cells (EPCs), which drive blood vessel formation through vasculogenesis, met in Kobe for this one-day event. EPCs represent a potentially important resource in re-vascularization therapies and in the study of the development and maintenance of the circulatory system.



EPC Biology Conference

1st Italy-Japan Meeting on Vertebrate Organogenesis (April 24 – 26)

Organizers: Shinichi Aizawa, Roberto Di Lauro and Antonio Simeone

This two-day program, held in Ischia, Italy, featured talks on multiple aspects of organogenesis ranging from genetic networks regulating somite formation to molecular pathways involved in the development of sensory organs and the brain.



1st Italy-Japan Meeting on Vertebrate Organogenesis

RIKEN-SIBS Meeting (November 17)

Organizers: Shinichi Aizawa and Naihe Jing

This one-day was held at the Shanghai Institutes for Biological Sciences in China, bringing together scientists from a number of institutes throughout China and Japan to build closer ties and opportunities for collaboration between these two nations in the study of stem cells and animal models of human disease.



RIKEN-SIBS Meeting

3rd Asia Reproductive Biotechnology Society Meeting (November 29 – December 1)

Organizers: Bui Xuan Nguyen, Nguyen Van Thuan and Teruhiko Wakayama

This meeting, co-hosted by the Vietnamese Academy of Science and Technology and the CDB and held in Hanoi, Vietnam, was attended by more than 200 researchers, exhibitors and dignitaries from Vietnam, Japan, Thailand, South Korea, China and Taiwan. Participants from outside the region also joined in the discussion of recent advances in assisted reproduction, animal fertility, nuclear transfer and early embryogenesis.



3rd Asia Reproductive Biotechnology Society Meeting

International Affiliations

The CDB is engaged in programs of cooperation with research organizations both within the Asia-Pacific region and around the world. These cooperative activities primarily involve individual researchers and laboratories. Details of overseas institutes that have formal agreements for collaboration with the CDB are listed below:



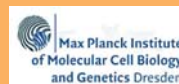
**The University of Texas
Graduate School of
Biomedical Sciences at
Houston, Texas, USA**

TEMASEK LIFE SCIENCES LABORATORY

**Temasek Life Sciences
Laboratory Limited,
Singapore**



**National Centre for Biological
Sciences, TIFR, Bangalore,
India**



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



**McGill University Faculty of
Science, Montreal, Canada**



**National Research Center for
Environment and Health,
Neuherberg, Germany**

Members of the CDB are also active in the administration of a number of international and regional organizations, including the International Society of Developmental Biologists (ISDB), the Asia-Pacific Developmental Biology Network and the Asia Reproductive Biotechnology Society (ARBS).



<http://apdbn.org/>



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



<http://www.cdb.riken.jp/arb/index.html>



2006 CDB Symposium

Logic of Development: New Strategies and Concepts

April 11 - 13, 2006

The 4th CDB annual symposium, on the theme, "Logic of Development: New Strategies and Concepts," drew an audience of more than 160 participants to the CDB for a full program of talks, poster discussions and discussion on April 10-12. The annual symposium series, which was launched in 2002, was established as a forum for addressing diverse aspects of developmental biology and the mechanisms of regeneration and aims to promote the free, timely and borderless exchange of research achievements. This year's event was co-organized by the CDB's Asako Sugimoto, Hiroki R. Ueda and Shigeo Hayashi along with Stephen Cohen of the European Molecular Biology Laboratory (Germany). The three-day program of talks and poster presentations focused on emerging concepts and approaches to the study of fundamental developmental questions, including the surprisingly diverse and important roles of microRNAs; theory, modeling, analysis and synthesis; evolutionary aspects of developmental systems; and functional genomics.

Session 1 [Micro RNA Function 1]

Victor Ambros (Dartmouth Medical School, USA)
René Ketting (Hubrecht Laboratory, Netherlands)

Session 2 [Micro RNA Function 2]

Eric A. Miska
(University of Cambridge/ The Wellcome Trust/
CR UK Gurdon Institute, UK)
Detlef Weigel
(Max Planck Institute for Developmental Biology, Germany)

Session 3 [Symmetry Breaking]

Tadashi Uemura (Kyoto University, Japan)
Yasushi Okada (The University of Tokyo, Japan)

Session 4 [Cellular and Theoretical Basis for Morphology]

Hirokazu Tsukaya
(The University of Tokyo/
National Institute for Basic Biology, Japan)
Shigeru Kondo (Nagoya University, Japan)

Session 5 [Quantitative Analysis of Dynamic Systems]

Atsushi Miyawaki
(RIKEN Brain Science Institute, Japan)
Shuichi Onami (Keio University, Japan)

Session 6 [From Analysis to Synthesis]

Ron Weiss (Princeton University, USA)
Hiroki R. Ueda
(RIKEN Center for Developmental Biology, Japan)

Session 7 [Cellular Logic of Morphogenesis]

Pernille Rørth
(European Molecular Biology Laboratory, Germany)
Emmanuel Farge (Curie Institute, France)

Session 8 [Lessons from Evolution]

Detlev Arendt
(European Molecular Biology Laboratory, Germany)
Kiyokazu Agata (Kyoto University, Japan)
Ralf J. Sommer (Max-Planck Institute, Germany)

Session 9 [Micro RNA Function 3]

Haruhiko Siomi (The University of Tokushima, Japan)
Stephen Cohen
(European Molecular Biology Laboratory, Germany)

Session 10 [Functional Genomics; Cell-Based Approach]

John Hogenesch
(The Scripps Research Institute, USA)
Nicolas Bertin (Dana-Farber Cancer Institute, USA)

Session 11 [Functional Genomics; Organism-Based Approach]

Asako Sugimoto
(RIKEN Center for Developmental Biology, Japan)
Duncan Davidson (MRC Human Genetics Unit, UK)

2007 CDB Symposium

Germ Line versus Soma: Towards Generating Totipotency

March 26-28, 2007

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

Robert E. Braun (Univ. of Washington, USA)
John J. Eppig (The Jackson Lab., USA)
Margaret T. Fuller (Stanford Univ., USA)
Hiroshi Handa (Tokyo Inst. of Technology, Japan)
Edith Heard (CNRS/Curie Inst., France)
Thomas Jenuwein (IMP, Austria)
Minoru S. H. Ko (NIH, USA)
Satoru Kobayashi (NIBB, Japan)
Ruth Lehmann (New York Univ., USA)
Tom Misteli (NIH, USA)
Akira Nakamura (RIKEN CDB, Japan)
Toru Nakano (Osaka Univ., Japan)
Hitoshi Niwa (RIKEN CDB, Japan)
Wolf Reik (The Babraham Inst., UK)
Gary Ruvkun (Harvard Med. School, USA)
Mitinori Saitou (RIKEN CDB, Japan)
Hans Schöler (Max-Planck-Inst., Germany)
Geraldine Seydoux (Johns Hopkins Univ., USA)
Susan Strome (Indiana Univ., USA)
Azim Surani (Univ. of Cambridge, UK)
Mark Van Doren (Johns Hopkins Univ., USA)
Shinya Yamanaka (Kyoto Univ., Japan)
Richard A. Young (MIT, USA)
Yi Zhang (Univ. of North Carolina at Chapel Hill, USA)

For more information, contact:

RIKEN, Center for Developmental Biology (CDB)
CDB Symposium 2006 Secretariat
Research Promotion Division
2-2-3, Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan
Tel: +81-78-306-3009 Fax: +81-78-306-3039
E-mail: sympo2006@cdb.riken.jp
<http://www.cdb.riken.jp/sympo2007/>

CDB Seminars

The CDB has made especial efforts to develop a full and diverse program of invited seminars by scientists from around the world. To date, the Center has hosted more than 300 such talks, in addition to numerous meetings, internal forums and colloquia. The following speakers presented CDB Seminars in the period from January to December, 2006:

date	title	speaker
01-18	Genetic activity influencing lineage allocation and cell movement during mouse gastrulation	Patrick TAM
03-03	Prions and the immune system	Neil Andrew MABBOTT
03-08	Turning a half into a whole	Bruno REVERSADE
03-13	Unraveling the logic of olfactory circuit formation	Takaki KOMIYAMA
03-16	Hair follicle stem cells	George COTSARELIS
03-16	Characterization and isolation of stem cell enriched human hair follicle bulge cells	Manabu OHYAMA
03-17	Mammalian spermatogenic stem cells in vivo: origin, niche and modes of self-renewal	Shosei YOSHIDA
03-20	In silico identification and in vivo validation of an Ath5 regulated target gene battery	Jochen WITTBRODT
04-03	Cell movements and shape changes during early anterior patterning in the mouse	Shankar SRINIVAS
04-05	p63 and epithelial stemness	Makoto SENO
04-21	Assembly of an RNP complex for interacellular mRNA transport and translational control	Anne EPHRUSSI
05-08	Selective elimination of mRNA provides a novel paradigm for the regulation of meiosis	Masayuki YAMAMOTO
05-22	Culture adaptation and self renewal of human embryonic stem cells	Peter W. ANDREWS
05-30	Heterotrimeric G protein signalling and asymmetric cell division in <i>Drosophila</i>	Fengwei YU
05-30	Look who's talking! Complex regulation of the epithelial-mesenchymal transition in neural crest and breast cancer cells	Donald F. NEWGREEN
06-07	Function of Suppressor of Fused (Su(fu)) and Kif27 in mammalian Hedgehog signal transduction	Shigeru MAKINO
06-13	Dissecting the role of Rho-mediated signaling in contractile ring formation	Keiju KAMIJO
06-15	Functional analysis of UNC-18 and UNC-13 in neurotransmitter release and its transcriptional regulation by novel protein F09G2.9	Toshihiro SASSA
06-26	Sleep, feeding, and neuropeptides	Masashi YANAGISAWA
06-27	Finding the synaptic organizers	Hisashi UMEMORI
07-18	Development and regeneration of the inner ear: the beginning and the end	Andy GROVES
07-19	From a nucleus to an organ: Combinatorial regulation between T-box and Spalt genes, and the chromatin remodeling complexes in heart development	Jun K. TAKEUCHI
07-25	Functional analysis of human genetic variant of BDNF (Val66Met)	Francis LEE
07-28	Vertebrate limb patterning and innervation	Edward LAUFER
07-31	A gene regulatory network for retinal ganglion cell development in the mouse	William KLEIN
07-31	Vertebrate dorsal-ventral limb patterning	Randy JOHNSON
07-31	Genetic mechanisms of morphological diversity between species	Richard BEHRINGER
08-01	Establishing cell fate in the cerebral cortex	Carina HANASHIMA
08-08	Cell fate decision by tumor suppressor p53: live or die	Sam W. LEE

date	title	speaker
08-31	Regulation of Cubitus interruptus, the transcription factor on the Hedgehog signal transduction pathway	Mary Ann PRICE
09-08	Wnt signaling and dorsal axis formation	Janet HEASMAN
09-08	Cortical actin; the early embryonic skeleton	Chris WYLIE
09-11	Wnt signaling in neural crest development: from Induction to migration	Roberto MAYOR
09-12	Therapeutic applications of human embryonic stem cells	Stephen MINGER
09-13	Chemical and functional genomic approaches toward regenerative medicine	Sheng DING
09-14	Defining stem cell characteristics: Tools and approaches	Jon D. CHESNUT
09-19	How to get in shape: regulation of morphogenesis in the early <i>Xenopus</i> embryo	Herbert STEINBEISSER
09-20	Cell fate specification by polarity determinants	Sergei SOKOL
09-21	Cadherins as regulators of cell migration	Doris WEDLICH
09-21	Regulation of neural stem cells in the embryonic brain by Notch signaling	Ken-ichi MIZUTANI
09-27	Anchoring of spindle microtubules to spindle pole bodies in fission yeast	Mika TOYA
09-28	DWnt4 regulates the dorsoventral specificity of retinal projections in the <i>Drosophila</i> visual system	Makoto SATO
10-16	Molecular and integrative neuroscience research in the University of Helsinki	Heikki RAUVALA
10-16	The Finnish Academy Center of Excellence in Development and Evolution Research	Irma THESLEFF
10-25	Signalling and tissue interactions for anterior morphogenesis in the mouse	Patrick TAM
10-25	The ground state of pluripotency	Austin SMITH
10-26	The analysis of NF- κ B pathway in B cell development, maintenance and transformation	Yoshiteru SASAKI
10-27	Regulating cell division during the development and regeneration of the inner ear	Neil SEGIL
11-10	JNK activator DLK regulates axon growth and neuronal migration in the developing cerebral cortex	Syu-ichi HIRAI
11-20	Identification of factors that generate pluripotent stem cells from fibroblast culture	Shinya YAMANAKA
11-21	Molecular mechanism of microtubule dynamics in mitosis	Kazuhisa KINOSHITA
11-27	Amine neurotransmitter signaling in <i>C. elegans</i>	Satoshi SUO
11-27	Transgenic alternative-splicing reporters reveal tissue-specific expression profiles and regulation mechanisms in vivo	Hidehito KUROYANAGI
12-11	Evolution and development of the fish skeleton: Paleontology meets developmental biology	Tsutomu MIYAKE
12-14	A paradoxical role of HMGA proteins in tumor suppression and tumorigenesis	Masashi NARITA
12-19	Interface between normal and transformed cells	Yasuyuki FUJITA
12-21	Genetic analysis of neuronal migration in cerebral cortex	Kazuhiro TOYO-OKA
12-25	Formation of lamina-specific neuronal connectivity in visual system	Masahito YAMAGATA

RIKEN Activities

RIKEN is one of Japan's largest publicly-funded scientific research organizations, comprising a number of institutes and centers throughout the country. Seeking to capitalize on the strengths of the diversity of its research programs—which span physics, chemistry and the life sciences, as well as engineering and medical science—RIKEN strongly encourages scientists working at different locations to meet, communicate and forge collaborations. The CDB has taken an active approach to engagement with this multidisciplinary environment through its participation in programs intended to build links within RIKEN at the individual, laboratory and institutional levels. In 2006, RIKEN underwent an organizational performance evaluation by its Advisory Council and made new strides in developing international visibility for its research achievements.

Advisory Council Report

In 2006, the RIKEN Advisory Council (RAC), a body of distinguished international scientists (Zach W. Hall, Chair), compiled its evaluation and recommendations for RIKEN's scientific activities and research governance in the report, "RIKEN: Leading Japanese Science to Global Pre-eminence." The report commends "the overall quality of science at RIKEN, which compares favorably with that of other leading research institutions worldwide," and suggests that RIKEN "has a particularly important role to play in the development of science in Asia."

The RAC emphasized the importance of continuing to support fundamental research in diverse fields, strengthening the administrative infrastructure, and the implementation of the Noyori Initiatives. This set of initiatives calls for RIKEN to:

1. Increase its visibility, both domestically and internationally
2. Maintain its outstanding history of achievements in science and technology
3. Provide an environment that stimulates and motivates its researchers
4. Make contributions to society and mankind
5. Participate in and contribute to the advancement of culture



RIKEN RESEARCH



In June RIKEN launched RIKEN RESEARCH, a new publication in print and online intended to draw the world's attention to some of RIKEN's best research in a timely and easy-to-understand fashion. This magazine provides a central resource where up-to-date information on key achievements of the numerous RIKEN institutes and research centers can be found. The core component of RIKEN RESEARCH is its short, easy-to-understand 'Research Highlight' articles explaining for a broad scientific audience a sampling of the latest research articles published by RIKEN scientists.

<http://www.rikenresearch.riken.jp/>

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Tel.+81-29-836-9111 Fax.+81-29-836-9109

**Wako Main Campus
Wako Institute**

Discovery Research Institute
Frontier Research System
Brain Science Institute
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Yokohama Institute

Genomic Sciences Center
Plant Science Center
SNP Research Center
Research Center for Allergy and Immunology
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Tel.+81-45-503-9111 Fax.+81-45-503-9113

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Nagoya, Aichi 463-0003
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**Kobe
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2-2-3 Minatojima-minamimachi, Chuo-ku,
Kobe, Hyogo 650-0047
Tel.+81-78-306-0111 Fax.+81-78-306-0101

Harima Institute

1-1-1 Kouto, Mikazuki, Sayo, Hyogo 679-5148
Tel.+81-791-58-0808 Fax.+81-791-58-0800

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On The Cover

X-gal histochemistry of a cranial region from a mouse embryo expressing *lacZ* under the Ret promoter. Cell bodies and processes of Ret-expressing neuronal populations including sensory, sympathetic and para-sympathetic neurons are visualized by blue deposits. Photo credit: Hideki ENOMOTO