RIKEN Center for Developmental Biology

2005 Annual Report

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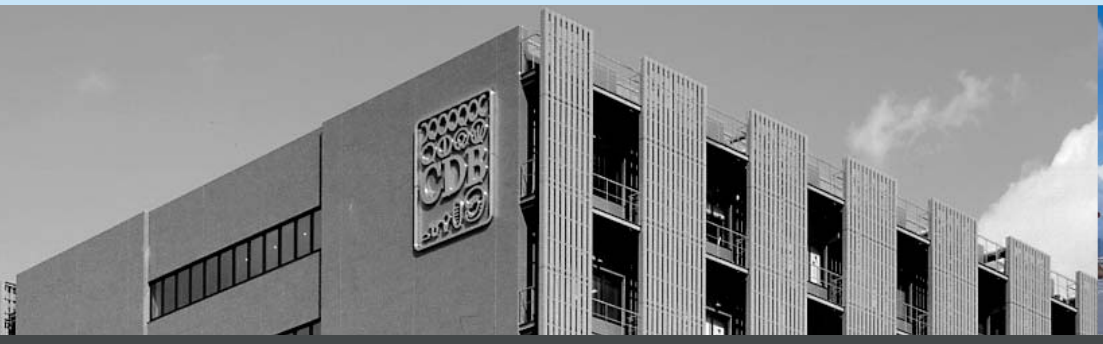
Contents



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 concluded its five-year term in March 2005.

RIKEN Center for Developmental Biology

April of this year marked the fifth anniversary of the establishment of the RIKEN Center for Developmental Biology under the auspices of the Japanese government's Millennium Project research initiatives. Looking back to that time, when the ground on which the CDB now stands was still nothing more than an empty plot on Kobe's Port Island, I recall both the great sense of possibility and the tremendous burden of responsibility shared by everyone in the first labs that signed on to this ambitious new project. Now, with our foundation-laying and growing pains behind us, the CDB is entering into a new phase of its history, in which we look to sustain the momentum that carried us through those remarkable days and continue building a tradition of pioneering life sciences research. The Millennium Project program concluded this year, but our goals remain consistent with its mission of exploring and understanding the mechanisms of animal development and regeneration.

Following a banner year in 2004, the research programs moved from strength to strength in 2005, reporting findings from across the spectrum of developmental biology and opening up new questions for investigation. A few areas saw particular progress, with a number of teams advancing our understanding of head development in mammals and several reports of techniques for guiding embryonic stem cells to differentiate into a range of cell lineages, including retinal neurons, mesendoderm and forebrain precursors. Mouse research in general came into its own this year, with CDB labs contributing to major new findings in the establishment of the germ line and to the first origins of cell differentiation in the early embryo. It is heartening to see that the investment of hard effort and long hours in the formative years of the CDB has paid such a handsome return, and I hope that you will find this report to be a helpful guide to some of the past year's research highlights.

Of course, research of this caliber cannot be conducted in isolation. The CDB backs its laboratories with funds and strong administrative support to encourage the holding of international seminars and meetings, and 2005 saw meetings held in conjunction with institutions in Taiwan, Hong Kong, Thailand, Germany and Finland, as well as an excellent two-day program focused on the developmental diversity of invertebrates. A scan of the author lists for articles published by CDB labs stands as testament to the internationality of the active research effort here, with collaborations between the CDB and labs in Europe, America and, increasingly, other parts of Asia now starting to bear fruit.

The growth of developmental biology and regenerative medicine in the Asia-Pacific region has been remarkable in recent years, and we have sought to encourage and support this trend in the interests of making these fields truly global in scope and in practice. We welcome the establishment of regional organizations such as the Asia-Pacific Developmental Biology Network and, beginning in 2006, plan to support a number of young scientists and graduate students with subsidies for travel to Japan to participate in our annual symposium. For longer-term guests and staff arriving from overseas, the CDB offers a wide range of support services to ease their transition into a new research and living environment, and by early 2006 we stand to see the proportion of our scientific staff coming from outside of Japan rise to above 10% of the total. I believe this increasingly global participation speaks both of the attention garnered by the Center's publication record and our growing reputation for having developed a research environment in which exceptional scientists from any background can feel equally at home.

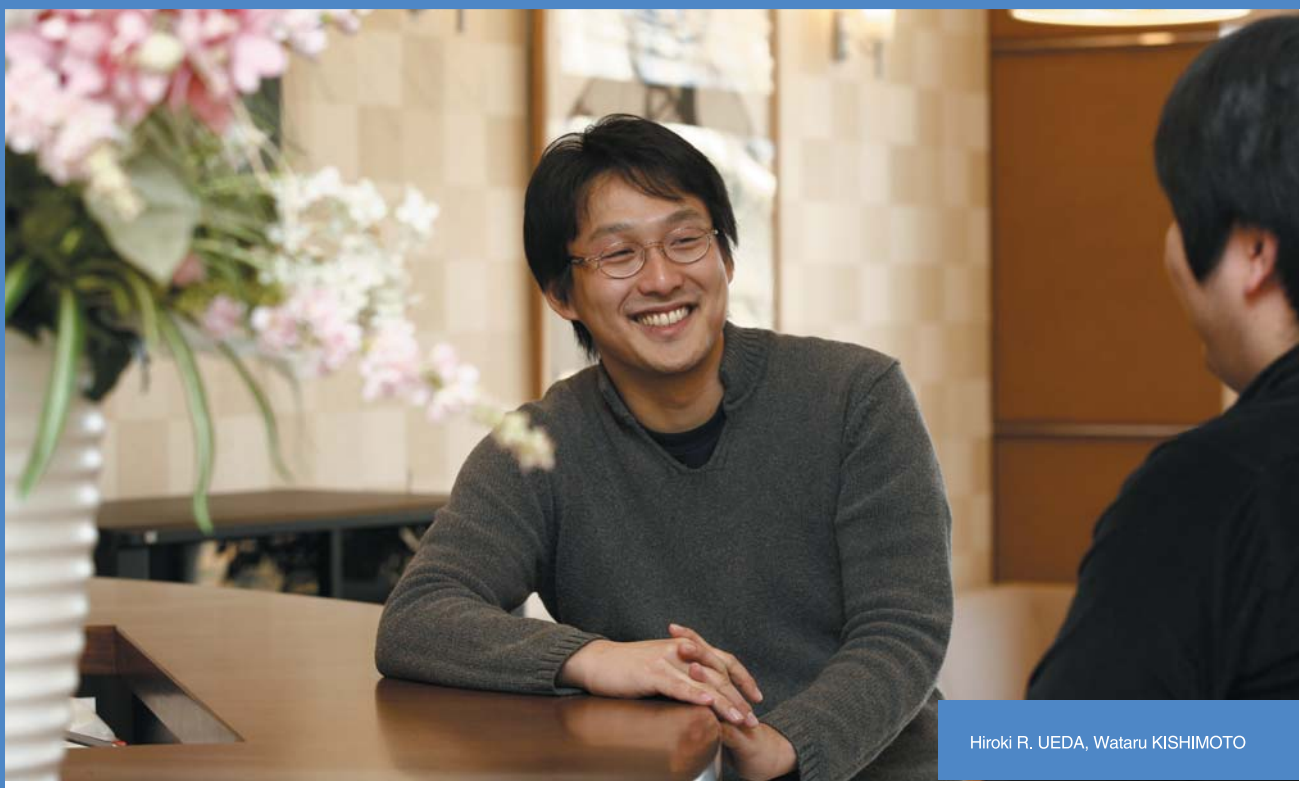
One of the primary objectives of the Millennium Project when it was launched in 2000 was to address the challenges of an aging society, one of Japan's most pressing issues for the economic, social and healthcare implications of its rapidly right-shifting demographic. For us in research, the problem of the decline in the average birth rate is compounded by a waning interest in science among younger generations, a quandary that confronts many other countries as well. While RIKEN is not an academic institution, we recognize the value of encouraging public understanding of and interest in science, and have developed a range of materials and programs in Japanese and English to introduce our research and the exciting frontiers of development and regeneration. Many of these can be found on our institute's website at www.cdb.riken.jp, and are available free for use in classroom and research presentations.

With the close of our fifth year, the CDB saw a number of transitions in its research staff, with two labs finding new homes at national universities, where we wish them continued success, and one new team joining the Center this summer. Encouraging mobility and opportunities has always been one of our goals, and it is gratifying to see that a number of CDB alumni have secured professorships in academia. As the Center enters a new phase of increased institutional maturity, we hope to continue to attract fresh new talent and foster the careers of our own staff as part of our ongoing mission to make real contributions to our field, and to the scientific endeavor in general. I hope that you find this report useful and informative, and wish you all the best for the year to come.

Masatoshi TAKEICHI
Director, RIKEN Center for Developmental Biology

At the heart of the clock

Key genetic regulators that help the body keep time



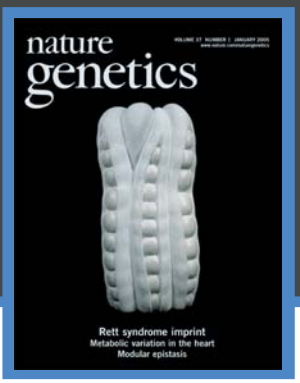
Hiroki R. UEDA, Wataru KISHIMOTO

Many of the mammalian body's systems are on a tightly controlled schedule, their activity rising and falling in response to the natural cycle of darkness and daylight, but also in response to internal genetic regulators ticking away in specific sites of the brain and other organs. These "clock" genes work in a complex and interconnected system of regulatory loops in which temporal regulators of genetic activity themselves may be regulated by other repressors and activators. This intricate network represents fertile ground for study by systems biologists, who seek to identify and analyze higher-order organizations and coordinated behaviors in sets of interacting biological components.

Scientists have been working for decades to elucidate the individual genetic components of these complex circadian systems in a range of experimental models. In a study published in the January issue of *Nature Genetics*, Hiroki R. Ueda (Team Leader, Laboratory for Systems Biology) reported the identification of what appears to be the central players of a network of genes that regulates daily biological rhythms in the mouse.

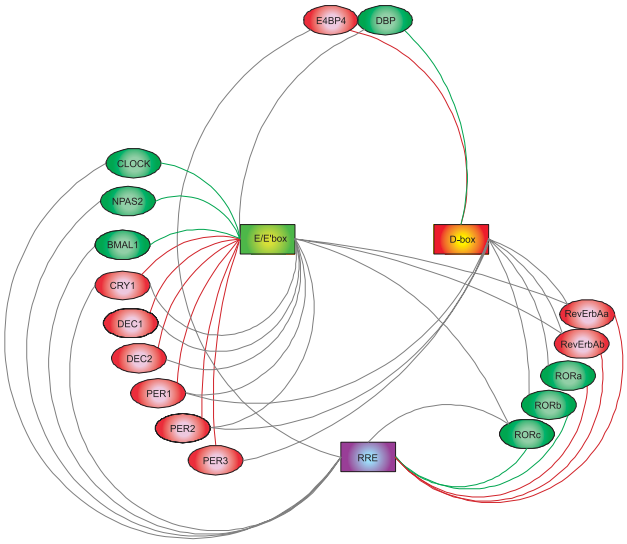
At least 16 clock and clock-related genes have been identified in the mouse, but the means by which they interact to achieve cyclical expression patterns had been a mystery. These 16 genes can be categorized into groups by their possession of one or more of three signature nucleotide sequences, conserved in human and mouse and linked to their times of peak expression. These three characteristic regions, named E/E'-box, D-box and RRE, are found on genes encoding transcription factors, which work to regulate the timing, site and amount of the production of RNA "messages" from gene sequences encoded in a cell's DNA. The Ueda team found that each of the three classes of gene regulated transcription at different times of day. Specifically, "morning" E/E'-box regions were found on nine genes, D-box "midday" transcription factors were located on seven genes, and six genes regulated by "night" RRE domains were identified. These three classes of transcription factors were also linked to the regulation of other genes in the 16-member network, with nine involved in morning transcriptional regulation, two in midday and five in night, allowing the team to devise a complex model of interacting regulatory loops in which individual genes both regulate and are regulated by other genes in the network.

Ueda H R, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y, Iino M and Hashimoto S. System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat Genet* 37:187-92 (2005).



Analysis of the network showed that the E/E'-box morning transcription factors were the most prevalent of the three types, both in terms of the genes they acted on directly and the input they received from other genes in the system, with nine instances of each. By demonstrating that E/E'-box regulation is the most highly connected set of "nodes" within the network, the Ueda study provides compelling evidence that these factors are likely to play the most central role in circadian regulation and therefore may represent attractive targets for those interested in determining and analyzing means of controlling the network as a whole. This conjecture was borne out by at least one experiment, in which the overexpression of a repressor of E/E'-box regulation resulted in the disruption of the rhythmicity of normally circadian promoters, an effect not seen when repressors of D-box or RRE regulation were overexpressed.

This detailed classification next allowed them to look for patterns in the activity of repressors and activators of clock gene expression, a search that revealed that different categories of genes tended to exhibit different temporal activity describable by two straightforward principles. If cycles of gene expression in this system are



Network of transcriptional factors involved in the regulation of the mammalian body clock.

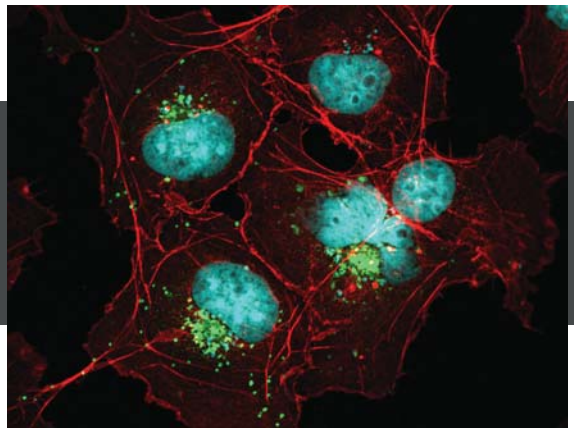
thought of as following the coordinated clockwise movement of hands around a dial, E/E'-box and RRE regulation could be characterized as "repressor precedes activator," with the repressor gene always remaining slightly ahead of the activator as they cycle throughout the day. In D-box regulation, a separate principle applies, in which expression levels of repressors remain in near-diametric opposition to those of activators, circling the dial in constant counterpoint. Although the exact means by which rhythmic oscillations in gene expression in human and mouse remain unknown, the "repressor precedes activator" mechanism appears to play an important role by delaying transcriptional activity, while "repressor antiphasic to activator" results in high-amplitude transcription.

The Ueda study is an important first step toward the identification of systems governing circadian gene activity in the mouse, but much remains to be done before this ornately structured and tightly controlled network is fully understood, as molecular signaling cascades, chromatin modifications, and the stability and localization of proteins are also thought to be involved in determining its cyclical rhythm. The benefits of such knowledge, however, could be great as well, for the ability to analyze and modulate normal and disturbed biological clocks in humans has potential clinical applications ranging from the development of drug administration regimens tailored to patients' physiological rhythms to new approaches to the treatment of circadian disorders such as insomnia and depression.

Shisa serves double duty

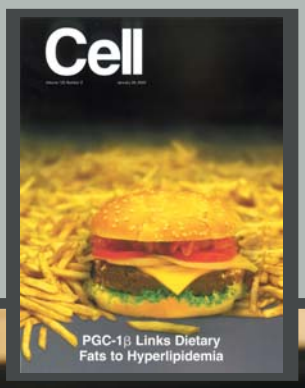
The network of regulatory factors involved in the development of the vertebrate head is an exquisitely tuned system that is baroque in its complexity. During early embryogenesis, arrays of activating and inhibitory factors work to mold an initially unstructured clump of cells into an increasingly recognizable body with a distinct back, belly, head and tail. The development of the head is enabled and guided by the activity of a region of cells called the organizer, which secretes inhibitors of multiple regulatory molecular pathways that affect the axial orientation and growth of the embryo; in a sense, the organizer serves as an active defense system against a host of factors that would otherwise frustrate the development of the nascent head. The activities of these secreted factors cross the boundaries of single cells, but it has been suggested that additional regulators of head development may be present and act autonomously within individual cells as well.

The Wnt and FGF signaling pathways are families of multifunctional molecules that work in cell growth and differentiation, and the patterning of tissues and anterior-posterior body axes. As with proteins in secretory pathways, following translation, Wnt and FGF ligands and their cognate receptors are processed by the endoplasmic reticulum (ER) where they are folded into their functional configurations and undergo modifications such as the addition of sugars to their peptide chains. Only after attending this "finishing school" are the proteins ready for transport to the surface to serve as transmembrane receptors or be released into the extracellular space. In an article published in the 28 January 2005 issue of *Cell*, Akihito Yamamoto of the Laboratory for Vertebrate Body Plan (Shinichi Aizawa, Group Director) and colleagues reported the identification of a novel factor, Shisa, which blocks Wnt and Fgf signaling at the endoplasmic reticulum by a mechanism previously unencountered in a developmental setting.



Confocal fluorescence image of COS cells expressing Frizzled (Fz; Green) and Shisa.

shisa mRNA is expressed in both the head organizer and the anterior neuroectoderm, the region from which the head emerges in embryos of the African clawed frog (*Xenopus laevis*), and has homologs in other vertebrates, including mouse, zebrafish and human. *shisa* encodes a secretory pathway protein with no known protein motif. In an early exploration of its role, Yamamoto found that its overexpression causes an expansion of the anterior territory, a property that earned the molecule its name. (The *shisa* is a form of sculpture common to Okinawa, representing a guardian lion-dog with a large head.) Further investigations of *shisa* function, using an experimental system known as animal cap assay, indicated that while Shisa has no discernible neuralizing activity, it acts as an anteriorizing agent that antagonizes both Wnt and FGF signaling. Loss of function studies revealed that Shisa is an essential factor for the proper formation of the head, especially in the presumptive head ectoderm during gastrulation.



Yamamoto A, Nagano T, Takehara S, Hibi M and Aizawa S. Shisa Promotes Head Formation through the Inhibition of Receptor Protein Maturation for the Caudalizing Factors, Wnt and FGF. *Cell* 120:223-35 (2005).

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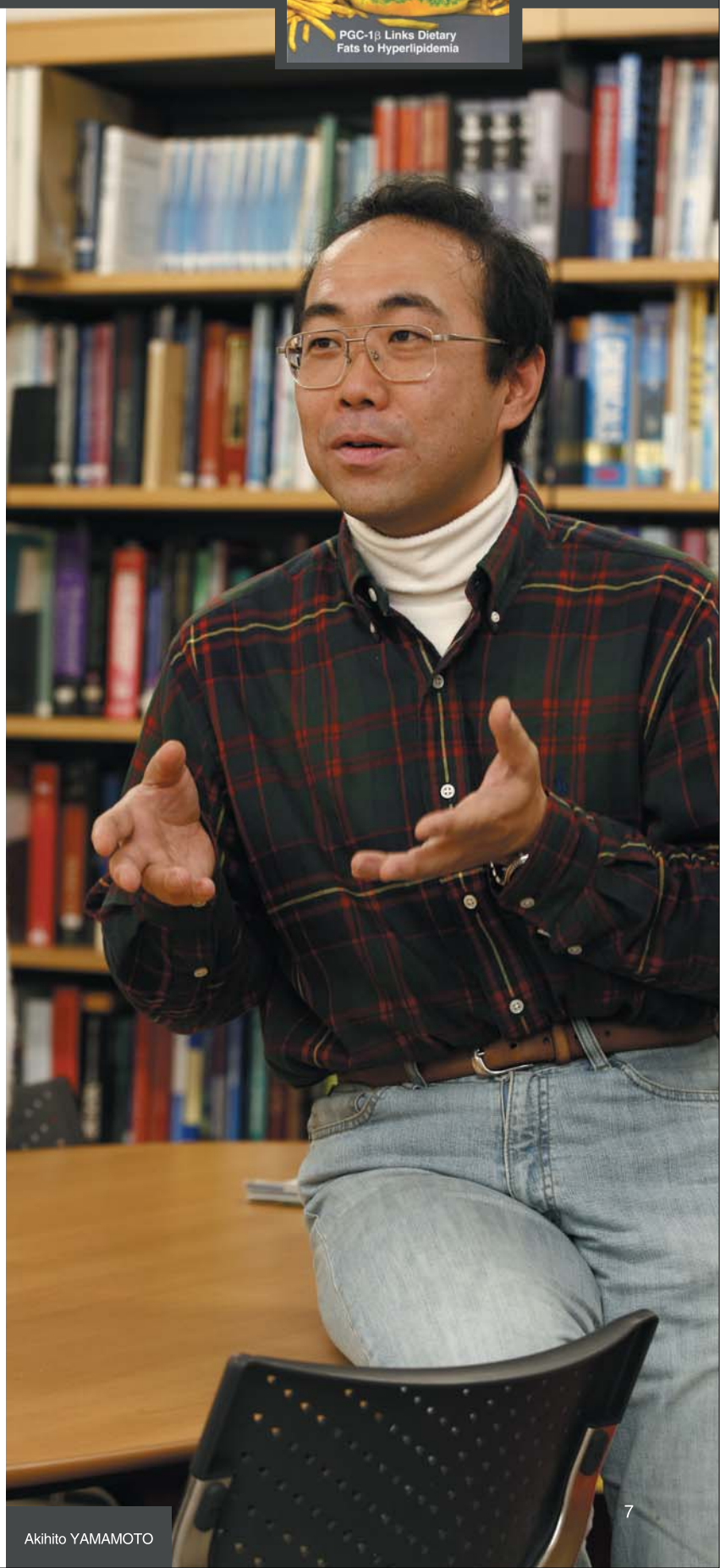
These initial in vivo studies suggested that Shisa is a secreted protein with a role as an inhibitor of Wnt and FGF signaling, which would have made it yet another molecule discharged by the cells of the head organizer. But a closer examination of Shisa's distribution within the cell revealed a distinct pattern of localization to the endoplasmic reticulum. Furthermore, in epistatic analysis, Shisa cell-autonomously inhibited signaling in receptor-expressing cells. When the team watched the activity of Frizzled (Fz, a Wnt receptor) in cells also expressing Shisa, they found that both proteins remained within the endoplasmic reticulum (Fz is normally transported to the cell's surface membrane). Looking for possible interactions, they found that Shisa specifically engages with a lower molecular weight species of Fz8, which they reasoned might be an immature version of the functional receptor protein, prior to its processing by the ER. Cells in which Shisa function was lost demonstrated higher surface expression of Fz and increased responsiveness to stimulation by Wnt ligands. These findings led Yamamoto et al to conclude that Shisa works by holding the unfinished Wnt receptor at the endoplasmic reticulum, interfering with its maturation and resulting in its degradation before it can establish itself as a functioning unit within the cell membrane.

Parallel investigations revealed similar ER co-localization with and inhibitory effects on an FGF receptor (FGFR), adding even more novelty and interest to the story, as FGF signaling has posteriorizing activity, but is also required for neural development. It is not yet known how Shisa balances the repression of posteriorization while allowing FGF-driven neural induction in the anterior region to take place, a question that invites further study. The protein structure of Shisa and the exact mechanism by which it binds to and exerts its inhibitory effects on two structurally dissimilar receptor proteins also remain unknown. Indeed, the identification of a molecule capable of regulating a pair of signaling pathways as central as Wnt and FGF by a novel mechanism seems destined to present new challenges of explication to cell and developmental biologists; as in any worthy exploration, the ultimate goal is not arrival, but a pushing back of the horizon.



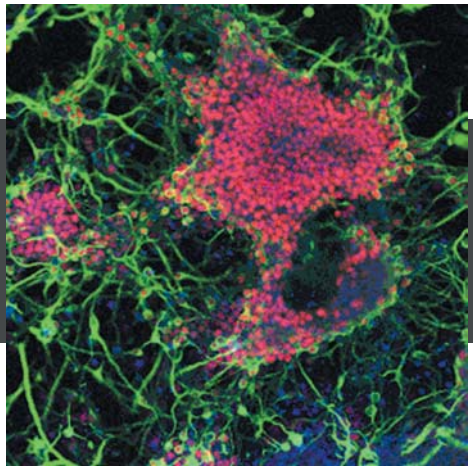
Akihito YAMAMOTO

Head first

Induction and regional specification of forebrain precursors from ES cells

The mammalian central nervous system is notoriously non-regenerative, and the treatment of disorders in which neural function has been compromised represents one of the greatest challenges remaining to modern medicine. Much attention has been paid in recent years to the promise of cell replacement therapy as a potential means to restore damaged nervous systems to health. This method, which involves the selective culture of cells for transplantation into patients deficient in a specific cell type, represents one of the best hopes on the horizon for patients suffering from a host of currently incurable afflictions.

Embryonic stem (ES) cells may make an especial contribution to the realization of the promise of regenerative medicine, as these cells have the potential to give rise to any of the body's myriad cell types. While researchers in recent years have developed methods allowing them to steer the differentiation of ES cells into a variety of neuronal types, efforts to induce the selective differentiation of precursors to the embryonic forebrain (called the telencephalon) have been frustrated by the strong tendency of neurons

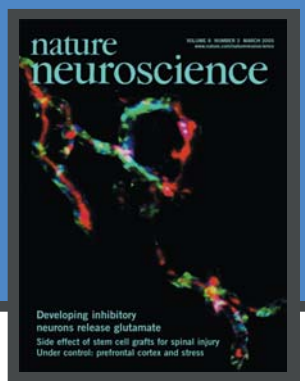


Telencephalic precursor cells induced by SFEB culture (Bf1 shown in red).

differentiated from ES cells using extant culture protocols to assume more posterior (caudal) neuronal fates. In a breakthrough achievement published in the February 2005 edition of *Nature Neuroscience*, Yoshiki Sasai (Group Director; Laboratory for Organogenesis and Neurogenesis) and colleagues announced their development of a technique enabling the highly selective differentiation of telencephalic precursors from mouse ES cells.

This is the latest in a series of methodological advances for the high-efficiency differentiation of neurons from ES cells by the Sasai laboratory. In previous reports, the group described the application of a method based on the ability of feeder cells (called stromal cell derived inducing activity, or SDIA) used in combination with various growth factors to steer mouse and primate ES cells to differentiate into dopaminergic, sensory, and enteric neurons at efficiencies of up to 90%. But these SDIA-based approaches failed to generate forebrain precursors (as distinguished by the expression of certain genes) at high frequency.

The group switched to a different tack to test the feasibility of inducing telencephalic precursor differentiation in vitro, using a culture technique that required neither feeder cells nor culture serum, a method they named SFEB (for Serum-free Floating culture of Embryoid Body-like aggregates). Embryoid bodies



Watanabe K, Kamiya D, Nishiyama A, Katayama T, Nozaki S, Kawasaki H, Watanabe Y, Mizuseki K and Sasai Y. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci* 8:288-96 (2005).

are agglomerates of stem cells that have been observed in traditional ES cell culture experiments. When Sasai and colleagues treated the SFEB colonies with antagonists of Wnt and Nodal, both of which are neural suppressing factors, they found that the cells showed neural differentiation at a near-perfect selectivity of about 90%. By adding an additional factor, Dkk1, to the cocktail, they were further able to steer nearly 40% of the cells down the path to a telencephalic fate (as evidenced by the expression of the Bf1 marker) – a first in the guided differentiation of ES cells.

They next sought to take the SFEB-cultured precursors one step further down the forebrain pathway by treating them with Wnt3a, a signaling factor that blocks neural differentiation early in embryogenesis, but interestingly promotes the adoption of a pallial (dorsal) fate in cells already committed to the telencephalic lineage. Their experiments bore out the hypothesis that the same would occur under SFEB culture, with significant dose-dependent increases in the population of cells expressing pallial markers following late-stage treatment with Wnt3a. Further experiments in which the SFEB aggregates were treated with Sonic hedgehog (Shh) for days 4-10 of culture sent the cells in the opposite direction, triggering an increase in the number of basal telencephalic neurons arising from the precursors.

The ability to generate the cellular forebears of the telencephalon from mouse ES cells represents a landmark in stem cell research. From a purely developmental perspective, the close mirroring of in vivo gene expression patterns by the externally induced effects seen in SFEB culture experiments provides new food for thought for scientists studying the genetic regulation of neurogenesis, particularly in its implications for the neural default model, which states that ectodermal cells tend to assume a neural fate in the absence of molecular messages to the contrary. And the proof-of-principle demonstration of the amenability of ES cells to forebrain differentiation and regional specification provides new avenues for biomedical researchers and clinicians to explore in the struggle to find cures for a range of human neurological disorders, including Huntington's and Alzheimer's disease, that affect the mind's highest functions.

Ayaka NISHIYAMA, Kiichi WATANABE, Daisuke KAMIYA

How the zebrafish (brain) got its stripes

Brain development is a territorial affair, with individual neurons vying for limited neurotrophic resources, groups of cells staking out turf and erecting various molecular signposts and fences to protect their claims, and entire neuronal populations that arise and fade away over the life of the animal. While this may sound like unneighborly behavior, the borders drawn in these developmental processes are of great importance in establishing the regions that give the brain its diversity of function.

In zebrafish, two distinct populations of neurons, primary and secondary, manifest during its ontogeny, with the primary neurons playing the vital role of enabling the larval fish to move, sense and respond to their environment immediately after hatching. These primary neurons form from regions known as proneuronal domains, which appear as three longitudinal stripes on the dorsal side of the embryo. The proneuronal domains can be visualized by staining for marker genes characteristic of proneural cells, such as *neurogenin1* (*neurog1*), *olig2* and *pnx*, and the mechanisms by which cells in these regions are specified to neuronal fates are fairly well understood. However, the process responsible for delineating the interproneuronal stripes, in which no proneural gene expression is observed, has remained largely a mystery.

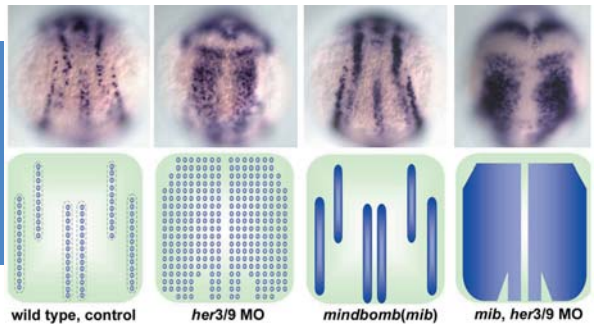
Young-Ki Bae and colleagues in the Laboratory for Vertebrate Axis Formation (Masahiko Hibi, Team Leader) set out to determine what might be responsible for this pattern of alternating proneural gene expression. Studies of proneuronal development in *Drosophila* have shown that cells adjacent to proneuronal neighbors begin to express a pair of genes, *Her/Hes* (for *hairy* and *enhancer of split-related*) genes, which encode transcriptional repressors to block neurogenesis in the cells in which they are expressed. Similar roles have been demonstrated for related families of genes in vertebrates, including mouse and amphibian (*Xenopus*). In some contexts, the activation of proneural suppressors occurs downstream in the Notch pathway, while other studies have shown that the position and width of proneuronal domains and intervening non-proneuronal bands seem to be regulated by positional information.

Young-Ki BAE

Bae Y K, Shimizu T and Hibi M. Patterning of proneuronal and inter-proneuronal domains by hairy- and enhancer of split-related genes in zebrafish neuroectoderm. *Development* 132:1375-85 (2005).

Bae et al looked at a pair of zebrafish *Her/Hes genes*, *her3* and *her9*, which are expressed in the three inter-proneuronal domains running parallel down the dorsal aspect of the zebrafish posterior neuroectoderm. Comparisons of *her3* and *her9* expression patterns against those of known proneuronal markers showed that both were restricted to the regions between proneuronal stripes, with *her3* showing a more limited range. They tested the effects of altered Bmp signaling on the expression of these genes by tracking their expression in mutants in which the interproneuronal domains spread as a result of interference with the Bmp pathway. These mutants showed corresponding expansions of *her3* and *her9*, indicating that the expression of these genes responds, either directly or indirectly, to positional information set up by Bmp.

They next examined the role of Notch signaling, which is known to play a role in the regulation of *Her* genes in many contexts in zebrafish development. Surprisingly, however, the team found that neither a mutation of the gene, *mind bomb*, which is involved in the activation of Notch signaling, nor the introduction of a chemical inhibitor of the Notch intracellular domain, nor an antimorphic form of the Delta protein had any effect on *her3* or *her9* in early neurogenesis.



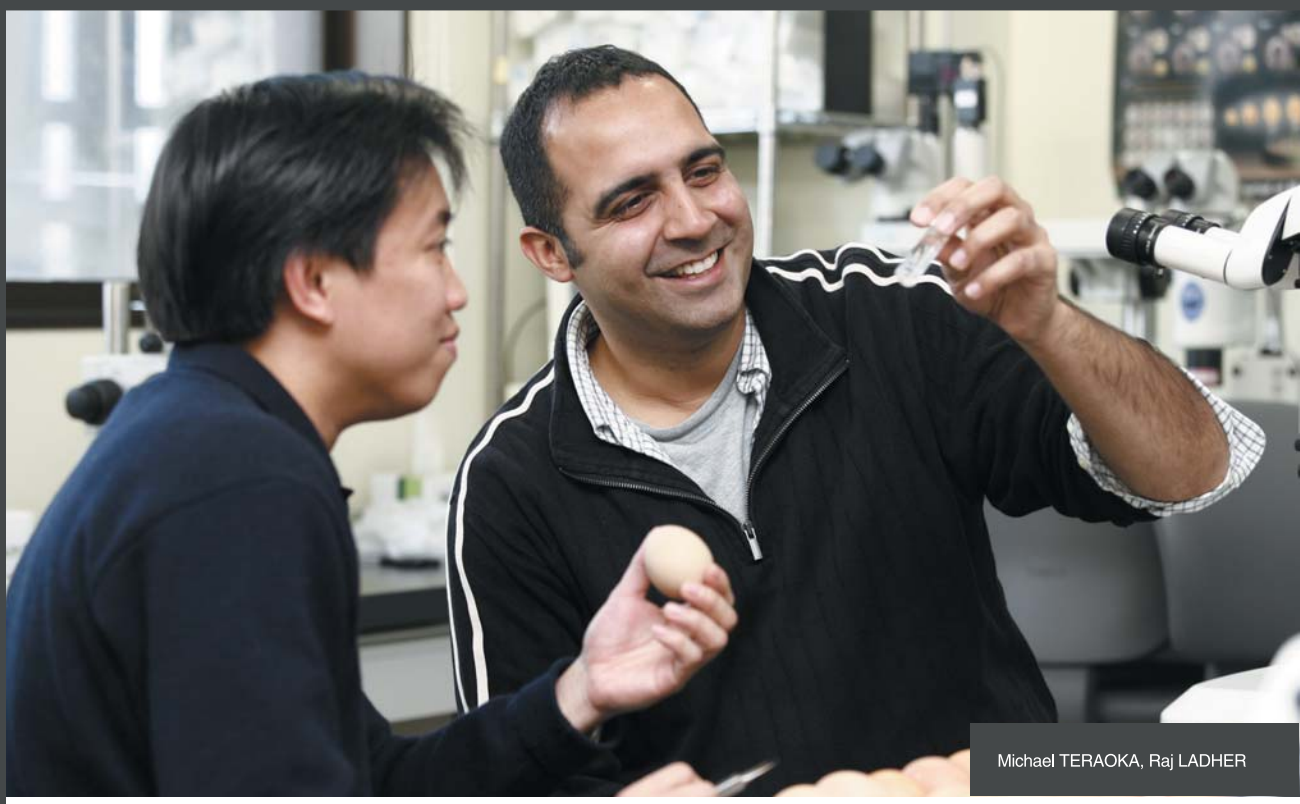
Changes in expression of the neuronal marker gene, *elavl3*, in her3/9 morphant, *mind bomb* and combined phenotypes. Wildtype shown on left.

Asking whether the interproneuronal regions might be determined secondarily by the proneuronal domains, the Hibi team tried misexpressing the proneuronal gene *neurog1*. They found that although *neurog1* could induce ectopic proneuronal domains, it had no impact on *her9*. (It did reduce *her3* expression, possibly through the upregulation of the gene for the Notch receptor, *delta*.) Similarly, morpholinos for *neurog1* and *olig2*, which inhibit the development of various primary neurons, had no effect on either *her3* or *her9*.

To investigate the function of *her3* and *her9*, Bae looked at phenotypes in embryos in which these genes' functions had been knocked down by antisense morpholino oligonucleotides, and discovered that loss of *her9* function resulted in ectopic expression of proneural signature genes in interproneuronal domains, as did knockdown of *her3*, although its effect was more spatially constrained. Combined knockdown of *her3* and *her9* function led to the loss of interproneuronal domains and induced ubiquitous neurogenesis in the neural plate. Furthermore, the inhibition of both *her3/her9* function and Notch signaling led to ubiquitous and homogenous expression of proneural and neuronal genes in the neural plate.

These findings led the lab to propose, in a report published in the February 2005 edition of the journal *Development*, a new model for the patterning of the embryonic zebrafish brain in which Notch and *her3/her9* play distinct roles in the organization of the alternating pro- and interproneuronal bands and neural specification. As previous reports have shown, Notch signaling works to limit the number of cells that adopt a neural fate by lateral inhibition within a given proneuronal domain, but it is the prepatterning of the neuroectoderm by *her3* and *her9* that give the zebrafish its earliest set of stripes.

Innermost secrets of inner ear induction



Michael TERAOKA, Raj LADHER

The ear's architecture is elaborate, with the three distinct components of outer, middle and inner ear coordinating to enable the senses of hearing, motion and balance. These otic subunits arise via separate genetic programs, the foundations of which are laid down very early in the embryo's development. The inner ear, which grows to become home to populations of signal-transducing hair cells used in audition and maintaining equilibrium, is a marvelously intricate and multifunctional structure. It derives from a small patch of embryonic tissue known as the otic placode, a thickened disc that appears in an ectodermal region that would otherwise be destined to become skin.

Studies in chicken and mouse have shown that the specification of this placode is the outcome of a pattern of tissue interactions between tissues from at least two of the three embryonic germ layers, mesoderm and ectoderm. This previous work demonstrated that, in both species, the adjacent regions of head mesoderm and neural ectoderm (or caudal hindbrain) must communicate and work together to achieve complete development of the otocyst. In the March 2005 issue of the journal *Genes and Development*, Raj Ladher (Team Leader, Laboratory for Sensory Development) and colleagues at the University of Utah have now determined a role for signals from the third germ layer, endoderm, in the initiation of the inner ear.

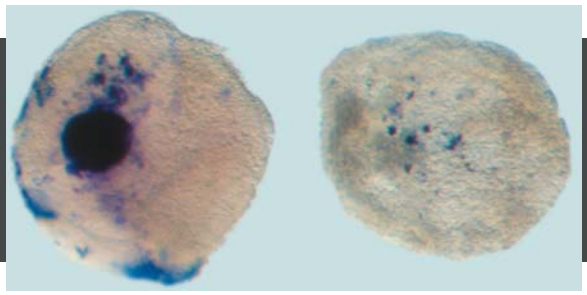
The study of ear development reveals great diversity in molecular agents across taxa, although most of that variety is confined to the FGF family of secreted proteins and receptors. In the chicken embryo, signaling by FGF19 from the cranial mesoderm induces the expression of WNT8c and FGF3 in the neural ectoderm, which then cooperatively induce the otic placode to form in a nearby non-neural ectodermal region. The mouse achieves the same ends by different means, using mesodermal FGF10 and FGF3 in the hindbrain to instruct the placode to form. Zebrafish embryos, meanwhile, take a third route, utilizing

Ladher R K, Wright T J, Moon A M, Mansour S L and Schoenwolf G C. FGF8 initiates inner ear induction in chick and mouse. *Genes Dev* 19:603-13 (2005).

FGF3 and FGF8 to engage and regulate otic induction. This last example sparked Ladher's interest, as there had been no reports of *Fgf8* expression in the parotic regions of either chicken or mouse, and, with his colleagues in Utah, he began to investigate the possible involvement of FGF8 in the induction of the ear in these species.

Tissue ablation studies in chick showed that endoderm makes a contribution to the initiation of otic development, which subsequent investigations indicated was due to its induction of mesodermal Fgf19. Looking in the subjacent endoderm at developmental stages corresponding to the start of the *Fgf19* expression, Ladher found that Fgf8 is indeed expressed in patterns suggesting a role in otic induction. The team found that exogenous FGF8 was sufficient to induce mesodermal *Fgf19*, then demonstrated its necessity by inhibiting *Fgf8* expression in vivo using RNAi, which resulted in the downregulation of *Fgf19* and failure of otic placode development.

In parallel studies in mouse, *Fgf8* was found to be expressed in a pattern consistent with a role in the development of the ear, with transcripts detected in relevant sites and at time-points synchronous to otic placode induction. Gene expression patterns indicated a possible overlap in function between Fgf8 and another FGF family member, Fgf3. Embryos entirely lacking *Fgf8* die prior to the initiation of ear development, so the team



Tissue that forms the inner ear expresses the transcription factor Pax2 (left); in tissues where Fgf8 is inhibited in the endoderm, the inner ear (as marked by Pax2 expression) does not form (right).

constructed a model, in which *Fgf8* expression is dramatically reduced and Fgf3 absent, to test the effect on otogenesis. They found that, at these low levels, *Fgf8* was unable to support ear development in the absence of *Fgf3*. This effect, however, did not translate to abnormalities in hindbrain development (which contrasts with the case for zebrafish, in which *Fgf3* and *Fgf8* also function redundantly), suggesting that in mouse the ear and the hindbrain are determined by different genetic routines. Examining the *Fgf3/8* double mutants more closely, the team saw that the phenotypes resembled those of *Fgf10* knockouts, a similarity which was substantiated at the molecular level by in situ hybridization studies showing clear reductions in *Fgf10* expression in the double mutant mesenchyme.

"What's interesting about these results is that they show the involvement of signals from all three germ layers in inducing the ear," says Ladher. "In both chick and mouse, endodermal FGF8 seems to be working as a molecular cue ball, setting off different combinations of serial and parallel interactions in the overlying mesoderm and ectoderm that ultimately result in the specification of the otic placode."

Asymmetric T cell division by dint of Wnt

Asymmetric division, in which a cell divides into daughter cells of different characters, is an indispensable process in the development of multicellular organisms. This process is important in many aspects of development in the nematode *C. elegans*, including the differentiation of a lineage of cells found in the tail region, named T cells. In a report published in the March 2005 issue of the journal *Development*, Hitoshi Sawa and colleagues in the Laboratory for Cell Fate Decision, and in labs at Osaka, Kobe and Keio Universities, described the requirement for a pair of molecules, LET-19 and DPY-22, thought to contribute to the formation of a multicomponent complex at work in T cell asymmetric division.

LET-19 and DPY-22 seem to achieve their asymmetry-regulating function by the transcriptional repression of target genes in the Wnt signaling cascade, a molecular pathway known to play roles in many aspects of development in a diverse menagerie of organisms, including *C. elegans*. In T cells, for instance, the factor TLP-1 has recently been shown to be regulated by the Wnt pathway resulting in its unequal expression in T lineage daughters. Loss of function of the genes *lin-44* or *lin-17* (which respectively encode proteins homologous to the Wnt ligand and its receptor Frizzled) interferes with normal asymmetric division, causing the failure of T.p cells to generate the phasmid socket cells that would usually be included in their progeny. The molecular means by which Wnt regulates the transcription of target genes in which to achieve this asymmetry, however, remain obscure.

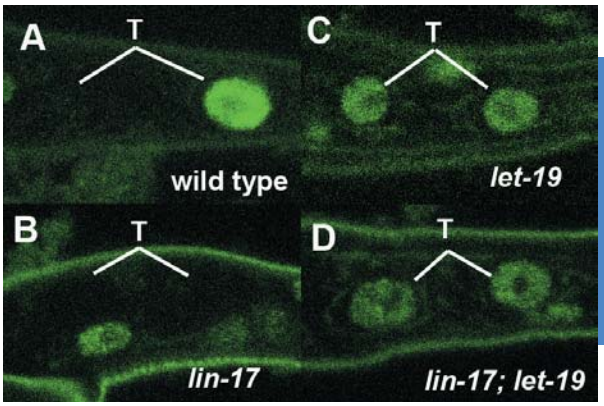
Sawa and colleagues first identified *let-19* and *dpy-22* in an analysis of worms in which the phasmid socket cells were absent. These phenotypes showed loss of asymmetry in T cell daughters similar to that seen in *lin-17/frizzled* mutants. Seeking to determine the mechanism by which *let-19* and *dpy-22* operate in this process, Sawa et al. looked at their relationship to a further pair of genes, *pop-1* and *tlp-1*, known to be expressed differentially in T.a and T.p cells, the anterior and posterior progeny of T cell division. In T.p cells, the localization of POP-1 is controlled by Wnt signaling, so the team used a construct fusing POP-1 with green fluorescent protein (GFP), allowing them to track the protein's distribution within cells. These studies revealed that mutations of neither *let-19* nor *dpy-22* affected POP-1 localization, ruling out the possibility of a regulatory role for these genes in that process.

Hitoshi SAWA

Yoda A, Kouike H, Okano H and Sawa H. Components of the transcriptional Mediator complex are required for asymmetric cell division in *C. elegans*. *Development* 132:1885-93 (2005).

They turned next to *tlp-1*, whose protein product is a downstream factor in the Wnt pathway expressed specifically in T.p but not in T.a cells. A study of *lin-17* mutants showed the expected defects in *tlp-1* expression. Looking at loss-of-function mutations for both *let-19* and *dpy-22*, Sawa found that in either of these mutants, *tlp-1* expression could be seen in both the T.a and the T.p daughter cells, reflecting the loss of their normally unequal distribution. Worms in which both *lin-17* and *let-19* were mutated showed the same phenotype as the *let-19* single mutant, indicating that *let-19* works downstream of the Wnt signal. Further studies using other cell lineages failed to produce any effects on patterns of *tlp-1* expression.

Pursuing the *let-19* and *dpy-22* connection to the Wnt cascade, Sawa next examined their effects in Pn.p cells, a series of 11 cells found running down the anterior-posterior axis on the ventral side of the nematode. P1.p and P2.p, the anterior–most of the cells, and P9.p~P11.p, the last three in line, undergo fusion with the hypodermis, leaving the remaining six cells in the center to act as precursors of one of the worm's organs of reproduction, the vulva. In this cohort, the gene *bar-1* encodes the *C. elegans* homolog of β-catenin, which serves to maintain the expression of LIN-39/Hox, an inhibitor of cell fusion. In *bar-1* mutants, fusion occurs ectopically, leading to a reduction in the number of vulval precursors. The team found that both the *let-19* and *dpy-22* mutants underwent less frequent cell fusion and seemed to repress the *bar-1* phenotype characterized by ectopic fusion. Contrastingly, *let-19* mutations did not suppress the *lin-39* phenotype, suggesting that *let-19* and *dpy-22* work by repressing *lin-39* downstream of *bar-1*. They looked for further involvement in other phenomena where Wnt signaling is a factor, such as endodermal induction and the formation of distal tip cells at the leading edge of the migrating gonad, but found that *let-19* and *dpy-22* function is specific to Pn.p and T cells.



T cells expressing *tlp-1::gfp* fusion protein. Wildtype cells (A) show expression in only a single daughter cell, while in *lin-17* mutants (B) expression is absent in both daughters. Patterns in *let-19* single mutant (C) and *lin-17/let-19* double mutant, in which both daughters express the fusion protein, are similar, suggesting *let-19* functions downstream of *lin-17*.

The gene products of *let-19* and *dpy-22* are homologous to mammalian MED13 and MED12, respectively, both of which are components of transcriptional regulatory "Mediator" complexes, which have been shown to play roles in the activation or repression of numerous genes. Two mammalian Mediator complexes, CRSP and ARC-L, are known, the latter being a larger, transcriptionally active complex that contains both MED12 and MED13. Co-immunoprecipitation assays demonstrated that LET-19 and DPY-22 bound other putative Mediator complex components, leading the team to surmise the existence of a pair of Mediator complexes in *C. elegans* as well, with LET-19 and DPY-22 acting as components of the counterpart to mammalian ARC-L.

The results of this thoroughgoing investigation point clearly to roles for LET-19 and DPY-22 in both the asymmetric division of T cells and the regulation of Pn.p cell fusion by the inhibition of Wnt target genes. Sawa et al speculate that activation of the Wnt (LIN-44/LIN-17) pathway results in the conversion of the ARC-L-like complex to one that resembles CRSP by causing the release of LET-19 and DPY-22, which suppress the expression of *tlp-1*. Normal asymmetry may arise in T cells where the ARC-L-like complex inhibits *tlp-1* in the anterior T.a cells, while the CRSP-like version lacking LET-19 and DPY-22 allows *tlp-1* to be activated in the T.p posterior daughter. Similarly, in Pn.p cell fusion, LET-19 and DPY-22 may inhibit the expression of *lin-39* in the absence of Wnt signaling, and conversely activating *lin-39* (and thereby repressing cell fusion) in the presence of Wnt signal. Although the means by which Wnt signaling and responsivity are regulated remain for further investigation, the Sawa study has shown how this prevalent and much-studied cascade may act as a kind of switch by adjusting the makeup of a protein complex responsible for regulating gene transcription.

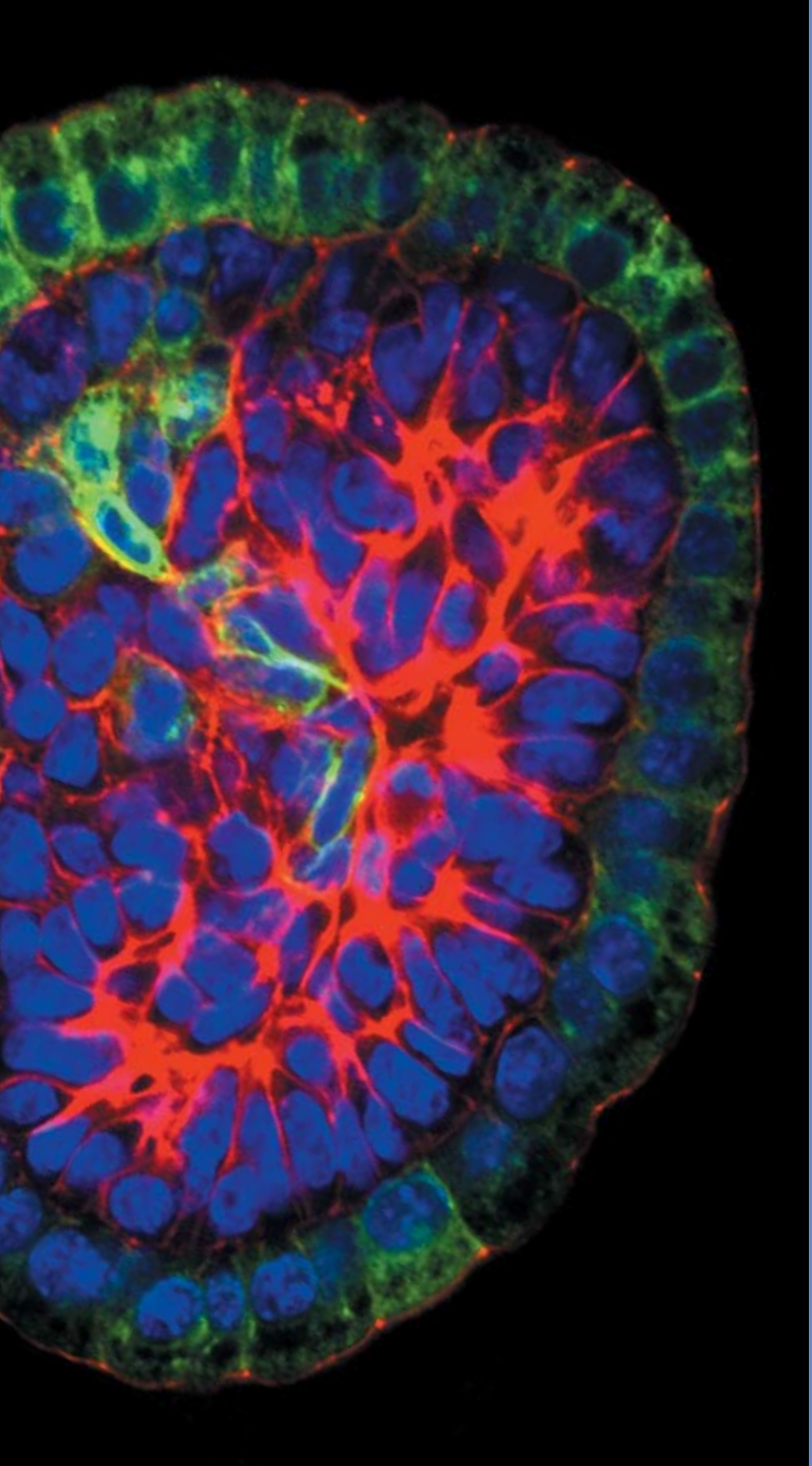
CDB Director wins Japan Prize

Masatoshi Takeichi, director of the RIKEN Kobe Institute and Center for Developmental Biology was named as one of the three Japan Prize honorees in 2005. These prizes, which are sometimes called the Japanese Nobels, are awarded by the Science and Technology Foundation of Japan (JSTF) to recognize "people from all parts of the world whose original and outstanding achievements in science and technology are recognized as having advanced the frontiers of knowledge and served the cause of peace and prosperity for mankind.

Dr. Takeichi was recognized for his work in the field of cellular adhesion, a set of processes by which individual cells are able to recognize other cells and form selective bonds with their appropriate counterparts. His identification of the first members of the cadherin family of calcium-dependent cell-cell adhesion molecules was a breakthrough that has allowed scientists to investigate in great detail the mechanisms by which complex multicellular structures form and hold together.

January-March Seminars

date	title	speaker
2005-01-05	Regulation of arborisation and synaptogenesis during the development of the zebrafish retinotectal projection by Slit-Robo signaling	Douglas CAMPBELL
2005-01-11	Novel modulators in Wnt and FGF signaling	Christof NIEHRS
2005-01-11	Cytokinesis cleavage plane positioning by the mitotic spindle	Henrik BRINGMANN
2005-01-11	Establishing cell polarity in the one-cell *C. elegans* embryo	Carrie COWAN
2005-01-17	Complex networks in biology: Are cells like the Internet?	Hawoong JEONG
2005-01-17	Mechanism of antigen cross-presentation by dendritic cells	Ichiro YAHARA
2005-01-18	Wnt signaling and the downstream pathway in lung development	Tadashi OKUBO
2005-01-21	Functional RNA	Yoshihide HAYASHIZAKI
2005-02-03	Migration patterning and lineage segregation of zebrafish trunk neural crest cells	Yasuko HONJO
2005-02-22	Deer antler regeneration: A system which allows full regeneration of mammalian appendages	Chunyi LI
2005-03-04	Regulation of centromeric transcripts and chromatin in *Arabidopsis thaliana*	Bruce P. MAY
2005-03-04	Regulation of heterochromatin formation in fission yeast	Derek GOTO
2005-03-07	Control of proliferation and senescence in melanocytes and melanoma	Colin GODING
2005-03-14	Genetic and electrophysiological analysis of zebrafish early behaviors	Hiromi HIRATA
2005-03-15	Myostatin: A modulator of skeletal muscle stem cells	Frank S. WALSH
2005-03-16	The steroid deficiency gene *ecdysoneless*	Marek JINDRA
2005-03-16	Nuclear receptor NHR-25/Ftz-f1/SF-1 is required during epidermal differentiation in *C. elegans*	Masako ASAHINA
2005-03-29	Regulation of cellular functions by Wnt signal network	Akira KIKUCHI



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Masahiko HIBI

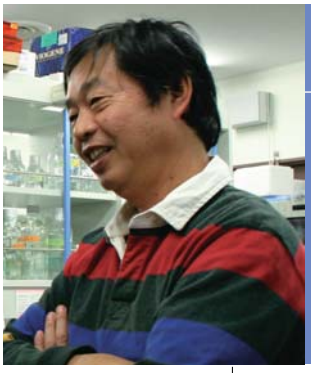
Shigeru KONDO

Shigeru KURATANI

Raj LADHER

Fumio MATSUZAKI

Transverse view of the emergence of the germ line (green) in the gastrulating mouse epiblast visualized by Blimp1-mEGFP reporter. The green cells surrounding the epiblast are visceral endoderm.



Vertebrate Body Plan

Shinichi AIZAWA Ph. D.

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

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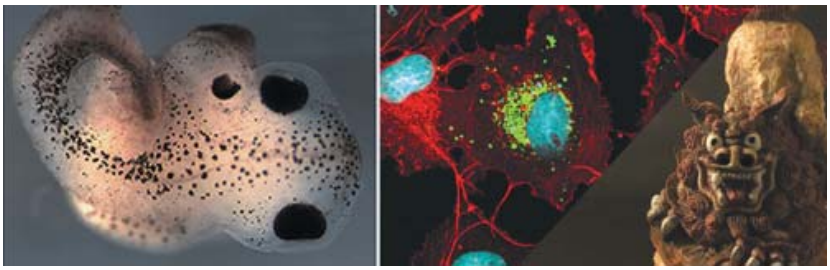
All vertebrate species share certain features in the development of the brain, in that all vertebrate brains comprise four regions – telencephalon, diencephalon, mesencephalon and metencephalon (or cerebellum) – an organizational trait that is thought to have appeared with the advent of the vertebrate 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.



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

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

Stem Cell Translational Research

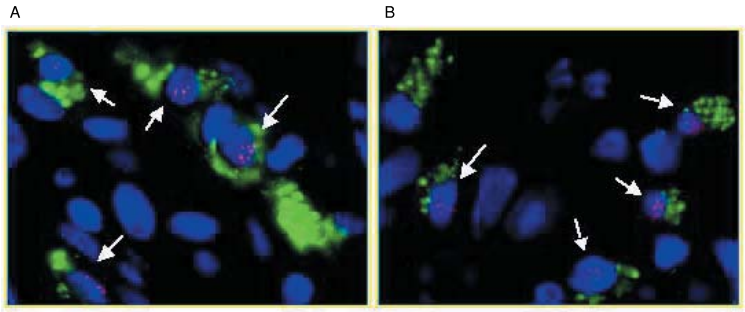
Takayuki ASAHARA M. D. , Ph. D.

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

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 vasculogenesis 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 PlGF gene transfer in myocardial infaction area. (green=bone marrow derived cells, red=GATA4 (in A), red=MEF2(in B))

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Publications

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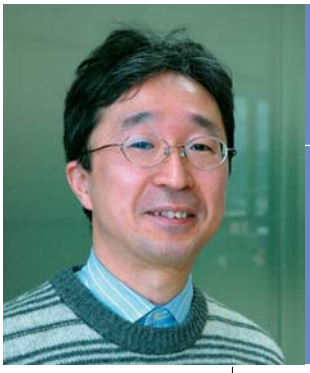

Neuronal Differentiation and Regeneration

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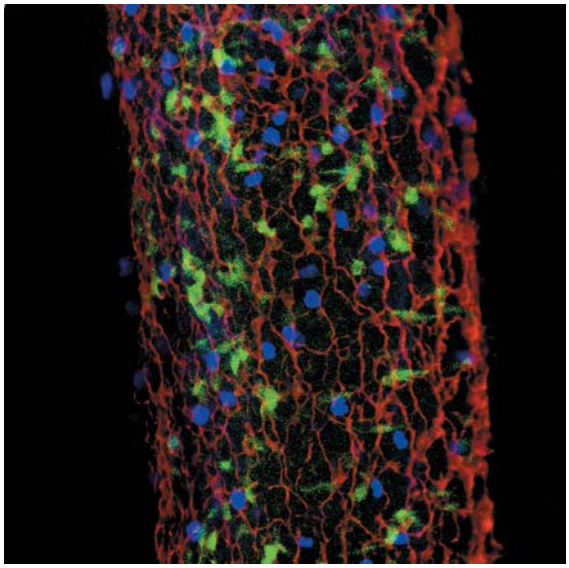
The architecture of the neural system, which underlies much of the dynamism of animal life, is frequently described as complex, but closer examination reveals great beauty as well. The construction of this exquisite and elaborate structure involves the birth, growth and death of countless individual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

Our laboratory is interested in questions fundamental to the development of the nervous system: determining the specific extracellular signals and cell autonomous routines that participate in the processes of neural development, and resolving the means by which diverse families of signaling molecules coordinate and interact at the molecular and cellular levels. To address these questions, we focus on a class of signaling molecules known as neurotrophic factors, most particularly, the GDNF Family Ligands (the GFLs). This family of neurotrophic factors includes four known members – GDNF (for Glial cell line Derived Neurotrophic Factor), Neurturin, Artemin and Persephin. The GFLs signal via receptor complexes formed by the RET receptor tyrosine kinase and one of four co-receptors, GRFα1-4. In vitro, these four receptors show differential affinities for specific GFLs, with GFRα1 showing the greatest ability to interact with the range of GFL family members. GFL signaling has been shown to affect neuronal growth and migration as well as axon guidance, and defects in this signaling system have been implicated in a number of congenital health problems.

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



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

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

Neural Network Development

Chihiro HAMA Ph. D.

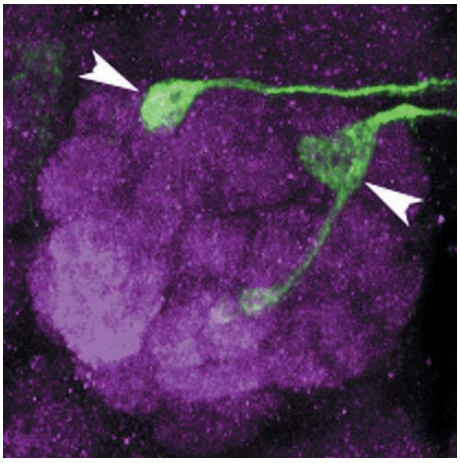
Chihiro Hama received his B. Sc. and M. Sc. from the University of Tokyo Department of Biophysics and Biochemistry and was awarded a Ph. D. from the same institution in 1985 for his work on the regulation of plasmid ColIb DNA replication by inc and repY. He spent the period from 1985 to 1988 as a post-doc 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. To improve our understanding of the molecular mechanisms underlying this circuit formation, we have conducted a mutant screen using fruit flies to identify the regulatory factors involved, and addressed the issue of how vesicle transport is involved in neurite differentiation.

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

To study the question of how 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. The genes responsible for these mutant phenotypes may be involved in axon guidance, synaptic targeting, glomerular formation or synapse formation. We have found one mutation of particular interest, which affects the asymmetric cell fate specification of ORNs that form pairs or clusters from single precursors during development and, as a consequence, change synaptic targets. It is hoped that analysis of this mutation will reveal a principle governing glomerular organization in the antennal lobe.

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



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

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Publications

Orihara-Ono M, et al. The slender lobes gene, identified by retarded mushroom body development, is required for proper nucleolar organization in *Drosophila*. *Dev Biol* 281:121-33 (2005).

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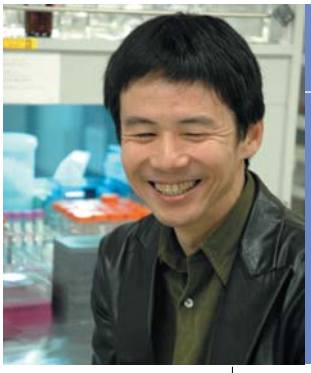

Morphogenetic Signaling

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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Publications

Shinza-Kameda M, et al. Regulation of layer-specific targeting by reciprocal expression of a cell adhesion molecule, capricious. *Neuron* 49:205-13 (2006).

Akimoto A, et al. Enhancer trapping with a red fluorescent protein reporter in *Drosophila*. *Dev Dyn* 233:993-7 (2005).

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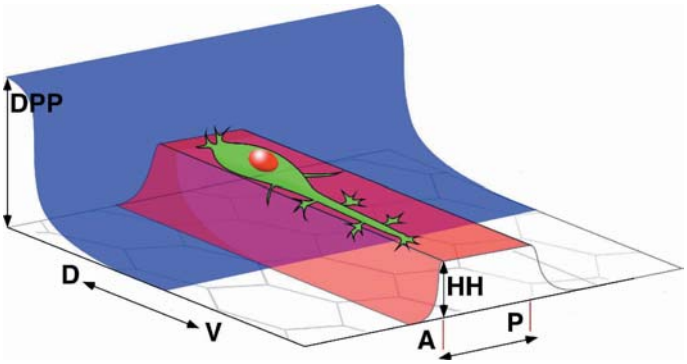
A range of vital developmental processes, including the abilities to build complex structures through selective cell adhesion, to move through the body, and to form epithelial tissues, rely on interactions between cells, and between cells and the extracellular matrix. The means by which cells are able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

The *Drosophila* tracheal system is a respiratory organ that delivers air directly to tissues through a network of finely branched epithelial tubes. Tracheal development begins by invagination of ectodermal epithelium that subsequently forms branches with specific types of cells migrating in stereotyped patterns. The branching patterns and cell fate are instructed by external cues, including FGF, WG and Dpp. We are studying the roles of those signaling molecules in the specification and migration of tracheal branches, as well as the mechanisms that coordinate cell movement and cell adhesion. We additionally use 4D confocal imaging of GFP-labeled embryos to study the dynamism of cell and organelle movement in living organisms. Using combinations of GFP markers and transcriptional enhancers of cell-specific expression, we have been able to capture movements of tracheal cells at resolutions sufficient to image cytoskeletal organization and cell adhesion structures in single cells.

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



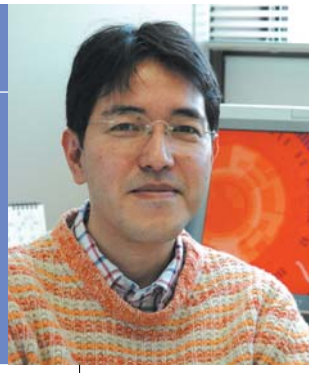
Model of Hedgehog (Hh) and Decapentaplegic (Dpp) roles in tracheal branching in *Drosophila*

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

Vertebrate Axis Formation

Masahiko HIBI M. D. , Ph. D.

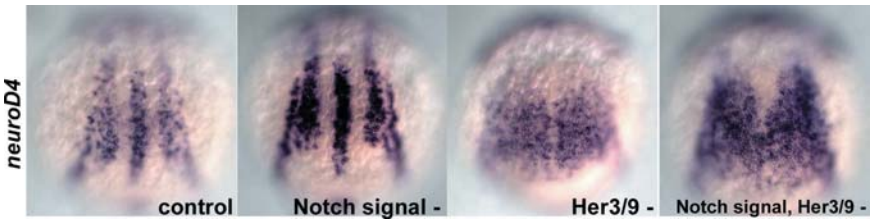
Masahiko Hibi received his M. D. from Hiroshima University School of Medicine in 1988, and his Ph. D. from the Osaka University Institute for Molecular and Cellular Biology in 1992. From 1992 to 1995, he worked as a postdoctoral fellow in Michael Karin's lab in the University of California, San Diego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



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 stepwise manner in vertebrates. These steps include neural induction, AP patterning and neurogenesis. In amphibian and teleost embryos, neuroectoderm is initially induced by BMP inhibitors, which are generated by the dorsal organizer. The induced neuroectoderm is characteristically anterior in character and is subsequently subjected to posteriorization. After neural induction and patterning, primary neurons are generated from proneuronal domains that are established as three longitudinal stripes along the anterior-posterior axis. The proneuronal domains are separated by the inter-proneuronal domains, which do not exhibit neurogenesis as occurs in the proneuronal domains. We have found that the zebrafish *hairy*- and *enhancer of split*-related genes *her3* and *her9* are expressed in the inter-proneuronal domains, and function as prepattern genes that control the patterning of the proneuronal and inter-proneuronal domains. We are also working to determine the mechanisms by which the AP axis of neural tissue is established.



Expression of the proneural gene neuroD4 in wild-type control, *mind bomb* (Notch signal-defective), *her3/her9* morphant (Her3/9-defective), and *her3/her9* mophant *mind bomb* (Her3/9 and Notch-defective) embryos.

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Publications

Muraoka O, et al. Sizzled controls dorso-ventral polarity by repressing cleavage of the Chordin protein. *Nat Cell Biol* (2006).

Hirata T, et al. Zinc-finger gene Fez in the olfactory sensory neurons regulates development of the olfactory bulb non-cell-autonomously. Development (2006).

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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 Prof. Shigekazu Nagata's lab at Osaka Bioscience Institute from 1994 to 1998 and in Prof. 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 CDB team leader in 2005.

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Publications

Setoguchi T and Kondo T. Nuclear export of OLIG2 in neural stem cells is essential for ciliary neurotrophic factor-induced astrocyte differentiation. *J Cell Biol* 166:963-8 (2004).

Kondo T and Raff M. Chromatin remodeling and histone modification in the conversion of oligodendrocyte precursors to neural stem cells. *Genes Dev* 18:2963-72 (2004).

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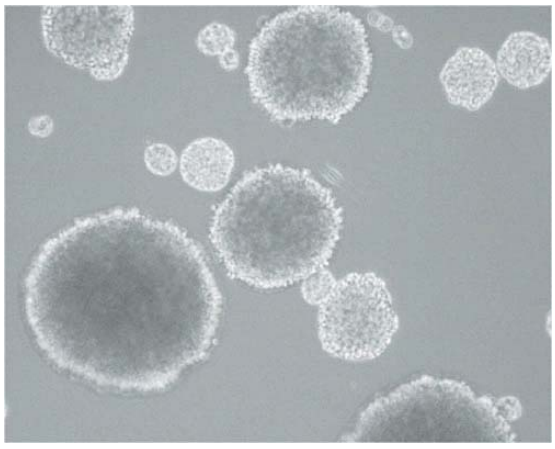
Mankind has long wondered whether it might be possible to regenerate body tissues and structures that have been lost. Recent research has shown that even the adult body contains a diverse range of tissue-specific somatic stem cells that serve to maintain the function and integrity of tissues, opening a promising 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 system, into neural stem-like cells, knowledge which it is hoped may lead to new techniques for the generation of neural stem cells. We are also interested in studying the characteristics of stem-like cells found in malignant tumors, with an eye toward the potential development of novel anti-cancer therapies.



Floating spheres generated from rat C6 glioma cell line

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

Evolutionary Morphology

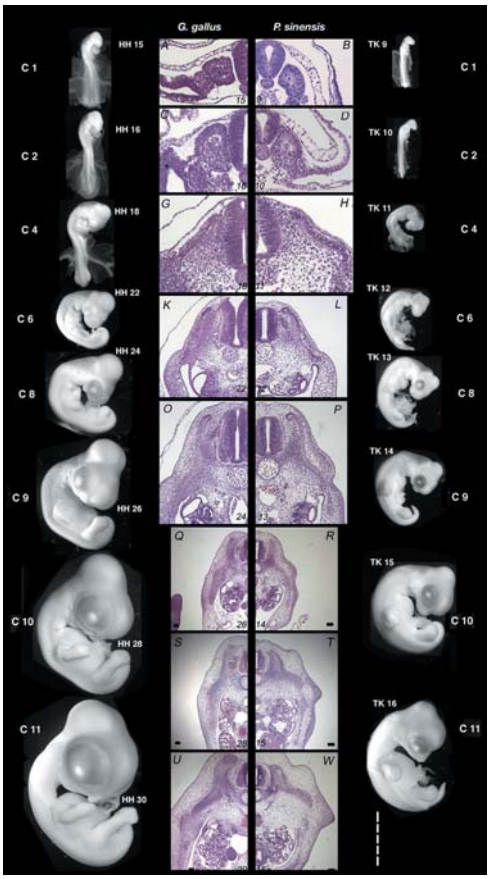
Shigeru KURATANI Ph. D.

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, Baylor College of Medicine, where he was engaged in molecular embryological research. He returned to Japan in 1994 to take a position as associate professor at the Institute of Medical Embryology and Genetics in the Kumamoto University School of Medicine. He moved to Okayama University to assume a professorship in the Department of Biology in 1997, where he remained until he was appointed team leader at the CDB. He was appointed group director in 2005.

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

Through the study of evolutionarily novel structures, our lab has identified changes in developmental mechanisms that have obliterated the structural homologies between organisms as evidenced in such novel structures as the gnathostome (jawed fish) jaw and the turtle shell. Study of lamprey jaw development is intended to shed light on the true origins of the vertebrate head, as lampreys lack a number of important features, such as jaws, that are possessed by gnathostomes. We aim to resolve the question of what primary factors 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 (*Pelodiscus*; right)

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Publications

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

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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 RIKEN CDB in 2002.

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Publications

Ladher R K, et al. FGF8 initiates inner ear induction in chick and mouse. *Genes Dev* 19:603-13 (2005).

Ladher R K and Schoenwolf G C. Neural Induction. *Developmental Neurobiology* (Plenum Press), eds. M. Rao and M. Jacobson. (2005)

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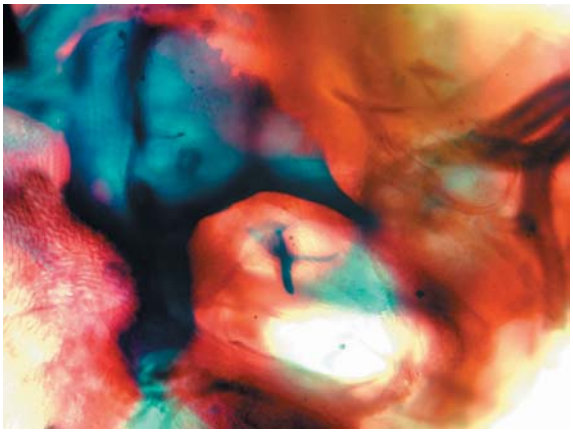
Organogenesis is a monumental undertaking. From situating a given organ at its correct location, to confirming that it is correctly engineered and constructed, to ensuring the necessary functional connections with other organs, the embryo has a daunting task before it; indeed, perhaps we should be surprised 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 differentiation of the otic placode, and in better understanding the cellular, molecular and embryological process responsible for this elegantly engineered organ of hearing.

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

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



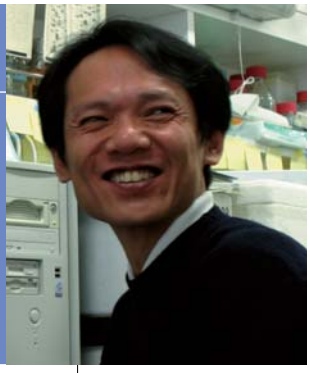
Alizarin Red (bone) and Alcian Blue (Cartilage) Staining of the 14 day old chick head. In contrast to the three bones found in mammals, the chicken middle ear consists of only one bone, the columella.

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

Cell Asymmetry

Fumio MATSUZAKI Ph. D.

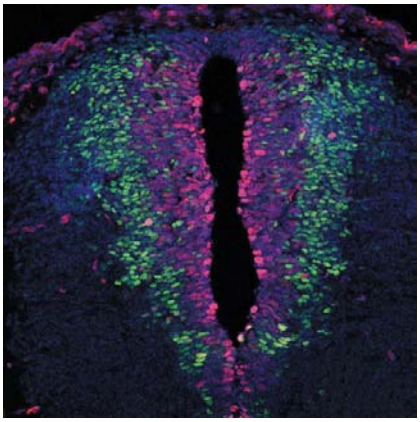
Fumio Matsuzaki received his B. Sc. from the Department of Biochemistry and Biophysics at the University of Tokyo in 1979, and his doctorate from the same institution in 1984, for his work on the characterization of the erythrocyte cytoskeletal structure. He spent the period from 1984 to 1988 as a postdoctoral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



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

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

The vertebrate brain comprises a considerably larger number of neurons arranged in a vastly more complex functional network than that in *Drosophila*. 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 asymmetric division is involved in neuronal fate decisions and in organizing the cellular architecture of the vertebrate brain.



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

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Izumi Y, et al. Differential functions of G protein and Baz-aPKC signaling pathways in Drosophila neuroblast asymmetric division. *J Cell Biol* 164:729-38 (2004).

Kawaguchi A, et al. Differential expression of Pax6 and Ngn2 between pair-generated cortical neurons. *J Neurosci Res* 78:784-95 (2004).

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Crest commencement

Pax3 and Zic1 co-activate neural crest differentiation

Takahiko SATO

Early in vertebrate development, the foundations of the nervous system are laid down in specific regions of the embryonic body. A sheet of epithelial tissue rolls into a cylinder, forming the neural tube, the structure that will give rise to the central nervous system. A migratory population of cells called the neural crest develops slightly later, before spreading throughout the body to create the peripheral and autonomic nervous systems, as well as a range of other tissues including facial cartilage and bone, the pigmented cells called melanocytes, and the adrenal medulla. Despite the importance of the neural crest, however, the molecular signals that function upstream in the multistep process of the specification and demarcation of its developmental field have so far remained a mystery.

A host of regulatory molecules, including members of the BMP and Wnt signaling families, have been implicated in this determination process, and a pair of transcriptional factors, *Foxd3* and *Slug*, has been identified as definitive markers of the presumptive neural crest, but the factors that define its exact boundaries have stayed out of reach. In a study published in the April edition of the journal *Development*, Yoshiki Sasai (Group Director, Laboratory for Organogenesis and Neurogenesis) and colleagues reported the identification of a pair of overlapping regulatory signals that seem to initiate the neural crest developmental program in the African clawed frog, *Xenopus laevis*.

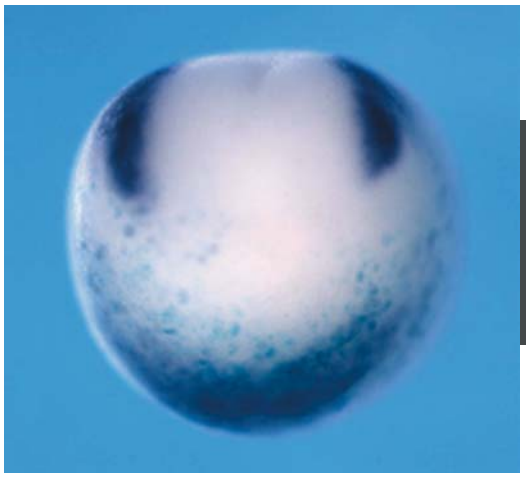
Earlier studies in the same laboratory had suggested a role for *Zic*-family factors in neural crest development, and they focused on *Zic1*, which is expressed in the dorsal ectodermal region of the gastrulating embryo, the site of prospective neural development. A second molecule, *Pax3*, shows a similar but distinct pattern of expression in about the same region and embryonic stages, which led the Sasai group to narrow their search to these candidates. Preliminary tests showed that an increase in BMP signaling, a potent neural inhibitor, suppressed the expression of both, while the suppression of BMP caused an expansion of their range toward the ventral side of the embryo. Conversely, the soluble factor Wnt caused *Pax3* and the presumptive neural crest marker, *Foxd3*, to be expanded beyond their normal anterior limits.

Sato T, Sasai N and Sasai Y. Neural crest determination by co-activation of Pax3 and Zic1 genes in Xenopus ectoderm. *Development* 132:2355-63 (2005).

They next looked at the effects of gain of *Pax3* and *Zic1* function in the developing frog, and found that both were able to trigger neural crest differentiation, as evidenced by the expression of *Foxd3* and *Slug* prior to the late gastrula stage, when those markers normally first appear, as well as in the typically non-neural ventral region. When misexpressed singly, both *Pax3* and *Zic1* showed the ability to trigger an ectopic expansion of *Foxd3* and *Slug* in the dorsal region, but that effect did not extend to the ventral side. On direct injection of both *Pax3* and *Zic1* into the ventral side of animal blastomeres from very early embryos, they found that the factors in combination could indeed induce neural crest markers even in the ventral side, indicating the potency and directness of their effect.

Sato et al followed up by studying how a loss of these molecules' function might affect the neural crest in otherwise normal embryos by injecting morpholino (MO) antisense oligonucleotides (a method of inhibiting the function of specific genes by interfering with the translation of the proteins they encode). The injection of either *Pax3* or *Zic1* MOs was sufficient to suppress the expression of the marker *Foxd3*, while the loss of function of either of the two factors had no discernible effects on the expression of the other, suggesting that both must be active to achieve normal determination of the neural crest.

Animal cap assays, which provide an in vitro model of many aspects of early *Xenopus* development, helped to clarify the details of the molecular interactions at work. Finding that *Pax3* alone failed to induce *Foxd3*, as it had in vivo, they began to search for the missing signals needed to achieve that effect. When they co-injected *Wnt3a* (a known factor in neural crest differentiation), they found not only that *Pax3* now strongly induced *Foxd3*, but also that *Zic1* began to be expressed. Injection of *Zic1* alone into untreated animal caps was able to induce *Foxd3*, but only weakly, an effect that was strongly complemented by co-injection with *Wnt3a*. Interestingly, the inductive action of these factors acting alone could be blocked by increasing the activity of the neural inhibitor, BMP4, but the combination of *Pax3*, *Zic1* and *Wnt3a* proved able to induce *Foxd3* robustly even in the face of an antagonistic BMP signal.



Ectopic ventral expression (bottom) of neural crest marker Foxd3 in stage 15 *Xenopus* embryo co-injected with Pax3 and Zic1 (anterior view).

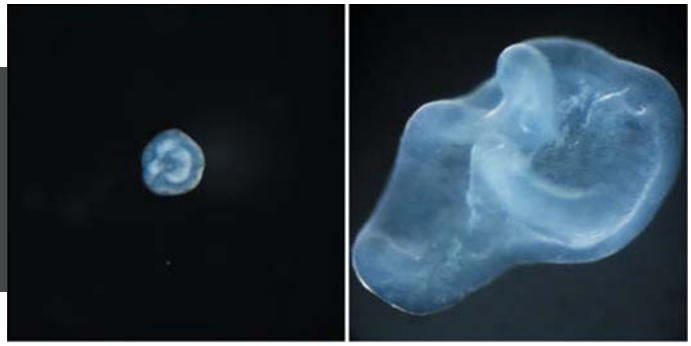
By interfering with gene function in dissociated cells, the group tested whether this co-activity between *Pax3* and *Zic1* in Wnt treated cells relied on external signals. Morpholino blockade of Zic1 in *Pax3*-injected and Wnt-treated single cells resulted in the loss of *Foxd3* induction, while cells exposed to all three signals continued to express Foxd3, indicating that the *Pax3, Zic1*, and *Wnt3a* effect is cell-autonomous. The critical role of the endogenous Wnt cascade was shown by the loss of *Foxd3* induction when Wnt signaling was disrupted by morpholino knockdown of β-catenin, a Wnt downstream factor.

This comprehensive and compelling set of evidence points strongly to a modus of neural crest differentiation involving the close cooperation of *Pax3* and *Zic1* in the presence of Wnt signaling in the pre-neural embryo. That this trio of signals operates even in the presence of inhibitory BMP signal suggests that the combination is a powerful determinant of the prospective neural crest, and the question of exactly how the Pax3-Zic1 partnership overrides BMP on the molecular level represents an intriguing subject for further study.

2b or not 2b a stem cell

During the development of the vertebrate retina, a whole spectrum of cells, including both neurons and glia, arise from a cadre of precursor cells, known as retinal progenitors. These progenitors are present throughout the developing neural retina, but show differential proliferation, with the cells at the outer fringes remaining in an undifferentiated, proliferative state for much longer than those in the central region. These dual capacities for proliferation and differentiation mark the marginal retinal progenitors as one form of stem cell, and as with all such cells, the means by which external factors exert control over the balance between this pair of functions remains an intriguing and important question.

Shinichi Nakagawa and colleagues in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) demonstrated that a signaling protein, Wnt2b, acts to maintain retinal progenitor cells in their undifferentiated state independently of well-known Notch-mediated lateral inhibitory signals. Nakagawa, who now heads his own research program on the RIKEN main campus (Wako, Japan), published his findings in the 18 May 2005 online edition of the journal *Development*.



Retinal explant expressing Wnt2b after one week of proliferation (right). Wnt2b inhibits differentiation, allowing continuous proliferation, thereby causing the explant to grow much larger than the control (left).

The Wnt family of proteins includes many secreted factors that function in a staggering range of developmental processes. In the formation of the retina, it was previously reported that Wnt2b is expressed in the anterior rim of the optical vesicle (the most marginal part of the nascent retina), which neighbors the stem cell-containing peripheral retina. Nakagawa focused on the possible involvement of this signaling factor in ensuring that the progenitor cells refrain from committing to more highly differentiated cellular fates, which is critical to their function as stem cells. Specifically, his team was curious to discover whether Wnt activity was restricted solely to the periphery of the neural retina, and whether Wnt2b was indeed directly responsible for the inhibition of differentiation. While they had showed both of these to be the case in previous work, they also made the interesting finding that Wnt2b's function as a repressor appears not to be linked to the function of a second signaling program, the Notch pathway, which is known to suppress neural and promote glial differentiation in the retina.

Nakagawa first looked at the effects of overexpressing either Wnt2b or Delta (the ligand that binds the Notch receptor) on cell proliferation in the developing retina and found that while Delta-expressing tissue had ceased growing after about two days, the Wnt culture continued to proliferate for eight or more, showing Wnt2b to be the likelier promoter of proliferation.

Next, the lab prepared Wnt2b and Delta tissue explants in which genes specific to neurons, glia and undifferentiated cells could be visualized by immunohistochemistry, and found that in both the Wnt2b and Delta explants the expression of neuronal markers was inhibited and that of undifferentiated cells was upregulated. But only the Wnt cells continued to proliferate, while cells in the Delta explant uniformly

Kubo F, Takeichi M and Nakagawa S. Wnt2b inhibits differentiation of retinal progenitor cells in the absence of Notch activity by downregulating the expression of proneural genes. *Development* 132:2759-70 (2005).

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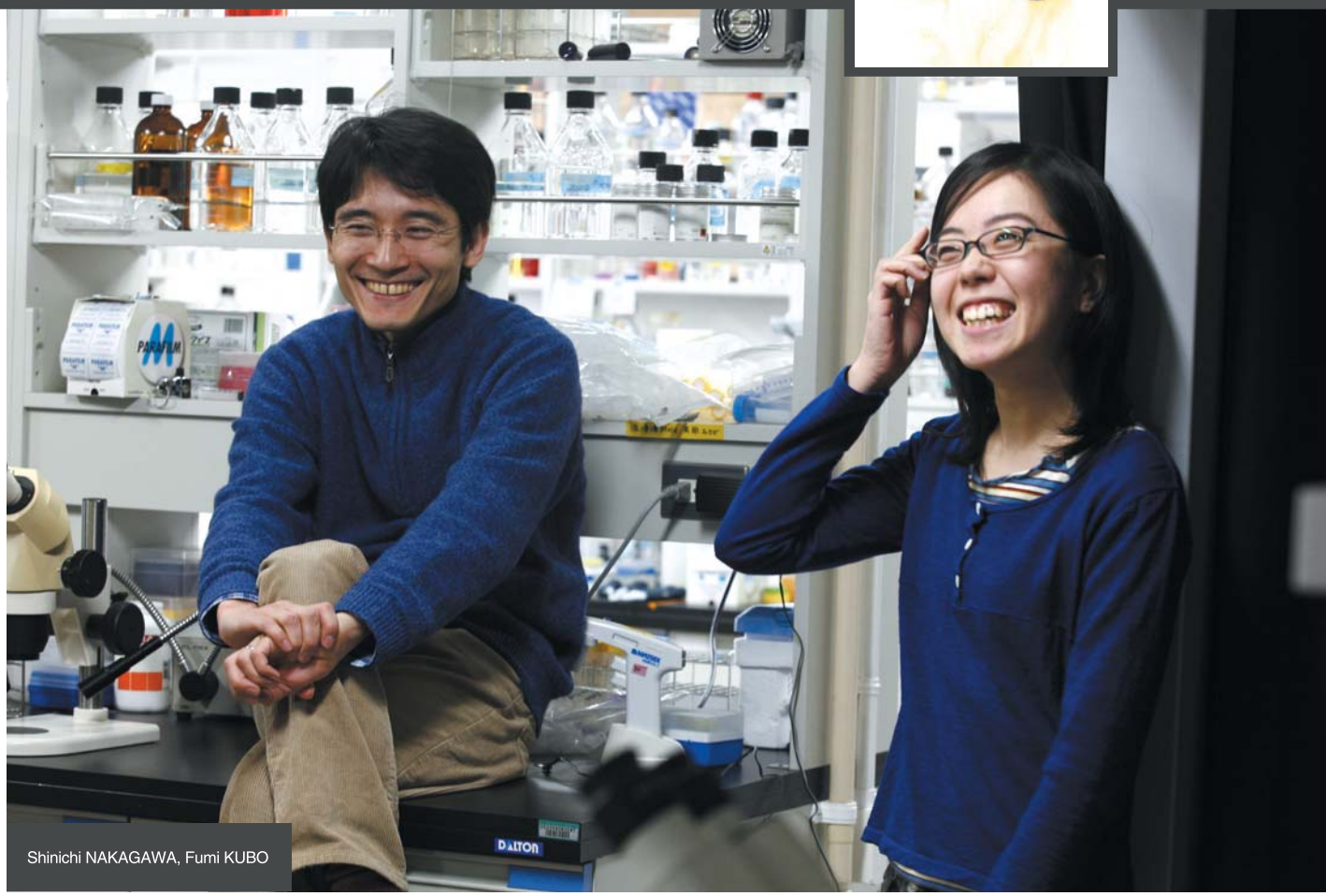


Shinichi NAKAGAWA, Fumi KUBO

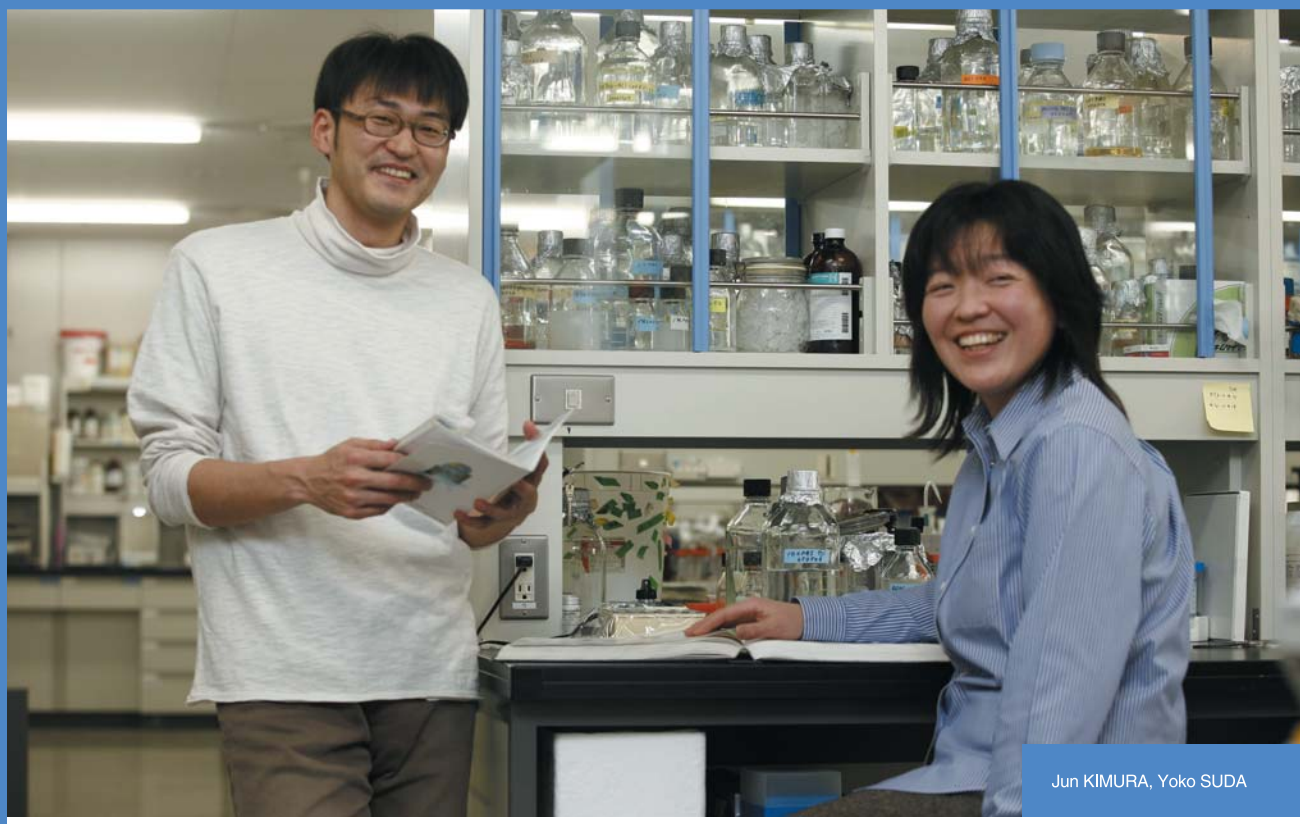
expressed glial markers. In essence, although both the Wnt and Notch pathways appeared capable of inhibiting differentiation into neurons, only Wnt seems able to keep the undifferentiated cells proliferating as well. The fact that Wnt2b was able to rescue cells from the premature neuronal differentiation caused by interference with the Delta/Notch cascade lent further credence to the idea that Wnt works by an independent mechanism in this population of cells.

The question then is, how does Wnt inhibit differentiation in retinal progenitor cells, if not via Notch? The Nakagawa team sought to address this question by looking at the relationship between the expression of Wnt2b and that of a number of proneural genes, which are essential for the differentiation of retinal neurons. They found that, in the Wnt2b explants, the activity of all proneural genes was significantly downregulated or absent, as indeed was the expression of Notch. These results suggest that Wnt signaling may be shutting down both proneural and Notch activity in retinal progenitors, enabling these cells to avoid differentiation into either neural or glial cells and to remain both uncommitted and proliferative.

This study of progenitor cell regulation dovetails nicely with the emerging emphasis on the role of the stem cell "niche," the name given to local microenvironments that are increasingly thought to play decisive roles in the determination of stem cell behavior. For now, in the developing retina, as in other contexts, the value of the environmental survey is being made clear at the cellular level.

Rethinking regionalization

Emx2 and *Pax6* at work in establishing the forebrain



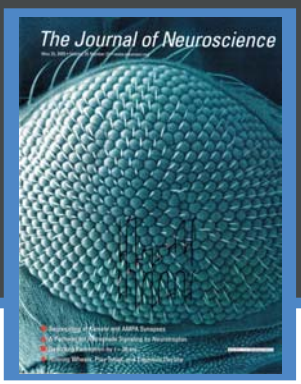
Jun KIMURA, Yoko SUDA

During embryogenesis, the vertebrate brain integrates multiple strata and primordial tracts into what has justifiably been described as the most complex biological system known. One conserved feature of this synthesis is the anterior-posterior sectioning of brain development, a process involving the three broadly distinct regions of fore-, mid- and hindbrain.

It has been proposed that the forebrain itself can be further subdivided into two regions: the rostrally located telencephalon (which will ultimately give rise to the cortex, the seat of many higher cognitive functions) and the more caudal (tail-ward) diencephalon, comprising, in rostral-caudal order, the prethalamus, thalamus, and pretectum. However, a recent study by Jun Kimura and Yoko Suda, a pair of research scientists in the Laboratory for Vertebrate Body Plan (Shinichi Aizawa; Group Director), indicates that this taxonomy of forebrain primordia may need to be revised. Their work, published in the May 25 issue of *The Journal of Neuroscience*, shows that, at a critical early ontogenetic stage in mice, the anterior neuroectoderm develops (as had been proposed) into midbrain, caudal forebrain and rostral forebrain primordia, but that the early caudal forebrain field includes a number of structures hitherto classed as rostral. This caudal forebrain anlage appears to be delineated by the expression of a pair of genes, *Emx2* and *Pax6*, working in conjunction with the more widely expressed mid- and forebrain determinants, *Otx1* and -2.

The authors had previously shown that in *Emx2*$^{-/-}$*Otx2*$^{+/-}$ double mutants, caudal forebrain primordia such as prethalamus, thalamus, and anterior pretectum were all lost. Extending those findings in this latest study, Kimura and Suda found that a number of rostral structures, including archipallium, cortical hem, choroid plexus, choroidal roof and eminentia thalami also failed to develop in *Emx2*$^{-/-}$*Otx2*$^{+/-}$mice, while others, such as neopallium, ganglionic eminences and commisural plate, are much less seriously compromised. Their work also revealed that this same set of caudal primordia was unaffected when *Emx2* was ectopically expressed throughout the entire forebrain and midbrain by being knocked into

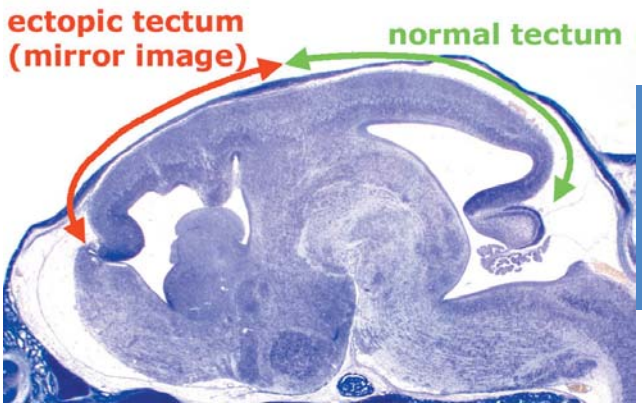
Kimura J, Suda Y, Kurokawa D, Hossain Z M, Nakamura M, Takahashi M, Hara A and Aizawa S. Emx2 and Pax6 function in cooperation with Otx2 and Otx1 to develop caudal forebrain primordium that includes future archipallium.
J Neurosci 25:5097-108 (2005).



the *Otx2* locus to yield *Otx2*$^{+/Emx2}$ mice. Further, *Emx2* was not expressed in the pretectum or thalamus, which led the group to question exactly how the caudal forebrain was specified in the *Emx2*$^{-/-}$ mutant.

Earlier work by another group had shown *Pax6* might serve as a redundant counterpart of *Emx2* in telencephalon development, for it was shown that at least one functional allele of either of these two genes is both necessary and sufficient to establish the cortex and surrounding structures. Kimura et al confirmed that the ranges of *Emx2* and *Pax6* expression largely coincide and, building on previous findings from their own lab, studied the cooperation of *Emx2* and *Pax6* with *Otx2* and *Otx1*. Their analysis of the activity of *Emx2* and *Pax6* during the earliest formative stages of forebrain revealed a possible disjunction between their roles in archipallium, where they are necessary for initial regionalization, and neopallium, in which they may function in later developmental processes.

Observations of the fate of the presumptive caudal forebrain in *Emx2/Pax6* double mutants tended to bear this hypothesis out. At the 6-somite stage, the caudal forebrain anlage in these mutants appeared to develop as normal, but its later development suggests that it somehow fails to be specified to its usual fate, and transformed instead into a mirror image of the tectum, a midbrain structure. This ectopic duplication may be the result of *Fgf8* expression in the overlying prethalamic roof, an effect that *Emx2* and *Pax6* might redundantly protect against in wildtype animals.



In the E18.5 *Emx2*$^{-/-}$*Pax6*$^{-/-}$ double mutant, ectopic tectum was formed in the region in which forebrain structures normally develop. This ectopic tectum developed as a mirror-image of the normal tectum.

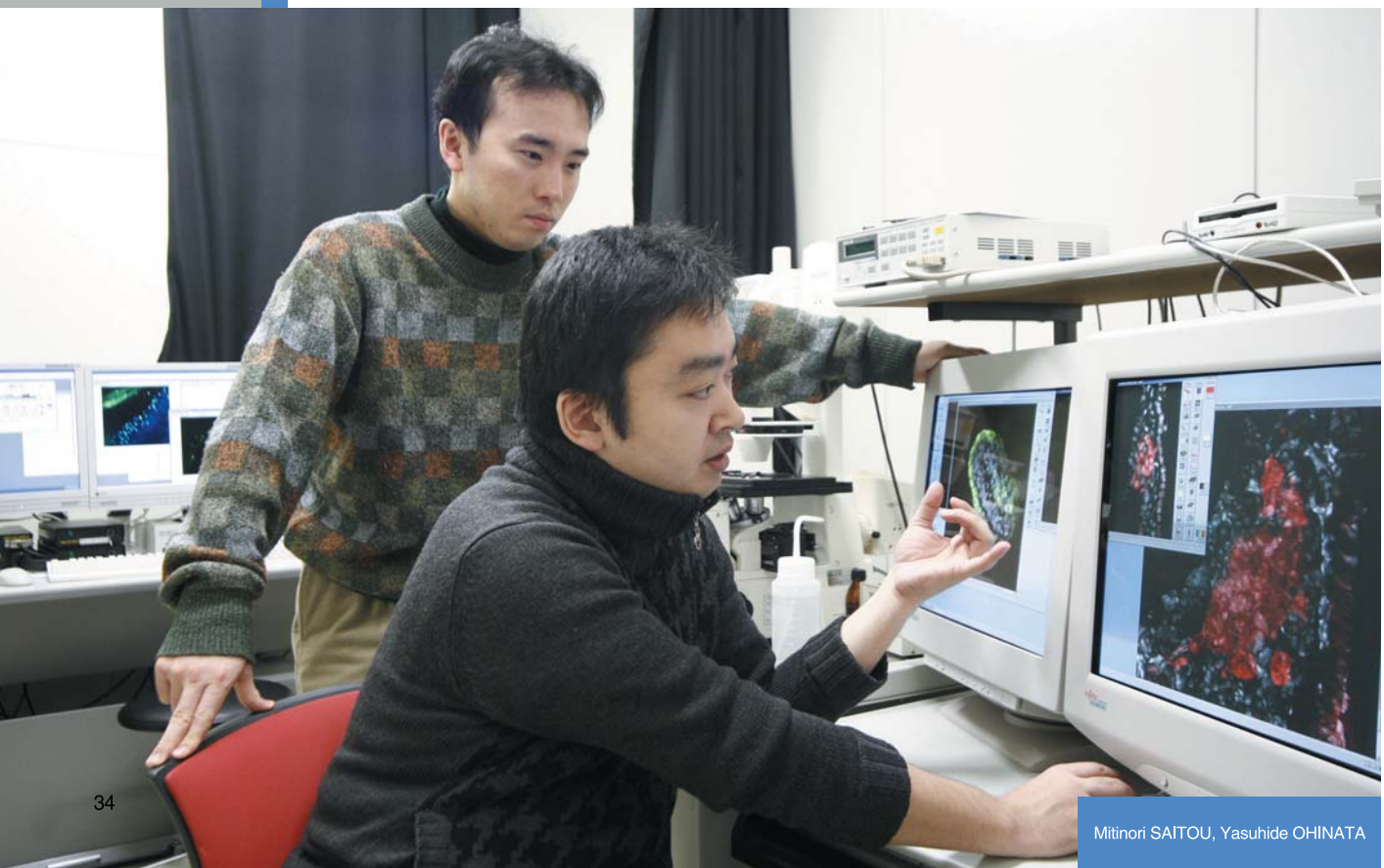
Questions of the emergence and ordering of the forebrain remain among the most hotly debated in developmental neurobiology and, given the significance of their end products (among other things, the centers of cognition and memory), will undoubtedly continue to attract great scientific interest in the future. This work by Suda and Kimura helps to clarify the genetic determinants of the earliest regionalization of forebrain precursors and provides stabler ground from which future explorations of this complex developmental arena might be launched.

The beginning of the beginning for the germline

Every cell in an individual's body contains identical genetic information, but only a very few are able to use that information to contribute directly to the creation of a new individual. Such cells, known as germ cells, differ from the somatic cells that make up the rest of the body in several very significant ways. Unlike somatic cells, the germ cells (which include both eggs and sperm) have the ability to shed and reacquire their epigenetic markings; to divide meiotically, thereby halving the normal complement of chromosomes; and to fuse into a zygote that can then give rise to every type of cell needed for the development of a new individual, a developmental capacity known as totipotency.

Germ cells in many species inherit their unique identities very early in development. In mice, the process begins with a small cohort of a few dozen primordial germ cells, or PGCs, which appear at the region that separates the extraembryonic tissue from the embryo proper, then migrate inward, traversing the hindgut endoderm on their way to colonize the gonads. The journey of these germline founders begins very early in the embryo's history, at a stage when the nascent mouse looks more like a tiny cylinder clinging to the uterine wall. It is believed that the germ cells' destiny is determined in large part in these early hours, with their initial segregation from their neighbors and the repression of signals that would otherwise prompt them to adopt a somatic fate.

In an article published in the 14 July 2005 edition of *Nature* magazine, Mitinori Saitou (Team Leader, Laboratory for Mammalian Germ Cell Biology), Yasuhide Ohinata and colleagues, working in collaboration with labs in the UK and the US, reported the identification of a new factor in the specification of PGCs. This molecule, Blimp1 (for B-lymphocyte induced maturation protein) appears to function earlier than any known factor in the determination of the germline in mice. A screen of cDNAs from individual PGCs at embryonic stage 7.5 yielded a number of promising candidates for germ lineage determinants, including Blimp1, first identified as a factor in the differentiation of B cells into immunoglobulin-secreting

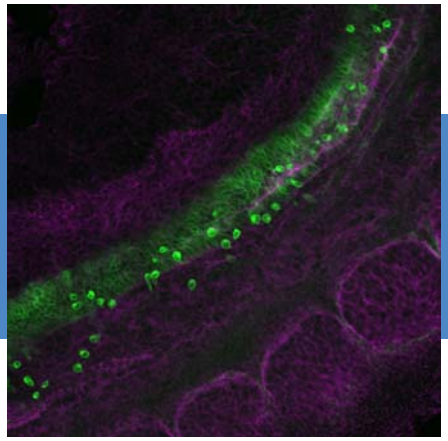


Mitinori SAITOU, Yasuhide OHINATA

Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, Barton S C, Obukhanych T, Nussenzweig M, Tarakhovsky A, Saitou M and Surani M A. Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* 436:207-13 (2005).

plasma cells, which circulate in the bloodstream and function at the frontlines of the body's immune response. "We thought it was intriguing to find that a factor functioning in such a terminal event as B cell differentiation might also be at work in the very earliest days of the embryo's development," notes Saitou, "It's always interesting to find cases where the same molecule turns up in two very different contexts."

The team next tracked *Blimp1*-positive cells by in situ hybridization, which revealed *Blimp1* expression in a thin swath of cells lying at the border between the extraembryonic ectoderm and the embryo itself. The onset of its expression was unexpectedly early, in the E6.25-stage epiblast, even before the process of gastrulation begins. Studies in which *Blimp1* expression was monitored by a fluorescent protein expressed under the control of *Blimp1* upstream elements confirmed that its expression began in a small number of cells in the epiblast which proliferated to about 40 cells by E7.5, nicely matching previous estimates of the number of founder PGCs. On tagging the *Blimp1*-expressing cells with the antibody for a second protein, stella, which is a definitive marker of nascent PGCs, the group found an almost perfect coincidence of the two proteins' expression, pointing to the strong involvement of Blimp1.



Blimp1-positive germ cells(green) at E9.5 migrating through hindgut endoderm towards future genital ridges. Note that hindgut endoderm is also expressing Blimp1.

Turning next to Blimp1 function, Ohinata et al created homozygous and heterozygous knockouts of the gene that let them study its full and partial loss of function. The effects of Blimp1 deficiency were broadly dose-dependent, with slightly more than half the normal number of PGCs in *Blimp1*$^{+/-}$ embryos and no normal-appearing PGCs in the null mutants, an effect that seemed to be linked to the failure of PGC genesis, rather than to cell proliferation or survival.

Even those cells that did arise in the loss-of-function mutants behaved differently than their wildtype counterparts, with stunted proliferation and failure to disperse and migrate being typical behavior of the mutant PGCs. The gene expression profiles of the Blimp1-deficient PGC-like cells were also abnormal. In normal development, PGCs begin to express *stella* while at the same time inhibiting the expression of *Hox-family* genes; in the mutants, few *stella*-positive cells were detected and *Hox* gene expression was prevalent.

"This is the earliest anyone has been able to trace back the origins of the mammalian germ cell lineage," says Saitou, "we're still not sure exactly how Blimp1 is causing this small cluster of cells to adopt this highly specialized fate, but we're looking forward to finding out whether there's any link to how it functions at the molecular level."

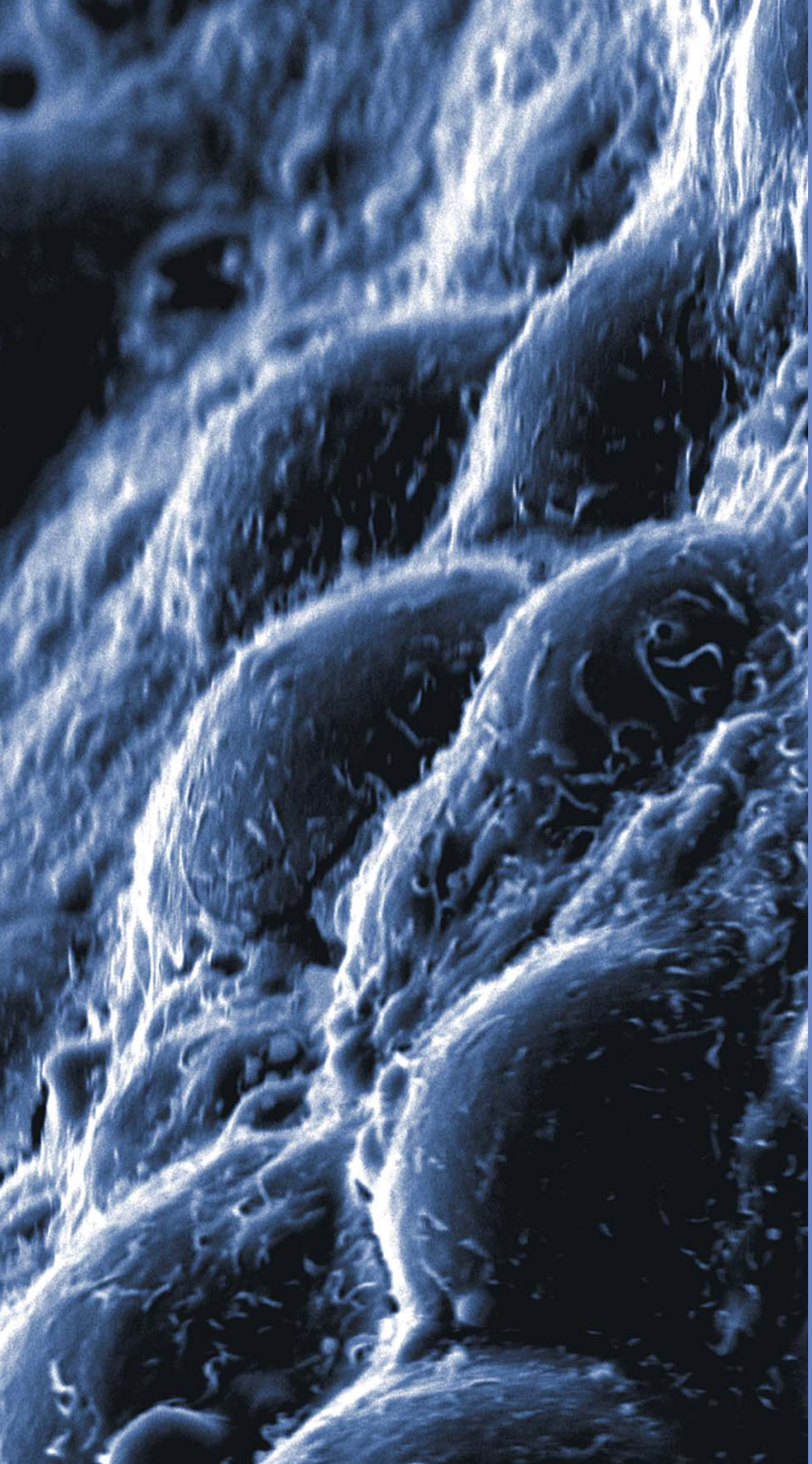
Tokyo Techno Forum gold prize

Hiroki R Ueda (Team Leader; Laboratory for Systems Biology) was named one of the three winners of the 2005 Tokyo Techno Forum 21 gold medal for his work on deciphering the network of genetic activity underlying biological clocks. These natural time-keeping mechanisms set an internal rhythm within the body that follows a cycle with a period of approximately 24 hours. As a graduate student, Ueda studied circadian genes in the fruit fly *Drosophila*, as well as the genetic regulation of the mammalian (mouse) biological clock.

At the CDB as well, Ueda's work has focused on the network of mouse clock genes. A collaborative study conducted with colleagues at Yamanouchi Pharmaceutical and the University of Tokyo led to the development of a "molecular timetable" of body time based on the sampling of gene expression levels at specific time points. Other work from his lab published in 2005 reported the identification of central players of a network of genes in the mouse that regulates daily biological rhythms.

April-June Seminars

date	title	speaker
2005-04-07	Organization of a sterol-rich membrane domain during cytokinesis in fission yeast	Tetsuya TAKEDA
2005-04-08	Development and evolution of a vertebrate innovation: The neural crest	David W. McCAULEY
2005-04-15	The mystery of animal design, a personal history of discovery	Peter LAWRENCE
2005-04-19	Neural induction in *Xenopus* requires early FGF signalling in addition to BMP inhibition	Patrick LEMAIRE
2005-05-16	Non-viral gene delivery in the chick embryo	Mike DeCASTRO
2005-05-25	Targeting of Arf-1 to the early Golgi by membrin, an ER-Golgi SNARE	Akira HONDA
2005-05-30	Functional ocular dominance plasticity revealed by the activity-regulated gene Arc	Yoshiaki TAGAWA
2005-05-31	Gastrulation through a primitive streak: cellular mechanics and signals	Claudio STERN
2005-06-06	Regulation of a DLK-1 and p38 MAP kinase pathway by the ubiquitin ligase RPM-1 is required for presynaptic development	Katsunori NAKATA
2005-06-07	Spatial-mechanical regulation of morphogenesis and malignancy	Valerie M. WEAVER
2005-06-07	c-kit and SSEA-1 define temporally and spatially distinct retinal progenitor subsets	Sumiko WATANABE
2005-06-08	Generation of a synthetic lymphoid tissue-like organoid using a stromal cell line and a biocompatible scaffold in mice	Sachiko SUEMATSU
2005-06-15	A paradigm change in circadian clock research: Genome-wide transcription rhythms driven by a protein-network-based clock in cyanobacteria	Hideo IWASAKI



Akira NAKAMURA

Jun-ichi NAKAYAMA

Shin-Ichi NISHIKAWA

Kiyoji NISHIWAKI

Hitoshi NIWA

Masaki OKANO

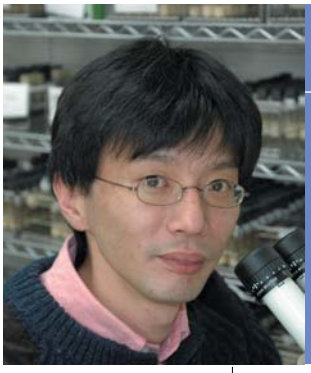
Tony PERRY

Mitinori SAITOU

Yoshiki SASAI

Hiroshi SASAKI

Scanning electron micrograph showing the surface of a chicken embryonic membrane.



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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Chiaki NAKAMOTO
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Hiroko SONOBE
Akie TANIGAWA

Publications

Boag P R, et al. A conserved RNA-protein complex component involved in physiological germline apoptosis regulation in *C. elegans*. *Development* 132:4975-86 (2005).

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

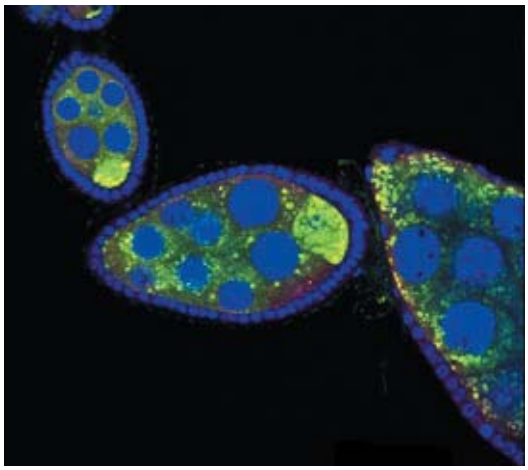
Nakamura A, et al. *Drosophila* Cup is an eIF4E binding protein that associates with Bruno and regulates *oskar* mRNA translation in oogenesis. *Dev Cell* 6:69-78 (2004).

Nakamura A, et al. Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during *Drosophila* oogenesis. *Development* 128:3233-42 (2001).

Germ cells are the only cell types capable of transmitting genetic information across generations, and their formation is characterized by unique developmental processes as well. In many types of animals, including the *Drosophila* fruit fly, the formation and differentiation of germ cells is controlled by mRNAs and proteins localized in a specific cytoplasmic region within eggs, 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 paticular interest is the mechanism of translational repression in germline development. In one example of this critically important form of regulation, the translation of the RNA for the maternal gene *oskar*, which functions in embryonic patterning and the formation of germline cells in *Drosophila*, is repressed during its transport to the posterior pole of the oocyte. We are now studying the function of 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 fruit fly germline development, we are also beginning to undertake investigations using the ascidian, *Ciona intestinalis*. Our team will explore the genetic regulation of ascidian germline development by characterizing promoter regions of germline specific genes and trans-acting factors that regulate germline specific gene expression.



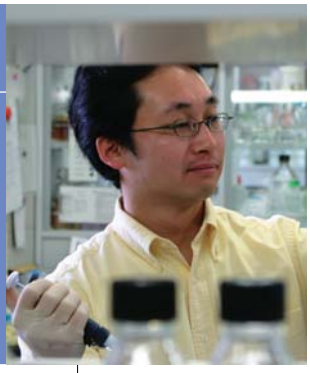
Maternal RNP particles, showing localization of DNA, Me31B and Cup proteins

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

Chromatin Dynamics

Jun-ichi NAKAYAMA Ph. D.

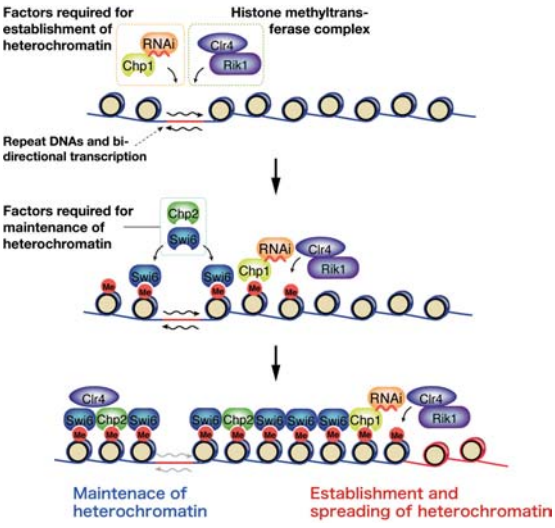
Jun-ichi Nakayama received his bachelor's, master's and doctoral degrees in bioscience from the Tokyo Institute of Technology, the last in 1999 for his work on the cloning and characterization of mammalian telomerase components. He spent the period from 1999 to 2001 as a postdoctoral researcher at Cold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



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

Our team investigates how modifications to the structure and configuration of chromatin (complexes of nuclear DNA and proteins that provide the structural basis of chromosomes) contribute to epigenetic gene regulation and how such modifications are transmitted over generations of cellular division by studying events at the molecular scale in the model organism, fission yeast (*Saccharomyces pombe*), and in cultured mammalian cells.

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



Model of heterochromatin formation

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

Stem Cell Biology

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

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

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

We are especially interested in the question of how cells maintain the never-ending self-renewal that sustains the organism as a whole. This will require, at the very least, solving the riddles of two essential processes. The first of these is the need for old cells destined for replacement to be able to disengage or otherwise be dislocated from their environmental milieux. The second is the requirement for preparing new cells in replacement. To investigate the first of these mechanisms, our group uses a system for labeling cells with special dyes, which allows us to monitor their location and behavior. We have also developed a system for differentiating embryonic stem (ES) cells in culture to study the second question of new cell production.

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

LacZ- labeled cells in developing mouse embryo

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Cell Migration

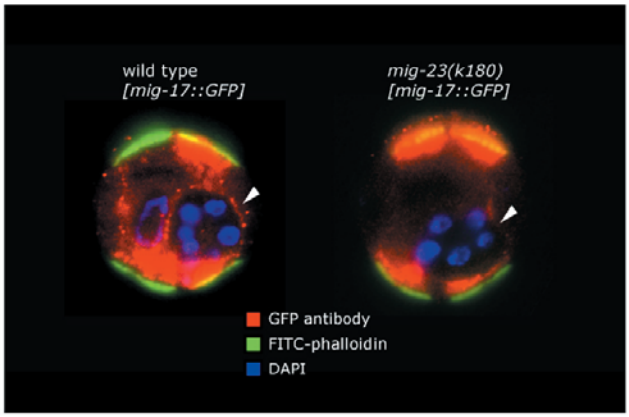
Kiyoji NISHIWAKI Ph. D.

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

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

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Pluripotent Cell Studies

Hitoshi NIWA M. D., Ph. D.

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

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Niwa H, et al. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* 123:917-29 (2005).

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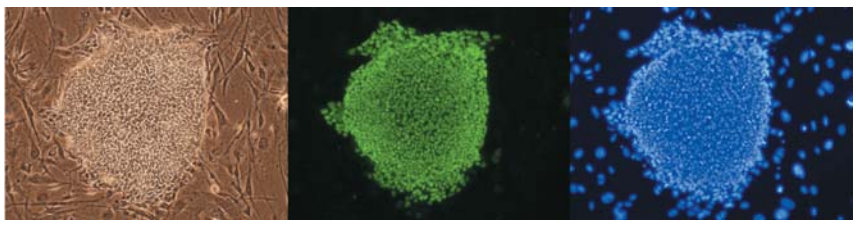
Pluripotency is a term used to describe the ability of certain types of stem cells that are capable of giving rise to at least one type of cell from all three germ layers – endoderm, mesoderm and ectoderm. Stem cells are also characterized by their ability to produce copies of themselves with similar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

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



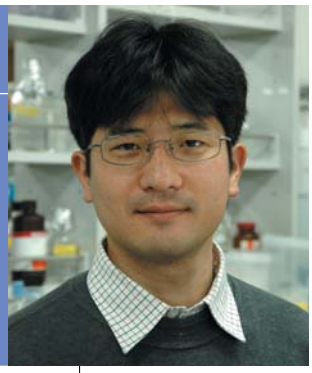
Monkey ES cells phase contrast image (left). Immunostaining for OCT3/4 (center). Nuclear staining by Hoechst 33458. (right)

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

Mammalian Epigenetic Studies

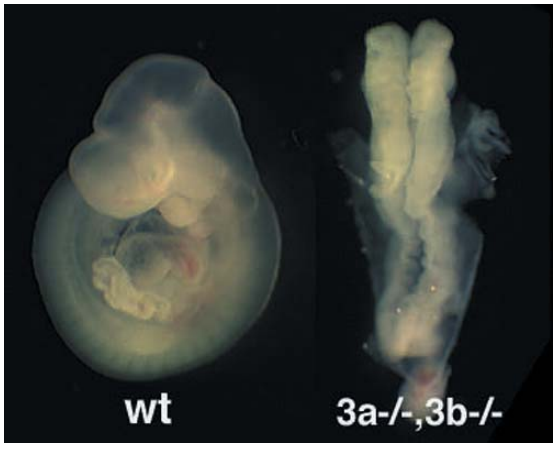
Masaki OKANO Ph. D.

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.



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

Our team studies epigenetics by focusing on a particular set of molecules that act in the methylation of DNA, with the goal of identifying their roles in the regulation of chromatin function in embryonic development and the processes of fate determination and plasticity in cell differentiation. We conduct phenotype analyses of mice in which various genes involved in DNA methylation have been knocked out, as well as biochemical and cell biological studies of embryonic stem (ES) cells to explore how dynamic changes in DNA methylation function as regulatory factors in mammalian embryogenesis and cell differentiation, and the means by which DNA methylation works to maintain cell proliferation and chromosome function. We anticipate that a deeper understanding of these questions will lead to new insights in the context-specificity of the DNA methylation process as well as the roles of methylation in development, health and regeneration.



Dnmt3a/3b double knockout mouse embryo at E9.5

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Mammalian Molecular Embryology

Tony PERRY Ph. D.

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

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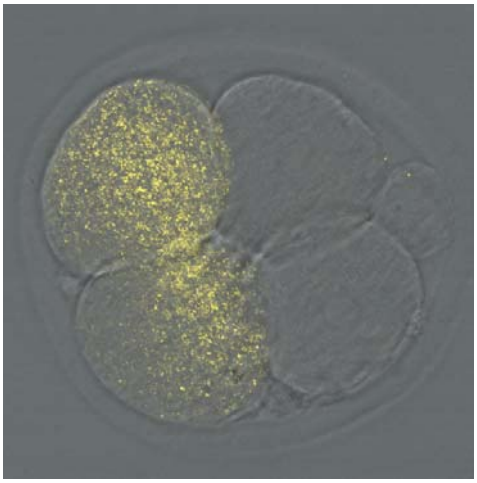
There can be few, if any, cells as specialized as sperm and egg; they appear unique (you can even see a mammalian oocyte with the naked eye) and don't typically divide by themselves. Yet when mammalian sperm and egg combine at fertilization, the single cell they generate is transformed within hours to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

Our laboratory combines molecular and cell biology with piezo-actuated micromanipulation of mouse gametes and embryos to study the events that occur immediately after sperm-egg union (oocyte activation) and their developmental consequences. One long-standing question concerns why oocytes don't begin to divide by themselves in the absence of a sperm. To address this, our group has developed a novel approach that yielded the revelation that the removal of a protein, which we dubbed Emi2, caused oocytes to progress through the cell cycle without stopping, as if the oocytes had been activated by a sperm. We then extended this to show that Emi2 works through Cdc20 and that both parthenogenetic activation and fertilization require Cdc20. This may be the first formal demonstration that the signaling responsible for MPF ablation has molecular components common to both parthenogenesis and fertilization, which is significant given the application of parthenogenetic activation in nuclear transfer and other current research.

In addition, we are interested in the interactions between sperm head components and the oocyte, 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 beneath the membrane of a sperm head reside macromolecular assemblies that include a nucleus (containing the paternal genome and associated proteins) and a surrounding cytoplasmic matrix, the perinuclear matrix, which rapidly comes into contact with the oocyte cytoplasm at fertilization and is an immediate source of paternally-contributed molecules that may modulate development. With a greater understanding of this development, our lab hopes to gain insights into the processes by which embryonic stem cells are formed and carcinogenesis is initiated.



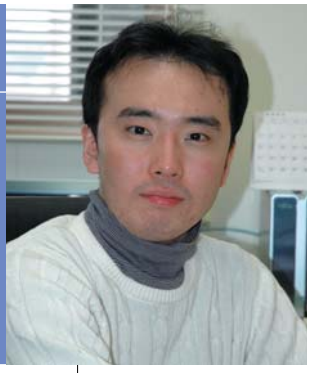
Twelve hours earlier, this 4-cell mouse embryo had just two cells. One of these had been injected with oocyte cytoplasm (containing fluorescent mitochondria so that it's easier to see). In the frog, this classic experiment causes the injected cell to stop dividing due to a long-sought CSF. The result: 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.

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

Mammalian Germ Cell Biology

Mitinori SAITOU M. D., Ph. D.

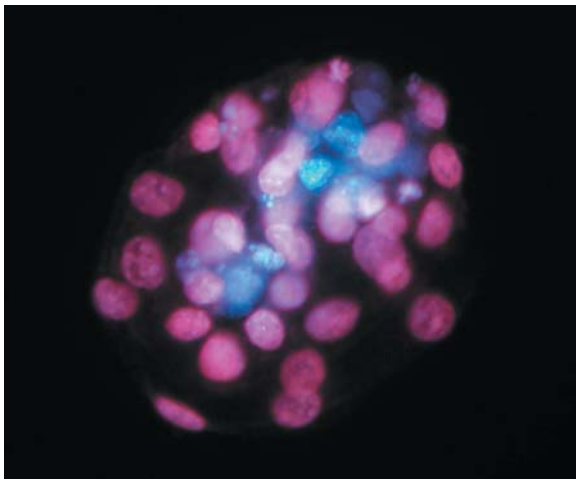
Mitinori Saitou graduated from the Kyoto University Faculty of Medicine in 1995, and was awarded an M. D. in 1999 for his study of the structure and function of mammalian tight junctions under Prof. Shoichiro Tsukita in the Kyoto University Graduate School of Medicine. After a brief fellowship in the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



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

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

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



A mouse blastocyst. Pink, trophectoderm. Blue, inner cell mass that subsequently forms epiblast from which all the somatic cells and the germ line will appear.

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Ohinata Y, et al. Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* 436:207-13 (2005).

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Organogenesis and Neurogenesis

Yoshiki SASAI M. D., Ph. D.

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

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Watanabe K, et al. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci* 8:288-96 (2005).

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Sasai N, et al. The neurotrophin-receptor-related protein NRH1 is essential for convergent extension movements. *Nat Cell Biol* 6:741-8 (2004).

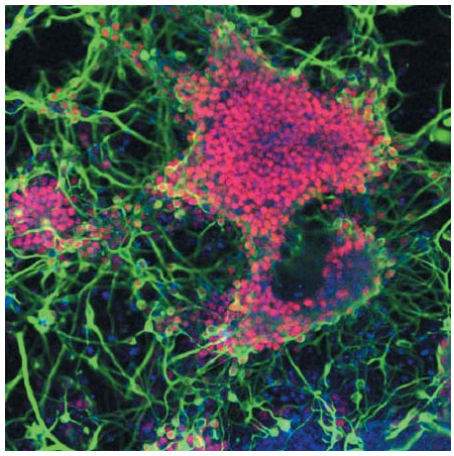
Mizuseki K, et al. Generation of neural crest-derived peripheral neurons and floor plate cells from mouse and primate embryonic stem cells. *Proc Natl Acad Sci U S A* 100:5828-33 (2003).

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)

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

Embryonic Induction

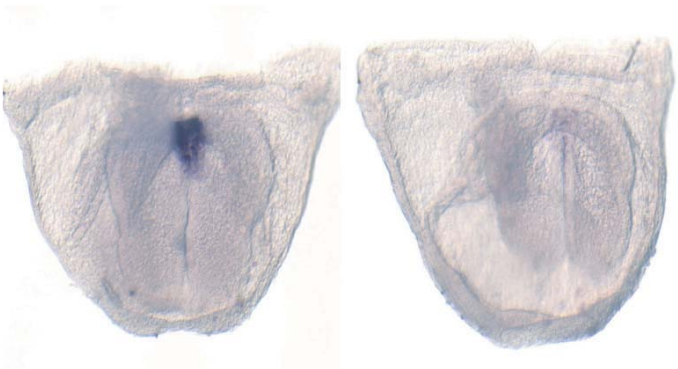
Hiroshi SASAKI Ph. D.

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

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

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

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



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

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

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Publications

Nishioka N, et al. Ssdp1 regulates head morphogenesis of mouse embryos by activating the Lim1-Ldb1 complex. *Development* 132:2535-46 (2005).

Sawada A, et al. Tead proteins activate the Foxa2 enhancer in the node in cooperation with a second factor. *Development* 132:4719-29 (2005).

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).

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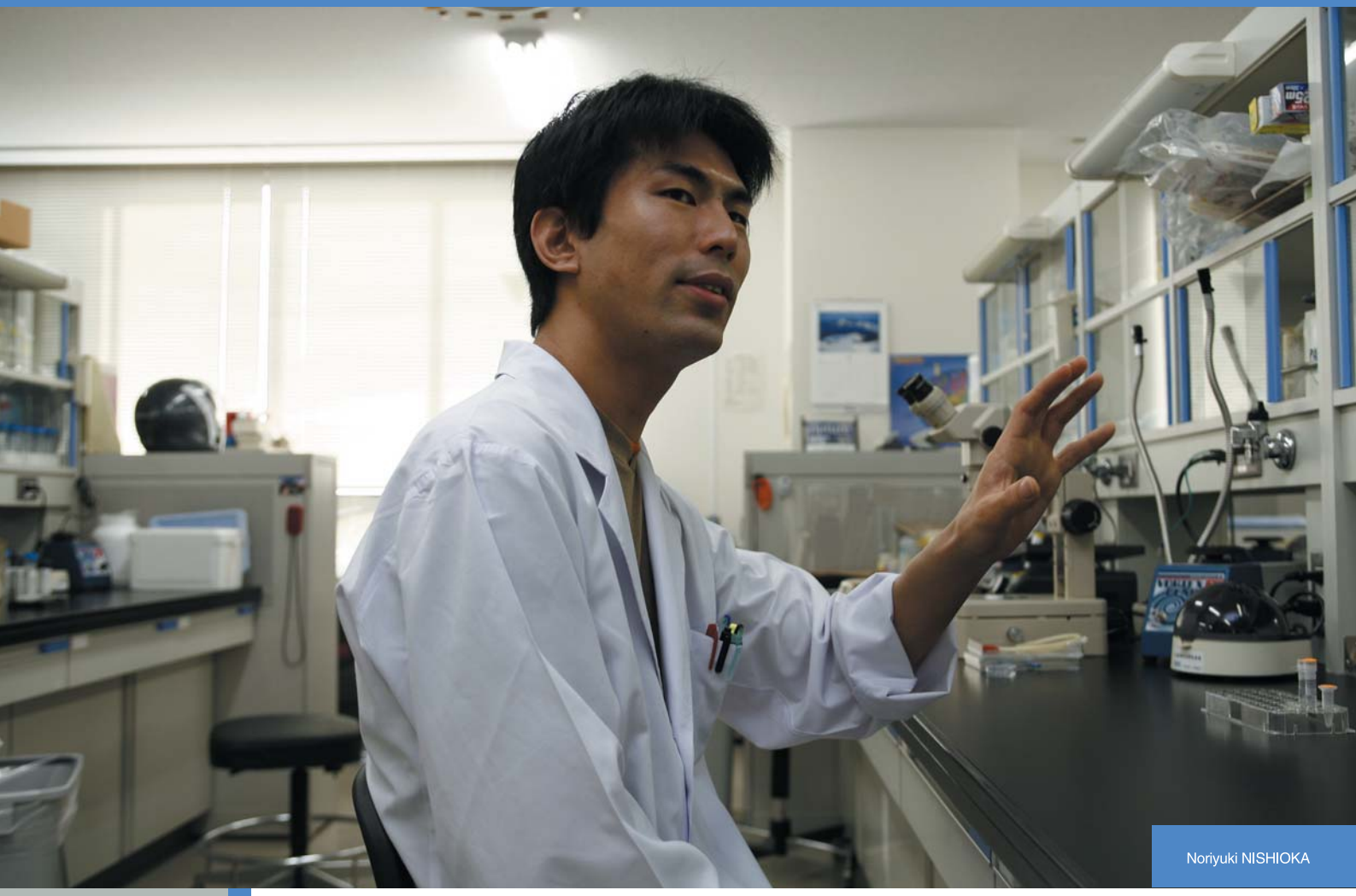
Headshrinker's secrets revealed

A role for Ssdp1 in head development



Noriyuki NISHIOKA

Early vertebrate embryogenesis is guided by the activity of signaling centers, patches of tissue capable of inducing the differentiation of surrounding regions and establishing many of the body plan's most basic features, such as the anterior-posterior and dorsal-ventral axes, the neural tube and the digestive tract. One such signaling center, known as the head organizer, plays a fundamental role in the ontogeny of the head by instructing the anteriorization and formation of head structures from the neural tube. A number of genes have already been identified as involved in the formation of the head organizer, but their specific functions and the interactions and spatiotemporal relationships that characterize these genes' expression patterns have yet to be worked out in their entirety.

In a research article published in the June 2005 issue of the journal *Development*, members of the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) reported new insights into the function of the transcriptional co-activator protein Ssdp1 in the later stages of head organizer development. The study, led by Noriyuki Nishioka, a research scientist in the Sasaki lab, in collaboration with labs in the Universities of Osaka, Hokkaido, Minnesota, and Texas and the NIH casts the genetic regulation of head induction in a fresh light by revealing one more component of the complex interplay between transcription factors needed to establish the structures of the head at the appropriate sites and developmental stages.

Nishioka N, Nagano S, Nakayama R, Kiyonari H, Ijiri T, Taniguchi K, Shawlot W, Hayashizaki Y, Westphal H, Behringer R R, Matsuda Y, Sakoda S, Kondoh H and Sasaki H. Ssdp1 regulates head morphogenesis of mouse embryos by activating the Lim1-Ldb1 complex. *Development* 132:2535-46 (2005).

The story began with a single mouse mutant, in which the embryo failed to develop any head structures whatsoever anterior to the ear, a hallmark of disturbed head organizer function. The mutation was discovered fortuitously during a transgenesis experiment involving an entirely different gene, which led the Sasaki team to surmise that they'd serendipitously interfered with a gene involved in head organizer formation or its activity and, dubbing this striking phenotype *headshrinker* (*hsk*), they set off at once to identify the gene in question. The results of fluorescence in situ hybridization (FISH), a method for tagging and mapping genetic genes to their chromosomal locations, showed that their target was a gene on chromosome 4 named Ssdp1 (for single-stranded DNA binding protein). The *headshrinker* phenotype results when Ssdp1 expression is downregulated and they found that they were able to rescue this phenotype by creating mice transgenic for *Ssdp1*, in which head organizer was apparently restored as pups were born with heads intact.

Nishioka et al next looked at head organizer development in the *hsk* mutants in closer detail and found that although early head organizer tissues such as the anterior visceral endoderm and anterior definitive endoderm formed appropriately, at day E7.5 the prechordal plate failed to take shape. When they next studied the effects of this prechordal plate defect on the neural ectoderm, they observed that all ectoderm-derived head structures anterior to the midbrain-hindbrain boundary were absent, suggesting that the prechordal plate works to suppress posteriorization of the neural ectoderm, a function important to the induction and maintenance of normal head development.

Failure of head development in mouse carrying the *hsk* mutation of the *Ssdp1* gene (right).

Previous studies had shown that another transcription factor–encoding gene, *Lim1*, is also essential for head organizer function, as indicated by the headless phenotype of Lim1 mutant mice. The Lim1 protein is a member of the LIM homeodomain family of transcription factors, and relies on its co-factor, Ldb1, which, interestingly, is also known to interact with Ssdp1. Intrigued by this connection, Nishioka hypothesized that Ssdp1 forms a ternary complex with Lim1-Ldb1, enhancing its transcriptional activator function. The team showed that all three of these factors are expressed in prechordal plate mesenchyme, and that simultaneous co-expression of Ssdp1, Lim1 and Ldb1 in vitro caused the marked upregulation of a prechordal plate-specific reporter gene. Indeed, even Ssdp1 alone caused dose-dependent transcriptional activation when expressed in the presence of Ldb1.

This series of experiments strongly points to a model in which a tripartite Ssdp1-Lim1-Ldb1 complex regulates head morphogenesis by driving the expression of genes in the late-stage head organizer, the prechordal plate. But the story doesn't end there as, in addition to its headlessness, the *hsk* mutant also exhibits a range of general defects in cell survival and proliferation and thoracic development, leaving questions of Ssdp1 function in those phenomena open to further study.

Poles apart

Asymmetry of cortical and nuclear localization of WRM-1

The body's great wealth of cellular diversity is generated by round after round of cell divisions in which a parent cell gives rise to a pair of progeny of differing types, a process known as asymmetric cell division. This can be brought about in a number of ways, one of the most common of which is the segregation of components integral to the determination of cell fate to different regions of the dividing cell. On mitotic division, these localized cell fate determinants are apportioned only to one of the two daughters, influencing its essential character and setting it apart from its sibling. In a sense, although such cells share the same genome, in their appearance and behavior they are more akin to fraternal than identical twins.

In many species, the Wnt signaling pathway – a molecular routine used to regulate the transcription of certain genes – is known to play a role in instructing cells to divide unequally. This widespread role, however, manifests in subtly different ways in different taxa. The roundworm, *C. elegans*, features a version of the Wnt cascade that includes WRM-1, a homolog of the transcription factor, β-catenin. In species from fly to man, this molecule is a dual agent, serving important functions in both cell adhesion, in which it forms a complex with transmembrane cadherins, and Wnt signaling, where its accumulation and degradation are determined by upstream events governed by the binding (or failure to bind) of Wnt to its receptor at the cell membrane. The roundworm homolog WRM-1, however, does not appear to follow this general pattern; it has no known role in cell adhesion, it may not be phosphorylated by GSK3 as in other species, and its intracellular localization and its function in the regulation of cell polarity have remained obscure. In an article published in the 1 August 2005 issue of *Genes and Development*, Hisako Takeshita and Hitoshi Sawa (Team Leader, Laboratory for Cell Fate Decision) described an intriguing pattern of localization for WRM-1 and a second molecule, the MAP kinase homolog LIT-1, before and after asymmetric mitosis in *C. elegans* cells that helps to clarify the picture of roundworm cell polarization.

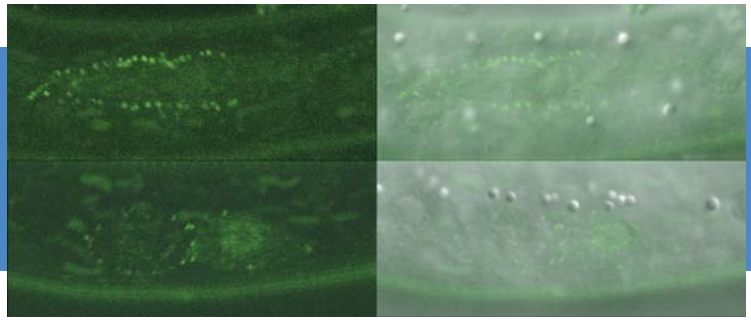
C. elegans cell divisions follow highly stereotyped and well-characterized patterns, making it relatively simple to detect aberrations from the norm. Using several strains of worms in which a region required for the function of the *wrm-1* gene had been deleted or conditionally interfered with, Takeshita and Sawa looked at cell division in these animals and found disruptions in the typical patterns of cell progeny. Similar results were obtained in studies of cell divisions in multiple cell lineages in mutants for the gene *lit-1*, or is activator *mom-4*, indicating that the majority of post-embryonic cell divisions in *C. elegans* are regulated by a set of similar mechanisms.

Hisako TAKESHITA

Takeshita H and Sawa H. Asymmetric cortical and nuclear localizations of WRM-1/β-catenin during asymmetric cell division in C. elegans. *Genes Dev* 19:1743-8 (2005).

They next fused a gene encoding green fluorescent protein (GFP) to *wrm-1* and *lit-1* to allow them to visually track the sites to which their protein products localized. In many of the cell pairs, they found that the nucleus of the posterior daughter showed a higher WRM-1 or LIT-1 GFP readout. However, during cell division, including telophase, the stage at which mitotic division nears completion and the two nuclei are cordoned off into opposing halves of the dividing cell, both WRM-1 and LIT-1 could also be detected at the anterior cortex. Interestingly, WRM-1 was also seen in both nuclei at this stage, suggesting that it is exported from the anterior nucleus during the latest phase of mitosis.

Wondering about how this shift in distribution occurred, the Sawa team used a technique known as fluorescence recovery after photobleaching (FRAP), in which all or part of a GFP-tagged cell is flashed with a bright light capable of eliminating its fluorescence, and then monitored to determine when and where its green glow is restored. After photobleaching the entire cell early in mitosis to show that GFP activity is not recovered when universally bleached away, they next applied light bursts to the anterior half of the cells, or to the posterior half of cells undergoing division, and found that in either case the recovery of both WRM-1 and LIT-1 fluorescent reporter activity was limited to the nuclei of the resulting cellular progeny – a sign that both the anteriorly or posteriorly localized moieties of these factors ultimately make their way to the posterior nucleus. Subsequent tests lent further weight to the idea that, in the case of LIT-1 at least, the redistribution was achieved by a higher rate of export from the anterior nucleus.



Asymmetric localization of WRM-1/β-catenin in the V5.p cell. Anterior is to the left and ventral to the bottom. Left panels are confocal images of WRM-1::GFP fusion protein and right panels are merges of GFP and Nomarski images. Top panels show localization on the anterior cortex before the division. Bottom panels shows localization in the posterior nucleus after the division.

Perhaps their most interesting finding came when they looked at WRM-1 and LIT-1 dynamics in a mutant for the *wnt* homolog, *egl-20*. In these mutants, prior to division the localization of WRM-1 and LIT-1 in the cortex became randomized, but by telophase these factors always localized to the nucleus on the opposite side of the dividing cell, regardless of the cortical starting point. Cross testing showed that while WRM-1 was required for localization of LIT-1 to the cortex and nucleus on opposite sides of the mitotic cell, the MOM-4 LIT-1 pathway seems to regulate only the nuclear localization of WRM-1.

From this compelling set of observations, Sawa and Takeshita developed an elaborate and complex model of asymmetry dynamics in the post-embryonic roundworm, in which Wnt signals remove WRM-1 from the posterior cortex, freeing it to travel through the cytoplasm to the nucleus. The cortical polarity thus established may then go on to regulate the asymmetry of the mitotic nuclei, in which differences in anterior and posterior nuclear export rates result in the LIT-1 WRM-1 complex remaining exclusively in the posterior nucleus as the cell splits in two.

Let there be light-sensing cells

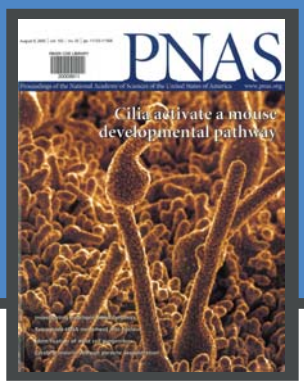
Humans are visual animals. Our reliance on sight is immediately apparent on considering the impact the loss of that ability can have on a person's range of everyday functions. The eye's marvelous ability to sense light depends on the function of a population of cells in the retina, known as photoreceptors, which comprises the familiar "rod" and "cone" shaped cells responsible for contrast and color vision. And, as is generally true of neural cells, photoreceptors regenerate poorly or not at all, making retinal degenerative disorders, in which this specific group of cells is affected, both intractable to currently available therapies and promising candidates for treatment by regenerative medicine. In this emerging field of medicine, clinicians and researchers seek to replace cells that have been damaged or lost and so restore physiological function, as well as to develop systems for studying human diseases by modeling them in vitro. But access to pure, safe and plentiful supplies of specific types of cell remains one of the primary hurdles to be overcome before the promise of this young discipline can be realized.

In the 2 August 2005 online edition of the *Proceedings of the National Academy of Sciences*, Yoshiki Sasai (Group Director, Laboratory for Organogenesis and Neurogenesis) and colleagues at the CBD and Kyoto University reported the world-first achievement of a method for inducing the differentiation of neural retinal precursors and photoreceptors from embryonic stem cells (ES cells) in mouse.

The Sasai group has published several previous studies showing the successful differentiation of a range of specialized neurons, including dopaminergic, enteric, and forebrain, from ES cells. Although these pluripotent stem cells are, by definition, capable of giving rise to any type of cell in the body, limiting that potential to the generation of only a single type of cells requires close and detailed guidance. In the laboratory, this takes the form of highly controlled methods of ES cell culture. In this latest

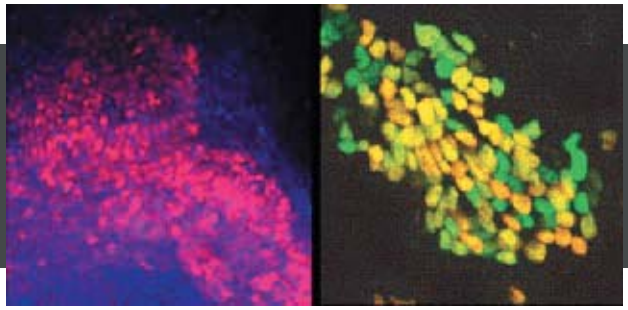
Hanako IKEDA

Ikeda H, Osakada F, Watanabe K, Mizuseki K, Haraguchi T, Miyoshi H, Kamiya D, Honda Y, Sasai N, Yoshimura N, Takahashi M and Sasai Y. Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. *Proc Natl Acad Sci U S A* 102:11331-6 (2005).



work, Sasai et al tweaked a protocol they had previously developed, called SFEB (for Serum-free Floating culture of Embryoid Body-like aggregates), which they showed earlier this year could yield neural differentiation at efficiencies of up to 90%.

Knowing that photoreceptors arise from a population of neural retinal precursor cells identifiable by specific patterns of gene expression, the group first looked for ways of mimicking in vitro the differentiation pathway followed by the developing embryo. After much experimentation with various combinations of culture conditions and growth factors, Hanako Ikeda (now at the Kyoto University Hospital Translational Research Center) discovered that by treating floating aggregates of ES cells with activin, fetal calf serum, and a pair of factors (Dkk1 and LeftyA) known to drive undifferentiated cells to a neural fate, she was able to induce their differentiation into cells bearing all the molecular hallmarks of neural retinal precursors at efficiencies as high as 16%. They dubbed the new method SFEB/DLFA.

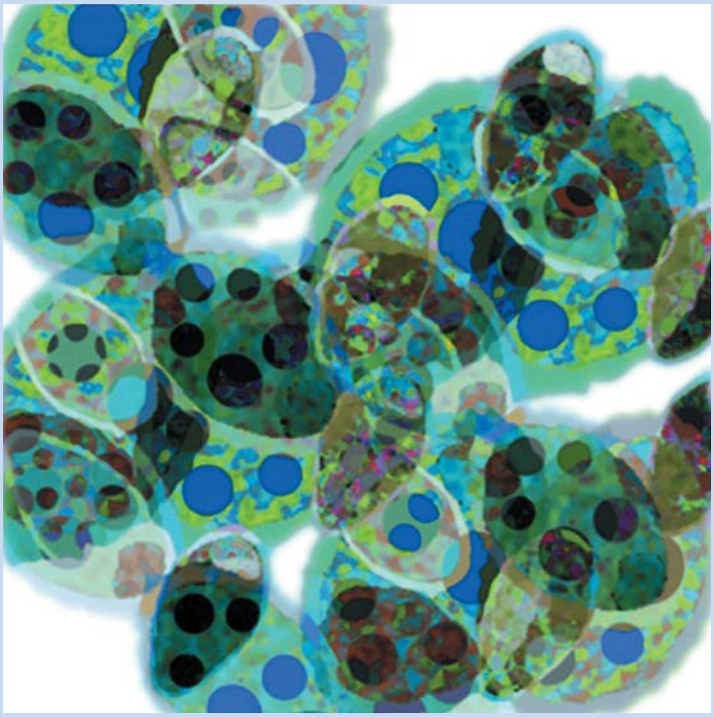


Cells derived from mouse ES cells using SFEB/DLFA culture. Cells express neural retinal precursor markers Rx (left, indicated in red) and Pax6 (right, green). Site of co-expression appears as yellow (at right).

Further tests yielded even more exciting results. On being cultured together with retinal cells from embryonic mice, the neural retinal precursors gave rise to large numbers (around 14% of the entire cultured aggregate) of cells expressing rhodopsin and recoverin, two molecules known to be specific to photoreceptors in vivo. Cultured with explants of embryonic neural retina, these ES cell-derived rhodopsin-positive cells behaved as photoreceptors would be expected to, integrating into sites in the neural retina explant where these cells normally appear.

This set of findings represents a significant advance, both as a demonstration of the importance of microenvironmental influences on embryonic cell decision-making, and as a proof-of-concept of a much sought after means to selectively induce photoreceptors from ES cells in culture. For patients and physicians confronting a range of disorders that damage the retina and threaten eyesight, such as age-related macular degeneration and the complications of diabetes, this should be welcome news indeed. Much research remains to be done before ES cell-derived photoreceptors can be brought to bear on human health problems but, as a first step, this work by Sasai and colleagues exemplifies the noblest aspirations of any science: to shed light and to offer hope.

Art & Science fest



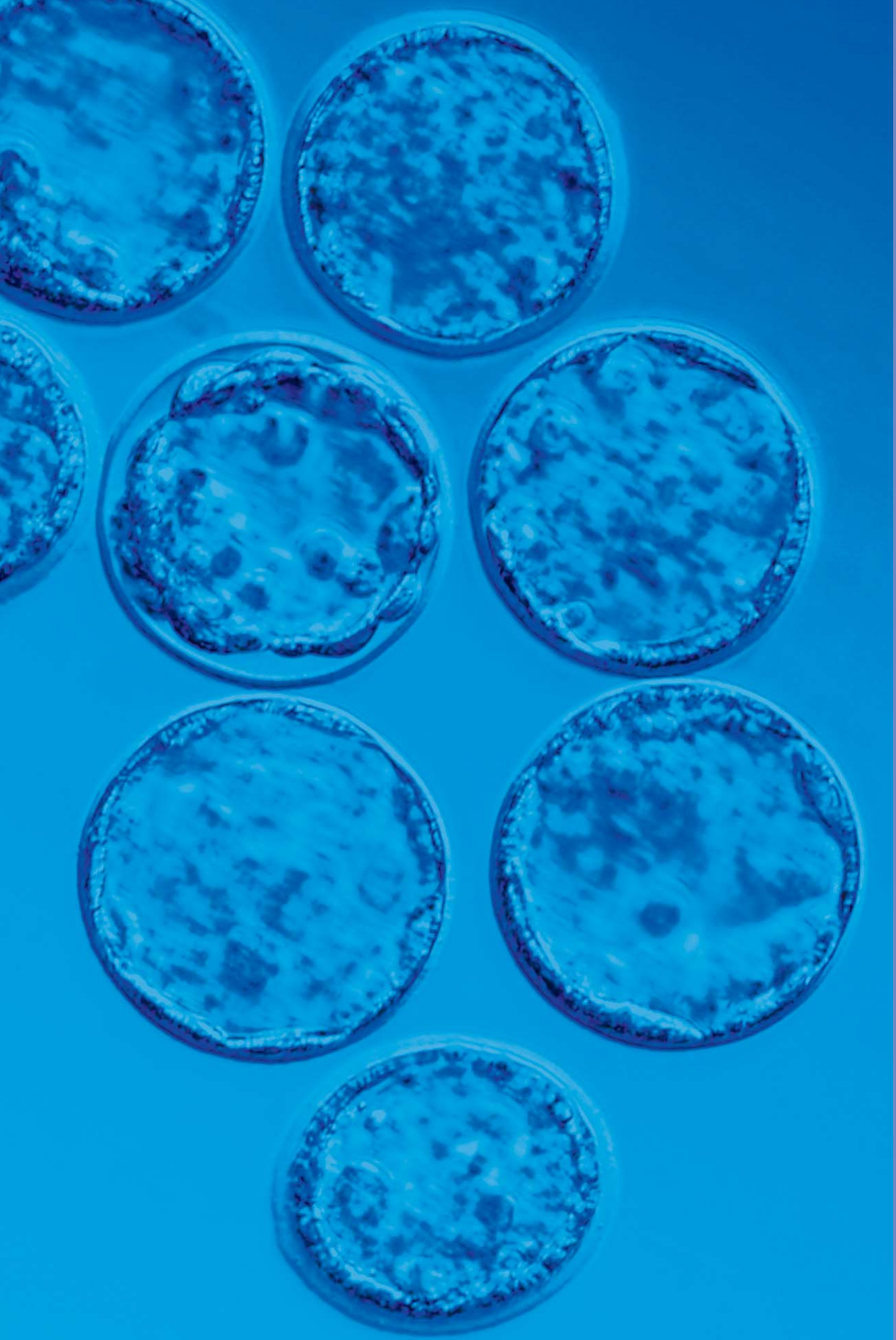
Ayako TSUBOYA, Arsnote Lab

This year's RIKEN public lecture brought together graphic designers and visual artists to exhibit their interpretations of images and phenomena from the world of science. The exhibition, titled RIKEN Art & Science, was held on October 11th in conjunction with the yearly public lectures held by the institute to encourage public awareness and understanding of science.

Many of the works on display were designed by members of a creative project named Arsnote Lab, who rendered microscopic images of cells and tissues into a vivid array of mosaics, panoramas and studies in color. Faculty from the Kobe Design University also contributed arrangements of biological structures and organisms curiously arrayed around figures from Hindu mythology. Several pieces featured images provided by CDB labs, from *Drosophila* germ line cells visualized in a fancifully colored negative space, to mouse olfactory tissue made almost three-dimensional through the subtle interplay of neon glows against a darkened field.

July-September Seminars

date	title	speaker
2005-07-15	IKKα and the control of epidermal differentiation	Michael KARIN
2005-07-22	Fate regulation of neural precursor cells in the developing mouse brain	Yukiko GOTOH
2005-08-30	A role for DOCK7, a novel activator of Rac, in neuronal polarization	Mitsuko WATANABE-UCHIDA
2005-09-27	Chromatin remodelling during mammalian spermatogenesis	M. R. S. RAO
2005-09-27	Genes specifically expressed in primate visual and association areas: their implication for formation and evolution of the primate neocotex.	Tetsuo YAMAMORI
2005-09-29	DNER acts as neuron specific Notch ligand during Bergmann glial development	Mototsugu EIRAKU
2005-09-30	Mechanisms of asymmetric cell division in *C. elegans* embryos	Pierre GONCZY



Hitoshi SAWA

Guojun SHENG

Asako SUGIMOTO

Yoshiko TAKAHASHI

Masatoshi TAKEICHI

Hiroki R. UEDA

Teruhiko WAKAYAMA

Shigenobu YONEMURA

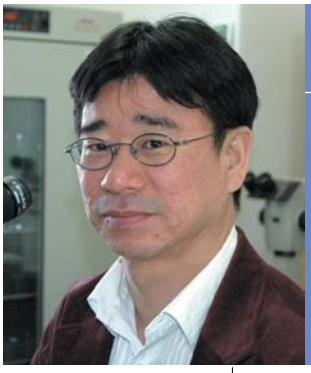
Animal Resources and Genetic Engineering

Genomics Support Unit

Leading Project Research Units

Migrations

Cloned blastocysts derived from somatic cell nuclei, treated with TSA



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.

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Assistant
Tomoko NAKASHIMA

Publications

Kagoshima H, et al. The *C. elegans* RUNX transcription factor RNT-1/MAB-2 is required for asymmetrical cell division of the T blast cell. *Dev Biol* 287:262-73 (2005).

Kidd A R, 3rd, et al. A β-catenin identified by functional rather than sequence criteria and its role in Wnt/MAPK signaling. *Cell* 121:761-72 (2005).

Takeshita H and Sawa H. Asymmetric cortical and nuclear localizations of WRM-1/β-catenin during asymmetric cell division in *C. elegans*. *Genes Dev* 19:1743-8 (2005).

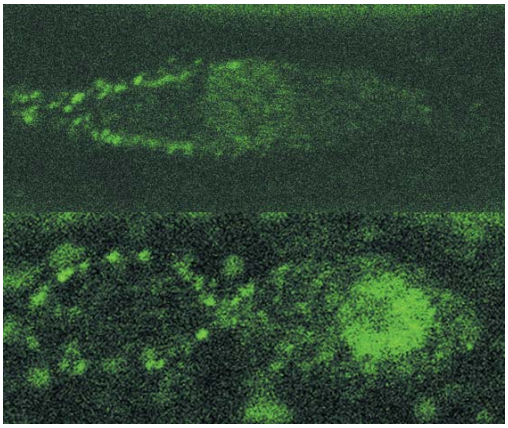
Yoda A, et al. Components of the transcriptional Mediator complex are required for asymmetric cell division in *C. elegans*. *Development* 132:1885-93 (2005).

Zhao X, et al. *tcl-2* encodes a novel protein that acts synergistically with Wnt signaling pathways in *C. elegans*. *Dev Biol* 256:276-89 (2003).

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

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

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



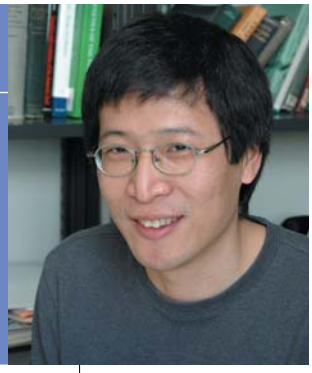
Asymmetric localization of WRM-1/β-catenin before (top) and after (bottom) division

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

Early Embryogenesis

Guojun SHENG Ph. D.

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

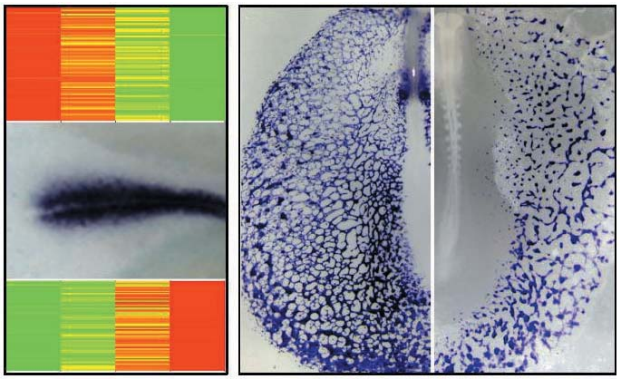


We study the molecular and cellular mechanisms of gastrulation, an early developmental process conserved throughout the animal kingdom. During gastrulation, three germ layers (ectoderm, mesoderm and endoderm) are formed from a uniform layer of epiblast cells. Each of these germ layers undergoes further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

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

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

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



Left panel: 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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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, et al. Tow phases of astral microtubule activity during cytokinesis in *C. elegans* embryos. *Dev Cell* (2006, in press).

Furuya M, et al. The *C. elegans* eyes absent ortholog EYA-1 is required for tissue differentiation and plays partially redundant roles with PAX-6. *Dev Biol* 286, 452-63 (2005).

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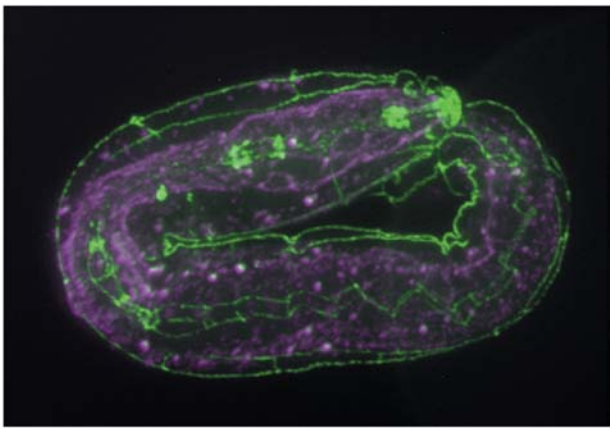
The cooperation between groups of genes encoded in an organism's genome is essential to the development of complex structures from a single cell, the fertilized egg. Our laboratory has adopted the nematode *Caenorhabditis elegans* as an experimental model. This organism provides an extremely useful system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

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

We are now beginning to perform live imaging and other cell biological studies of discrete sets of genes identified in our phenomics analyses. One focal topic is the regulation of mitosis and meiosis 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 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 segregation of cell fate determinants. We are interested in how mitosis, meiosis, and differentiation are regulated interactively in the context of embryonic ontogeny. And in a concurrent project, we have also initiated studies on cell shape change during morphogenesis. By studies such as these, we hope to provide a more detailed picture of the regulation of dynamic processes by networks of genes.



A *C. elegans* embryo at late stage of morphogenesis. Green: cell boundary of epidermal cells (MH27 mAb). Magenta: outer surface of pharynx and intestine (KT14 mAb).

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

Body Patterning

Yoshiko TAKAHASHI Ph. D.

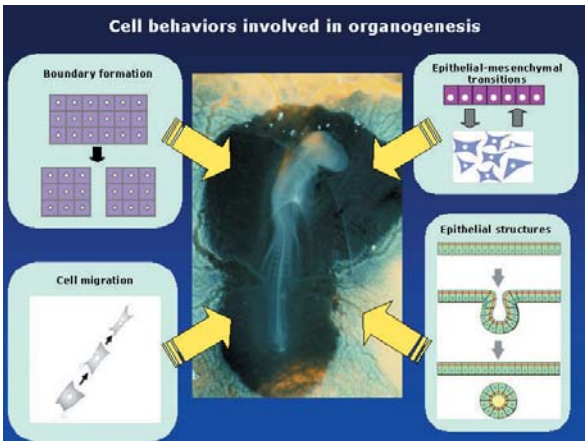
Yoshiko Takahashi received her B. Sc. from the University of Hiroshima, before moving to the Kyoto University Department of Biophysics where she received her master's and doctoral degrees in developmental biology. She pursued consecutive postdoctoral fellowships in developmental biology at the Institut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

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

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

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

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



Cell behaviors involved in organogenesis.

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Cell Adhesion and Tissue Patterning

Masatoshi TAKEICHI Ph. D.

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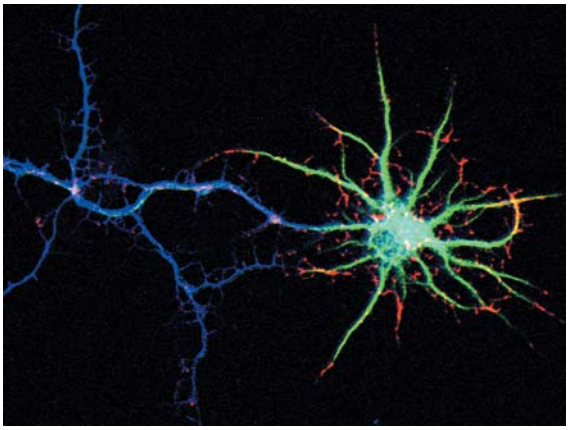
The general research focus of our lab is the study of the cellular and molecular basis of the mechanisms by which animal cells are organized into precisely ordered multicellular structures, such as tissues and neural networks. We are particularly interested in the roles played by cell-cell adhesion and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

Cell-cell adhesion is dynamic and reversible, which is important for regulating many types of morphogenetic cell behavior. Cadherin activity is modulated by interactions with cytoskeletal or signaling factors mediated by members of the catenin family of cadherin-associated proteins. We are studying the mechanisms underlying the cross-talk between cadherins and cytoskeletal or signaling systems, with the goal of uncovering novel regulatory mechanisms specific to cell-cell adhesion.

We are also interested in the mechanisms underlying the formation of neural networks, particularly interneuronal recognition during synapse formation. The cadherin/catenin complex is localized in synaptic contacts, and different cadherin subtypes are expressed by different neurons of the brain. We are now investigating ways to determine how synaptic contacts are regulated by this adhesion system, how this process is involved in the physiological regulation of synaptic activities, and the role of neuron type-specific expression of cadherin subtypes in neural network development. We are also interested in the roles of other cell surface molecules, such as OL-protocadherin and nectins, in interneuronal recognition processes.

A third area of interest to our lab is the mechanisms by which animal tissues are organized through the processes of dynamic cell rearrangement, such as cell migration and relocation. Using the neural retina and brain cortices as model systems, our team is attempting to determine how cell migration and positioning are controlled by cell adhesion and signalling molecules during the formation of laminar structures in these tissues.



An early hippocampal neuron in culture, triple-immunostained for nectin-3 (red), tau (blue; axon marker), and MAP2 (green; dendrite marker). Nectin is a subfamily of Ig-superfamily molecules, which can regulate cadherin localization and activities.

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

Systems Biology

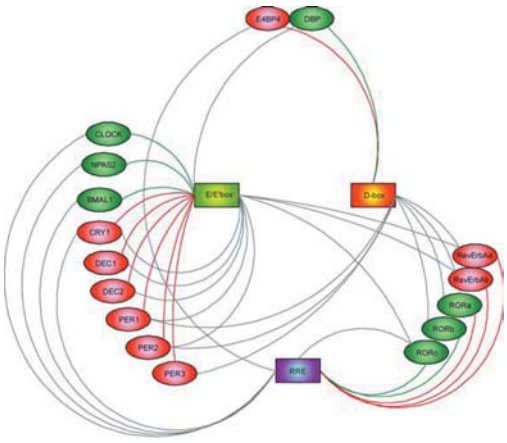
Hiroki R. UEDA M. D. , Ph. D.

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

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

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

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



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

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

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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 producing the world's first cloned mouse. He was appointed to an assistant professorship at the same institution in 1998, and moved to the Rockefeller University as a research assistant professor in 1999. He spent a year as a researcher at Advanced Cell Technology before returning to Japan to take his current position at the RIKEN CDB.

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Kishigami S, et al. Epigenetic abnormalities of the mouse paternal zygotic genome associated with microinsemination of round spermatids. *Dev Biol* 289:195-205 (2006).

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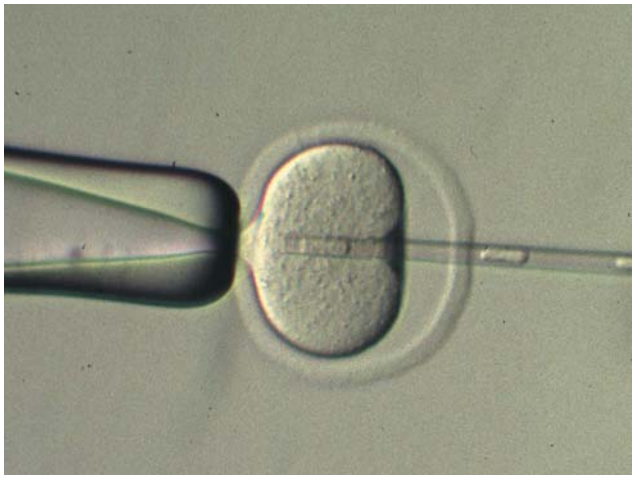
A limitless number of clones of an animal can be generated from its somatic cells. Only a few years ago, such a statement would have belonged to the realm of science fiction, but now thanks to advances in the technology known as micromanipulation, which allows researchers to work with individual cells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

The cause of these defects is thought to be due to imperfections in the nuclear transfer technique used to remove the nucleus from a host cell and replace it with another from a donor, imperfections which presumably lead to total or partial failure of reprogramming, the process in which a cell's developmental potential is reset to a totipotent state (such as is exhibited by a naturally fertilized egg). However, the specific details of the technical flaws, the ways in which they lead to reprogramming failure, and possible solutions to the problem all remain completely unknown. Despite these challenges, cloning continues to be a biotechnology of great promise, given its potential applications in the field of regenerative medicine, such as the generation of embryonic stem (ES) cells by nuclear transfer, which may one day allow 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.



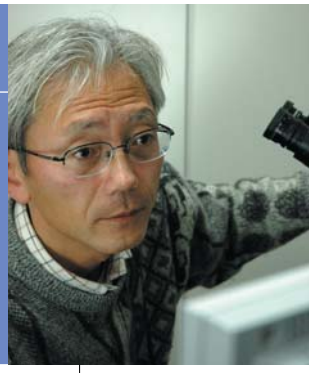
Transfer of a somatic nucleus into an enucleated egg

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

Cellular Morphogenesis

Shigenobu YONEMURA Ph. D.

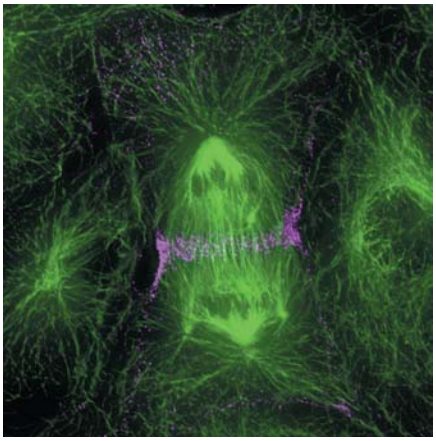
Shigenobu Yonemura received his B. Sc., M. Sc. and Ph. D. from the University of Tokyo, earning his doctorate in 1988 for thesis work under Prof. I. Mabuchi. He spent a year as a postdoctoral fellow at the same institution before moving to pursue a fellowship at Johns Hopkins University from 1989 to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



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 by taking 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 Rho in cells and tissues. Animal cells cleave by forming a furrow between the two daughter nuclei following nuclear division. We have found that Rho accumulates at the putative furrow region in a microtubule-dependent manner prior to the initiation of furrowing. This is the first evidence that Rho localization is regulated by microtubules. Further, we have determined the signaling pathway connecting microtubules and Rho by knocking down a number of microtubule-associated proteins. Regulation by Rho also appears 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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Nishimura Y and Yonemura S. Centralspindlin regulates ECT2 and RhoA accumulation at the equatorial cortex during cytokinesis. *J Cell Sci* 119:104-14 (2006).

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Animal Resources and Genetic Engineering

Shinichi AIZAWA Ph. D.

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Hitoshi MIYACHI
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Mayumi MIZUSHIRO
Kaori NASU

Publications

Furushima K, et al. Characterization of Opr deficiency in mouse brain: subtle defects in dorsomedial telencephalon and medioventral forebrain. *Dev Dyn* 232:1056-61 (2005).

Hara T, et al. Deficiency of protein kinase Calpha in mice results in impairment of epidermal hyperplasia and enhancement of tumor formation in two-stage skin carcinogenesis. *Cancer Res* 65:7356-62 (2005).

Hiratsuka S, et al. Vascular endothelial growth factor A (VEGF-A) is involved in guidance of VEGF receptor-positive cells to the anterior portion of early embryos. *Mol Cell Biol* 25:355-63 (2005).

Kassai H, et al. Farnesylation of retinal transducin underlies its translocation during light adaptation. *Neuron* 47:529-39 (2005).

Terada K, et al. A type I DnaJ homolog, DjA1, regulates androgen receptor signaling and spermatogenesis. *Embo J* 24:611-22 (2005).

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.

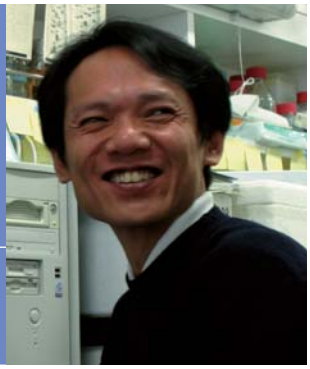
The LARGE team continues to expand its services and initiate new programs, notably the generation of target vectors from sequence information alone, and the independent production of novel genetically-modified constructs, a drive that generates on the order of 40-50 new mutant strains per year. Such 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.

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

Genomics Support Unit

Fumio MATSUZAKI Ph. D.



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

Genome Resource and Analysis

Hiroshi TARUI Ph. D.

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

Staff

Subunit Leader
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Osamu NISHIMURA
Kaori TATSUMI
Junko UEDA

Assistant
Chiharu TANEGASHIMA

Functional Genomics

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

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

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Research Scientist
Takeya KASUKAWA

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Junko NISHIO
Kenichiro UNO

Assistant
Ikuko TADA

Leading Project Research Units

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

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

Cell Plasticity

Mitsuko KOSAKA Ph. D.

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

Staff

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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 and cartilage. The regeneration of solid organs, such as liver or pancreas, remains 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. This approach ultimately allowed us to identify and characterize stem cells in such organs. We now seek to achieve a better understanding of the underlying stem cell biology and the development of basic technologies that will enable us to realize the promise of regenerative medicine for patients suffering from health conditions involving these organs.

Staff

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Momotarou ISHIKAWA
Yuji OSHIMA

Part-Time Staff
Keiko SUEYOSHI

Assistant
Yumie KITA

Migrations

The CDB celebrated its fifth anniversary in 2005, giving occasion to a number of transitions in its research programs and staff.

Evolutionary Regeneration Biology

Kiyokazu AGATA Ph. D.

Kiyokazu Agata, one of the Center's founding Group Directors, led the Laboratory for Evolutionary Regeneration Biology from June of 2000 to March of 2005. He has taken a new appointment as Professor in the Kyoto University Department of Biophysics, where he continues his research into the biological mechanisms of regeneration.

Positional Information

Shigeru KONDO Ph. D.

Shigeru Kondo served as Team Leader for the Laboratory for Positional Information from February 2001 to March of 2005, when he took an appointment as Professor at the Nagoya University Graduate School of Science. His research focuses on demonstrating the mathematical basis of pattern formation in development, and using mathematical models as predictive tools to aid in the identification of genes and molecules involved in the generation of spatial structures.

Evolutionary Morphology

Shigeru KURATANI Ph. D.

Shigeru Kuratani joined the CDB as Team Leader of the Laboratory for Evolutionary Morphology in February 2001 and has made a number of impressive contributions to our understanding of how evolutionary changes affect developmental processes. He was promoted to Group Director in June 2005.

Cell Lineage Modulation

Toru KONDO Ph. D.

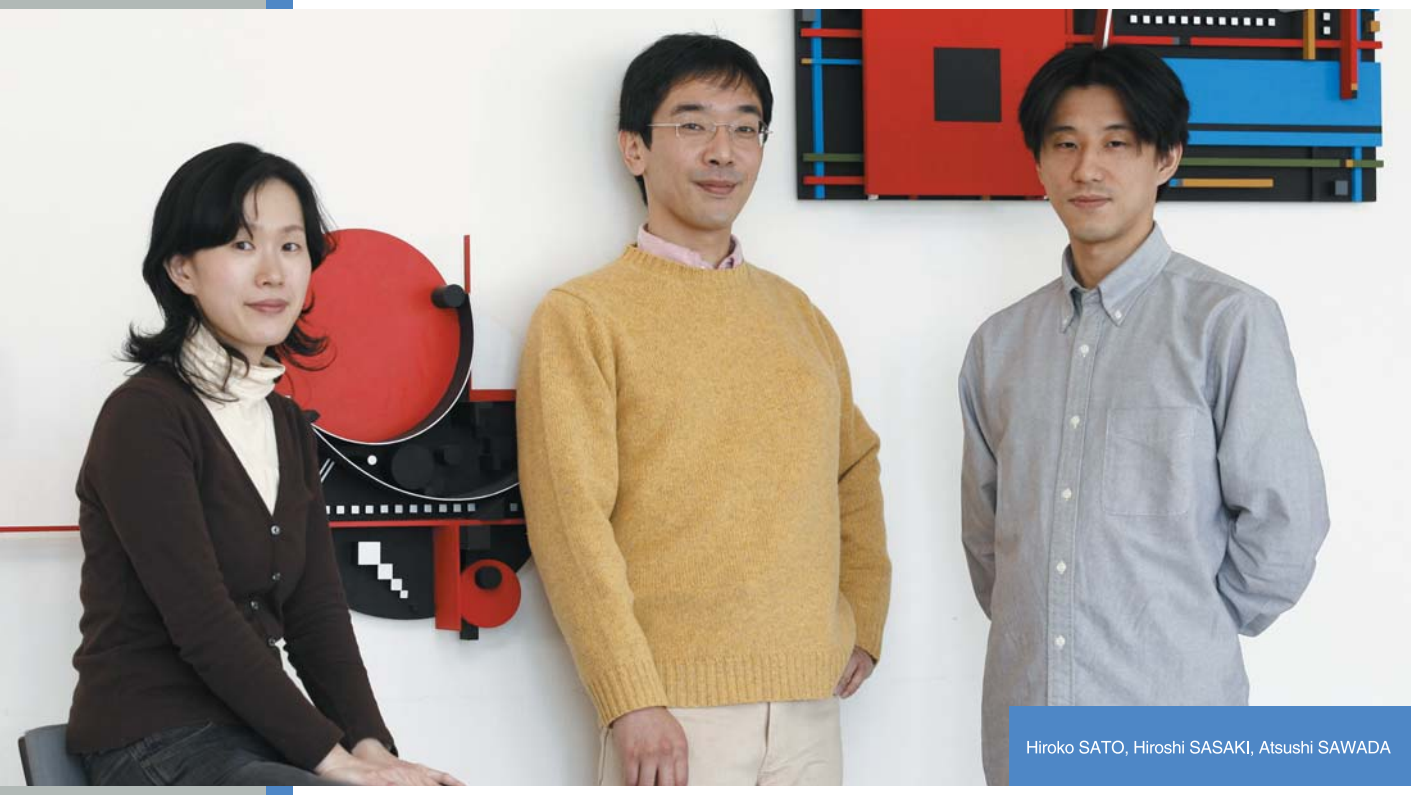
Toru Kondo joined the CDB in April 2005 as the Team Leader of the Laboratory for Cell Lineage Modulation, moving from a position as Group leader in the Centre for Brain Repair in the University of Cambridge (UK). His team will focus on deciphering the reacquisition of "stemness" using the dedifferentiation of oligodendrocyte progenitor cells (OPCs) into neural stem-like cells as its main model.

Tead start

Activation of the *Foxa2* enhancer in the node

The great diversity of vertebrate forms is the result of developmental processes that shape the body as it grows, an intricately orchestrated activity that relies on signals from centers within the embryo capable of directing tissue movement and differentiation. Perhaps the best known of these signaling centers is the "organizer," first identified by Hans Spemann and Hilde Mangold in the newt as a region capable of directing neighboring tissues to adopt specific fates. Studies in other organisms including frog, chick, and mouse have shown that organizing centers in these species all share certain properties and characteristics as they guide the embryo through gastrulation, in which it sorts itself into three germ layers. Perhaps even more interestingly, many organizers themselves comprise a diverse and dynamic assembly of cells, marked by specific patterns of gene expression and playing a continuously changing series of roles as the embryo matures.

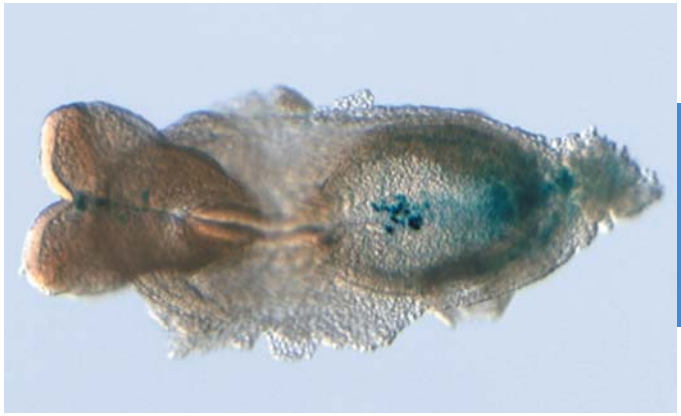
In the mouse, the organizer forms at the anterior end of a structure known as the primitive streak, about six and a half days after conception. Throughout embryogenesis, the organizer plays home to a number of cell populations, first coordinating the emergence of the head before assuming a new identity as the "node," which regulates trunk development beginning at about embryonic day 7. The node remains a morphologically distinct region thereafter, and contains cells that serve as the progenitors of the notochord, a rod-like stretch of mesoderm that underlies the nascent central nervous system along the midline of the body and itself serves as a signaling center regulating the dorsoventral patterning of the trunk. It is characterized by the expression of a number of node-specific molecules, one of the most significant of which is *Foxa2*, a transcription factor known to regulate the development of other midline signaling centers as well. Given its prevalence and importance in organizing the embryonic midline in all taxa studied, the question of the regulation of *Foxa2* expression is one of widespread interest to developmental biologists. In an article published in the November 2005 issue of *Development*, Atsushi Sawada of the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) and colleagues in the CDB and Osaka University reported an advance in the understanding of how *Foxa2* gene expression is driven in the node.



Hiroko SATO, Hiroshi SASAKI, Atsushi SAWADA

Sawada A, Nishizaki Y, Sato H, Yada Y, Nakayama R, Yamamoto S, Nishioka N, Kondoh H and Sasaki H. Tead proteins activate the Foxa2 enhancer in the node in cooperation with a second factor. *Development* 132:4719-29 (2005).

Building on previous knowledge of a *Foxa2* enhancer specific to the node and notochord, the team made transgenic mice in which a core-element sequence containing a motif critical to the enhancer's activity was used to drive the expression of β-galactosidase, enabling them indirectly to track *Foxa2* activation over space and time. Equipped with a monitor of enhancer activity, they studied patterns of expression in the embryo and noticed an intriguing similarity to the footprint of Wnt/β-catenin, a molecular pathway involved in many aspects of cell signaling and differentiation. Co-transfection of canonical Wnt factors with the core element (CE) into embryonic carcinoma cells revealed that Wnt does act upstream of the *Foxa2* enhancer, albeit in an indirect manner.



Node-specific expression of Foxa2 is achieved by a core-element sequence of the enhancer.

Looking at the CE more closely, Sawada et al found that its function relies on a pair of three-nucleotide regions in its 27-base sequence, suggesting that the cooperation of two transcription factors is essential for the enhancer's ability to activate *Foxa2* expression in the node. Now curious, they used the CE sequence as bait in a yeast one-hybrid screening against a mouse cDNA library, intended to isolate factors that interact with the core element. A pair of members of the TEAD family of transcription factors turned up in many of the positive clones. *Tead* genes and their cofactor Yap65 are expressed widely in the embryo during the E6.5 to E8.5 stages, including in regions of *Foxa2* activity, marking them as promising candidates for further study, which was borne out by transfection of members of the Tead family and the Yap65 cofactor into carcinoma cells. All four Tead factors appear to be involved in the regulation of the *Foxa2* enhancer core element, although at varying intensities, with Tead1 and Tead4 showing the strongest activity. Importantly, this Tead effect was found to be independent of the Wnt/β-catenin pathway, whose precise molecular nature remains unknown, but is thought to be linked to the CE 5' region.

Looking next in vivo, the Sasaki team electroporated a repressor form of Tead into transgenic mouse embryos and found that interference with Tead function disturbed both *Foxa2* expression and the normal development of the notochord. Parallel studies in zebrafish showed that Tead proteins regulate Foxa2 in the embryonic shield, an organizer with similar function to the mouse node, in fish as well.

The pervasive expression of Foxa2 and Tead in multiple embryonic regions and stages suggests that both play fundamental and varied roles in development, but their convergence at the relatively narrow spatiotemporal window of the node/notochord indicates that their functional interaction there is highly context-specific. The evidence from the Sasaki study provides a compelling case for an induction of the Foxa2 enhancer by Tead in cooperation with a transcription factor downstream of Wnt that is localized exclusively to the node, which may serve to explain the unique activity of this particular incarnation of the organizer. The identity of Tead's co-agent remains to be found, but these latest steps forward by the Sasaki team have brought the picture of vertebrate embryonic induction that much clearer into focus.

Push and pull factors

Contrary signals guide A-P axis specification

Chiharu KIMURA-YOSHIDA, Isao MATSUO, Hiroshi NAKANO

Early mammalian embryos lack direction; that is, their cells are not organized along the distinct axes that characterize the adult body. But after just a few days of development, the embryo rearranges itself so that it has the first makings of a front and a back end, a top and a bottom, and left and right sides. In order to organize itself along these axes, the embryo relies on a map of molecular signals that guide cells as they differentiate, divide, migrate and interact with each other to assemble into a body.

The anterior-posterior (AP) axis, which corresponds roughly to the head-to-tail midline in the adult mouse, is one of the first to form, developing soon after the implantation of the blastocyst into the uterine wall and the commencement of gastrulation. In this process, a group of visceral endodermal cells near the distal tip begin to move up one side of the cylindrical embryo to form the prospective anterior, while at the same time a second region of gene expression in the ectoderm proximal to the point of implantation shifts down the opposite side to lay the foundations of the embryo's posterior. This axis rotation is known to require the function of the homeobox gene *Otx2*, which plays such a fundamental role in the formation of the anterior that mice embryos lacking the gene develop entirely without heads. However, the specifics of the genetic mechanisms that underlie axis conversion have not been fully worked out. Chiharu Kimura-Yoshida and members of the Head Organizer project led by Isao Matsuo (Laboratory for Vertebrate Body Plan; Shinichi Aizawa, Group Director), have now found a possible answer to that question. In an article published in the November issue of *Developmental Cell*, Kimura-Yoshida et al. describe the involvement of canonical Wnt signaling and Wnt signaling antagonists in guiding cell migration of the distal visceral endoderm (DVE) during the axis conversion process.

The link between Wnt signaling and axis rotation was suggested by previous reports that the Wnt antagonist *Dickkopf1* (*Dkk1*) was absent from the distal visceral endoderm of *Otx2* null mutants. The canonical Wnt pathway has been implicated in axis specification in frogs as well, an effect mediated by β-catenin, which is stabilized following Wnt activation and enters the nucleus to switch on the expression

Kimura-Yoshida C, et al. Canonical Wnt signaling and its antagonist regulate anterior-posterior axis polarization by guiding cell migration in mouse visceral endoderm. *Dev Cell* 9:639-50 (2005).

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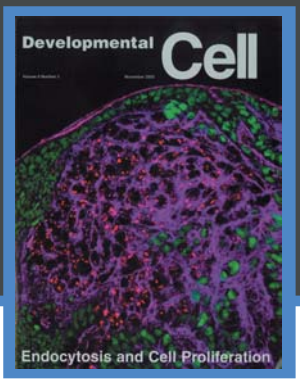
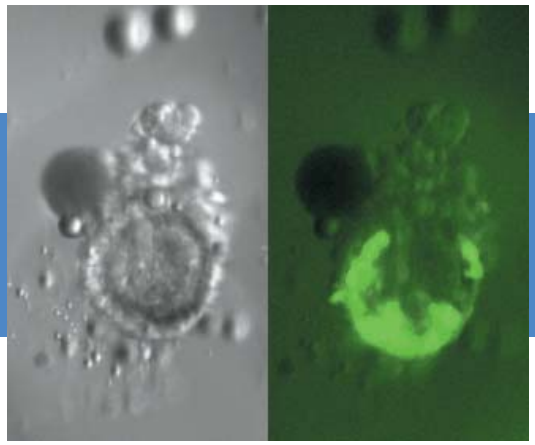


of target genes. Dkk1 acts as a Wnt antagonist by indirectly causing β-catenin to degrade before it reaches the nucleus, thereby preventing it from activating gene expression. Interestingly, Dkk1 is itself a downstream target of *Otx2*.

The group first looked at *Dkk1* expression and found that, following DVE migration, Dkk1 was present in the prospective anterior, but not the posterior, side of the visceral endoderm. They next made conditional knockin mice in which *Dkk1* cDNA was inserted into the *Otx2* gene locus, and found that axis rotation defects were rescued in these animals, even when *Otx2* itself was prevented from functioning. The anterior migration defect was also partially rescued in Otx2$^{-/-}$ mice heterozygous for β-catenin, which is a target of *Dkk1*'s Wnt-antagonistic effect.

Evidence of a role for Wnt inhibition in AP axis specification in hand, Kimura-Yoshida et al. tried misexpressing a canonical Wnt ligand in the epiblast and found that the mutant embryos lost their normal asymmetry in terms of both histology and gene expression patterns, demonstrating that ectopic Wnt signaling leads to failure of axis conversion. Looking more closely at the distribution of β-catenin, the group found that as the axis rotation began, β-catenin expression dropped sharply in the anterior visceral endoderm while it remained high in the prospective posterior. Tests in *Otx2* homozygous mutants showed that this asymmetric distribution in the visceral endoderm is dependent on Otx2 expression; specifically, that *Otx2* is required to reduce β-catenin levels in the anterior visceral endoderm, an effect likely mediated by Dkk1.



When a Dkk1-soaked bead was embedded in the prospective posterior side of the pregastrula mouse embryo, migrating visceral endoderm cells marked by Cerl-GFP altered direction and migrated to the same side of the bead.

The take-home message of this set of findings was that Dkk1 and Wnt signaling might be working at complementary cross-purposes, respectively serving to attract and repel migrating visceral endoderm and thereby steering it to its destination. In vitro studies of GFP-tagged cells showed that the labeled cells did indeed migrate toward Dkk1-soaked beads, while steering well clear of beads soaked in Wnt3a. This effect wasn't limited to the movement of the cells alone; after brief incubation in the presence of Dkk1, the visceral endoderm exhibited the same asymmetric β-catenin distribution seen in the developing embryo.

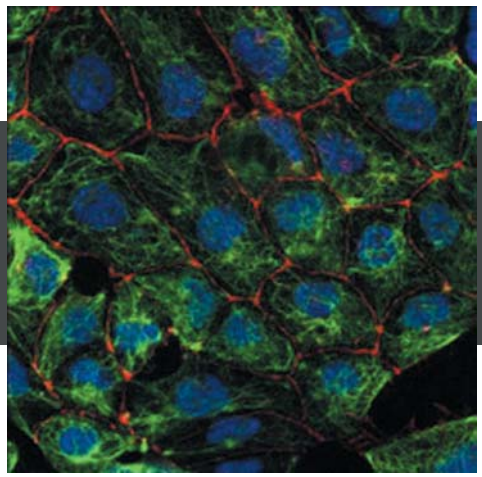
This work, which was conducted in collaboration with labs in Osaka and Sendai (Japan) as well as Oeiras and Faro (Portugal) represents a significant advance in the understanding of how gene expression works to establish one of the body's primary axes, via a mechanism that has been conserved widely throughout metazoan evolution. That the asymmetric allocation of β-catenin works in setting up an axis polarity in the mouse, as has been shown to be true in frogs, fish, chordates and ascidians, highlights yet again that nature is happy to rummage through its toolbox rather than constantly invent anew.

Fulfilling their bipotential

Differentiation of mesendodermal lineages from ES cells

Embryonic stem cells are noted for their pluripotency – the ability to give rise to every type of cell in the body – but it has so far proven challenging to steer them to differentiate into specific lineages in culture. A detailed knowledge of the mechanisms that guide the embryo in vivo can provide valuable clues toward achieving that goal, but again, much remains to be clarified in the understanding of development as well.

At 3.5 days after fertilization, the mouse embryo is a hollow ball, called a blastocyst, made up of an outer layer of trophectodermal cells that go on to form placenta and an inner cell mass (ICM) of pluripotent cells, with a natural ability similar to that of ES cells in vitro to generate all of the body's cells. These ICM cells are initially unspecified, but through a process known as gastrulation they gradually differentiate into the tissues of the three germ layers: ectoderm, mesoderm and endoderm. The development of the endodermal layer is a complicated affair involving a pair of sub-lineages known as visceral endoderm, which contributes to extraembryonic tissues, and definitive endoderm, which give rise to embryonic endodermal cells such as gut, liver, pancreas and lung. The complexity doesn't end there however, for a subset of the definitive endoderm is reckoned to function as mesendoderm, the precursor material that will ultimately give rise to both mesoderm and endoderm as the embryo matures. This ability has been confirmed in a number of species, including the *C. elegans* roundworm and the African clawed frog, *Xenopus laevis*, but mesendodermal bipotentiality remains putative in mouse and other mammals.



Endodermal-derived epithelial cells differentiated from mouse ES cells; Co-expression of E-cadherin (red), Foxa2 (blue), cytokeratin18 (green).

In a series of experiments using mouse ES cells, the Laboratory for Stem Cell Biology (Shin-Ichi Nishikawa; Group Director) demonstrated a means for inducing their differentiation both to visceral endoderm and, via an intermediary mesendodermal step, to definitive endoderm and mesoderm. This work now makes it possible for the first time to track these differentiation events in culture and to steer differentiation toward each of these lineages at high efficiency and to develop purified populations of cells sorted by their expression of surface antibodies. The studies were described in a pair of reports published separately in the October 2005 issue of *Development* and the December 2005 issue of *Nature Biotechnology*.

Looking at patterns of gene expression in the region of the early embryo from which the endoderm derives, they identified *goosecoid* (*gsc*) as a factor likely to be involved in endodermal differentiation and surmised it might have utility as an endodermal marker. The group next knocked-in a version of the gene tagged with a green fluorescent protein and experimented with the cells in vitro until they had a

Tada S, Era T, Furusawa C, Sakurai H, Nishikawa S, Kinoshita M, Nakao K and Chiba T. Characterization of mesendoderm: a diverging point of the definitive endoderm and mesoderm in embryonic stem cell differentiation culture.
Development 132:4363-74 (2005).

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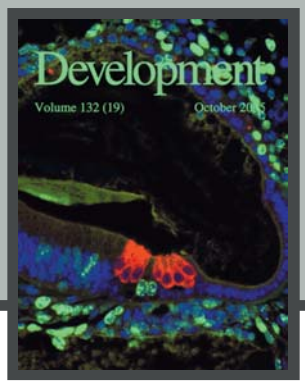

Yasunaga M, Tada S, Torikai-Nishikawa S, Nakano Y, Okada M, Jakt L M, Nishikawa S, Chiba T and Era T. Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells.
Nat Biotechnol 23:1542-50 (2005).

Cover image © Nature Publishing Group 2005

method of inducing differentiation that yielded a population of cells nearly all of which glowed green (signaling their expression of *gsc*). They next screened the gsc$^+$ colonies and determined that cells positive for goosecoid, e-cadherin and PDGFRa (gsc$^+$ECD$^+$aR$^+$) possessed the twofold potency to differentiate into both mesoderm and endoderm characteristic of mesendoderm in other species. Even more intriguingly, this mesendodermal population could be subdivided into gsc$^+$ECD$^-$aR$^+$ and gsc$^+$ECD$^+$aR$^-$ complements, which the group found differentiated into mesoderm and definitive endoderm, respectively. Turning to cells in which the *goosecoid* gene was not fused to express fluorescent protein, they demonstrated that the e-cadherin and PDGFRa and aR did their job equally well in genetically unmodified cells.

Armed with this knowledge, the lab subsequently created a cell line in which the goosecoid locus bore the gene for gfp and huCD25 was introduced into the *sox17* locus (*sox17* is a known marker for extraembryonic visceral and definitive endoderm). This double knock-in enabled them to sort the definitive (gsc$^+$sox17$^+$) from the visceral (gsc$^-$sox17$^+$) fractions as the cells differentiated and, from there, to develop culture media best suited to nudging cells toward either of these lineages. Comparing gene expression in definitive and visceral endoderm, they identified seven surface proteins whose patterns of expression differed between populations. One of these molecules, CXCR4, is expressed specifically in definitive endoderm, meaning that by creating a monoclonal antibody against this protein, Nishikawa's group was able to track differentiation in unadulterated ES cells, and to obtain pure populations of definitive endoderm by cell sorting.

The pluripotency that defines ES cells stands behind their allure as a source for populations of cells that might one day be used in cell replacement therapy. The research presented in these two recent articles first used genetically altered ES cells to study and identify surface markers characteristic of each lineage of interest. This allowed them next to follow the initial differentiative events of the mesendoderm in unmodified ES cells and, most importantly, to successfully purify the resulting populations, a feat that may in the future make it possible to obtain large quantities of clinically useful cell types from endodermal organs such as pancreas and liver. Indeed, by both providing the first clear evidence of a mesodermal stage in mammalian gastrulation and at the same time opening a path toward healthcare strategies of great promise, these studies stand as a testament to the power of bipotentiality.

Takumi ERA, Shin-Ichi NISHIKAWA, Masahiro YASUNAGA

Whence pluripotency?

Interaction between Cdx2 and Oct3/4 sorts out the early embryo

Differentiation, the process by which cells assume sets of properties and specialized functions, is one of the most fundamental mechanisms of development. In the embryo, cells progress from a less differentiated to a more specifically differentiated state, a maturation that generally results in the loss of the differentiated cell's ability to take on other roles. This trade-off between potential and lineage commitment begins very early in the life of the mammalian embryo, as shown by the loss of totipotency (the autonomous ability of a cell to develop into an organism entire) of cells after only a few rounds of division. This first differentiation event results in the segregation of the early, ball-shaped embryo (called a blastocyst) into two distinct tissue types: an outer layer of trophectoderm, which goes on to form placenta, and the inner cell mass (ICM), a population of cells that both serves as the wellspring for every one of the cells in the embryo proper and contributes additional extraembryonic tissue as well. Despite the primary importance of this pivotal event, its molecular underpinnings have remained a tantalizing puzzle to researchers for nearly two decades.

A significant portion of that puzzle has now been fit into place by researchers from the Laboratory for Pluripotent Cell Studies (Hitoshi Niwa; Team Leader), who described the interaction between a pair of factors, Oct3/4 and Cdx2, that sets up the trophectoderm/ICM divide. Working with colleagues from Kobe University, the Japan Science and Technology Agency, and Mount Sinai Hospital (Canada), the study proposed a new model for the genetic basis of the earliest instance of cellular differentiation in the December 2005 issue of the journal *Cell*.

In previous work with embryonic stem (ES) cells, which share many of the properties of cells from the blastocyst interior, Niwa discovered that he could induce the ES cells to differentiate into trophectoderm (something they do not normally do) by repressing the function of the transcription factor, Oct3/4. Other work has further recently identified a second molecule, Cdx2, as intimately involved in trophectoderm formation in the mouse blastocyst. With that finding as a cue, the Niwa team tested the effects of overexpressing Cdx2 in ES cells in vitro and found that this also could induce trophoblast stem (TS) cells under certain culture conditions. On injection back into a blastocyst, Cdx2-induced TS cells that had been lab-

Kadue TAKAHASHI, Rika YAGI, Hitoshi NIWA, Yayoi TOYOOKA, Daisuke SHIMOSATO

Niwa H, Toyooka Y, Shimosato D, Strumpf D, Takahashi K, Yagi R and Rossant J. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* 123:917-29 (2005).

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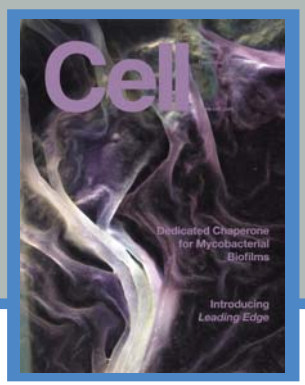
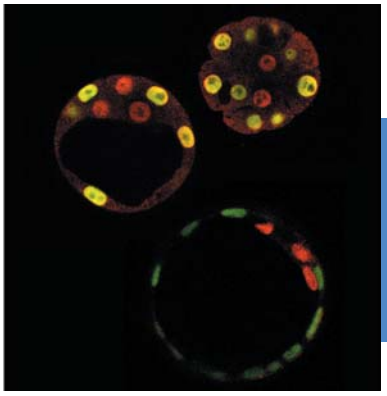


eled with a fluorescent transgene to allow them to be visualized were seen to give rise to placental cell lineages in the chimeric embryos, as evidenced by the green glow from the placenta when exposed to an excitation wavelength of light.

Prompted by their discovery to investigate the possibility of a relationship between Oct3/4 and Cdx2 (specifically, that they might act in a mutually inhibitory fashion), the team transfected ES cells with both molecules and observed the effects on genes normally activated by endogenous Oct3/4. They found that Cdx2 represses Oct3/4's transcriptional activating effects and, interestingly, that this repression depended on Oct3/4; in the absence of Oct3/4, no Cdx2-mediated inhibitory effect was seen on its downstream target genes. Intriguingly, Oct3/4 seems to exert a suppressive influence on Cdx2 as well. Cdx2 is thought to positively regulate its own expression in ES cells, but when these same cells are cultured with an Oct3/4 expression vector, this autoregulation is significantly damped.

This set of findings hinted that Cdx2 and Oct3/4 are locked in a mutually inhibitory relationship. Curious about the mechanics of this interaction, the Niwa team first labeled the two molecules to study their localization within cells and found that when both were expressed together, they relocated to inactive regions of the nucleus. Next, they performed immunoprecipitation analysis (a means of determining whether two molecules bind to each other) and found that Oct3/4 and Cdx2 do indeed co-precipitate, indicating a direct interaction on the molecular level.



Photomicrographs of late morula (top) early blastocyst (middle) and late blastocyst (bottom) stained for localization of Cdx2 (green) and Oct3/4 (red).

The story took on a new twist when Niwa and colleagues looked at ES cells lacking both Cdx2 and Oct3/4 function. Although Cdx2 was thought to be an inducer of trophectoderm, they discovered that, when Oct3/4 function is inhibited, even ES cells with homozygous deletions of Cdx2 can give rise to trophectoderm. The unexpected dispensability of Cdx2 in trophectodermal differentiation led them to inquire just what does Cdx2 actually do. Creating a line of cells in which Cdx2 expression could be controlled conditionally, they compared TS cells derived solely as a result of the down regulation of Oct3/4 with those actively induced by Cdx2 expression, and found that the TS cells lacking Cdx2 were deficient in their ability to self-renew, which is one of the hallmark properties of all stem cells. As it turns out, a second factor, Eomesodermin (Eomeso), is capable of inducing trophectodermal differentiation even in the absence of Cdx2; Eomeso, however, could not entirely compensate for Cdx2 function, and had no repressive effect on Oct3/4.

The Niwa team next observed how cells immunostained for Cdx2 and Oct3/4 behave in morula and blastocyst-stage embryos. In the early morula, both factors were detected in all cells' nuclei, but by the later 10-16 cell stage Cdx2 could only be found in some of the outermost cells (Oct3/4 expression remained pervasive). By the blastocyst stage, the segregation was complete with Cdx2 limited to the outer layer and Oct3/4 restricted to the interior. These findings present the strong possibility that the loss of Cdx2 expression in the inner cells of the morula may be the trigger for the territorial sorting of the Cdx2-expressing trophectoderm from the Oct3/4-expressing inner cell mass. Niwa suggests that this process may be the result of the mutual repression of the two molecules, but whether the dynamics of this sorting out are simply stochastic, or actively determined by cell polarity or size is yet to be determined. Other questions, such as whether this reciprocal inhibition is both necessary and sufficient to drive Cdx2 out to the morula periphery and the mechanism by which Oct3/4 maintains pluripotency in ES cells, also await answers. Answers that no doubt will stand on the solid foundations laid by this landmark series of experiments.

Aft agley

A prescription for reprogramming errors in nuclear transfer

Mammalian cloning remains a young field whose first fruits, such as Dolly the sheep and Cumulina the cloned mouse, are still less than a decade old. When Teruhiko Wakayama (Team Leader; Laboratory for Genomic Reprogramming) and colleagues at his former lab under Ryuzo Yanagimachi in the University of Hawaii successfully created the first cloned mouse by transferring the nucleus of an ovarian cumulus cell to an unfertilized egg, it was heralded as a major achievement in the face of the low efficiencies that dog the procedure even to this day. Since that time, researchers have tried altering the timing of the nuclear transfer (NT), tested an array of methods to activate the oocyte into receptivity, and experimented with a whole range of differentiated cell types as nuclear donors, but to little effect; the success rate of mouse cloning attempts to produce live offspring has languished at only about 2%.

A straightforward new tweak developed by the Wakayama lab, published in the 9 December 2005 edition of *Biochemical and Biophysical Research Communications*, presented the first significant increase in mouse cloning efficiency in recent years. This step forward was achieved by researchers who treated the NT zygotes with the histone deacetylase inhibitor, trichostatin A (TSA), thereby boosting efficiency to an unprecedented 6%.

The reasons for the difficulties in the cloning of mammals have been proposed to stem back to incomplete or incorrect reprogramming of the nucleus following transfer into the oocyte. The chromosomes of differentiated cells carry molecular markers that specify which genes are to be expressed and which shut down, helping to specify the patterns of gene expression, and so, the form and function of such cells. In natural fertilization, the information sets contained with nuclei of the sperm and egg are reprogrammed as the two cells fuse, enabling the ontogeny of a unique new individual. But when a differentiated nucleus is introduced directly into an oocyte as is done in cloning attempts, this reprogramming frequently appears to go awry. The molecular signatures involved are written in a script of methyl groups that adorn genes directly and the acetylation of histone complexes that package the DNA into tightly coiled bundles, but it is a mystery how the oocyte opens up this intricate code for revision.

Satoshi KISHIGAMI, Teruhiko WAKAYAMA

Kishigami S, Mizutani E, Ohta H, Hikichi T, Thuan N V, Wakayama S, Bui H T and Wakayama T. Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. *Biochem Biophys Res Commun* 340:183-9 (2006).

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Research scientist Satoshi Kishigami and Wakayama had previously observed hypermethylation of DNA following the transfer of immature round spermatids into oocytes, later finding that treatment of the oocytes with TSA prior to transfer eliminated this abnormality. This led them to conjecture that TSA might act to quell hypermethylation and improve the ability of transferred nuclei to be reprogrammed correctly.

Working first with cumulus donor cells, they experimented with different timing and dosages of TSA treatment and found that exposure to 5nM of TSA for 10 hours following nuclear transfer improved development to the blastocyst stage (a yardstick commonly used to measure NT success) to 75%, as compared to 20% in the control group, a result that also suggests that hypermethylation takes place in the first few hours following the activation of the recipient egg. Additional experiments using nuclei from tail-tip fibroblasts and spleen lymphocytes met with similarly improved success rates.

Cloned blastocysts treated with trichostatin A.

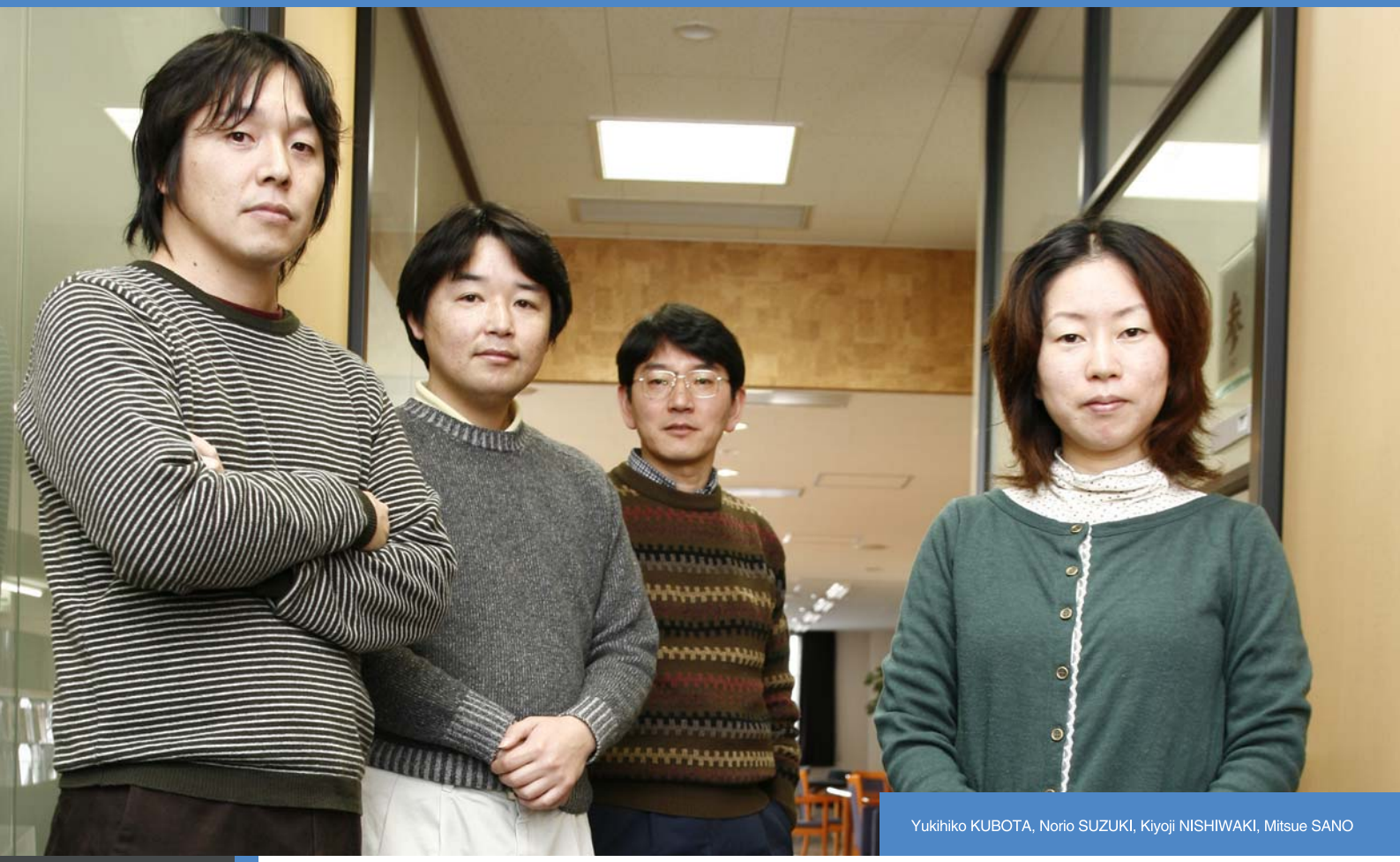
The positive effects of TSA treatment translated into higher live births as well, with 6% of all attempts leading to the birth of pups (against only about 1% for control). These animals were found to be healthy and normal in all respects, save for the enlarged placenta that typifies all cloned mice, and importantly, do not exhibit the obesity and shortened life expectancy seen in some other mouse clones in the past. Interestingly, when the donor nucleus was taken from an ES rather than a somatic cell, TSA had the opposite effect, and no clones developed to term. ES cells are in themselves excellent nuclear donors, as their DNA methylation is naturally low; unmodified cloning by ES cell nuclear transfer generally sees 2~6% success in producing live pups. The picture presented by these findings is that methylation may be a determinant of success that is highly dosage-sensitive.

TSA appears to be a positive addition to the technique for establishing ntES cell lines, which are embryonic stem cell lines derived from blastocysts created by nuclear transfer. TSA treatment increased efficiency by two or threefold, and the ntES cells so created were found to express all of the molecular markers characteristic of ES cells, including Oct3/4 and Nanog.

Cloning from somatic cells and the derivation of ES cells from nuclear transfer embryos represent extremely promising technologies, with applications ranging from the preservation of endangered species to the generation of cell populations for clinical use free of the risk of immune rejection. For those reasons alone, this advance in the efficiency of these procedures is of unmistakable impact. The underlying evidence suggesting a critical role for histone deacetylase inhibitors in reprogramming is also intriguing for the fundamental biological insights it provides. A more detailed examination of the precise means by which TSA augments the mechanisms of reprogramming awaits.

COG-driven machinery

Golgi complex function helps steer cell migration during *C. elegans* gonadogenesis



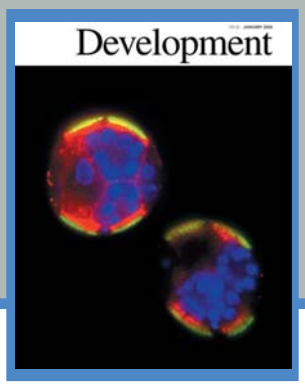
Yukihiko KUBOTA, Norio SUZUKI, Kiyoji NISHIWAKI, Mitsue SANO

The ability of cells to migrate, both individually and in coordinated groups, is a fundamental requirement for organogenesis. Migration takes place as the outcome of a collection of processes, from signaling by guidance molecules to rearrangements of the extracellular matrix and cytoskeleton, all of which must be meticulously choreographed and executed in order for cells to reach their appropriate destinations. The roundworm, *C. elegans*, provides a useful system for studying the dynamics of cell migration in its gonadal development, in which distal tip cells (DTCs) at the leading edges of two migrating arms wend their way through the larval body following contralateral U-shaped trajectories. Several labs have focused on how the developing roundworm is able to ensure that these gonadal pathfinder cells stay on course along these stereotyped routes, but the molecular mechanics have yet to be fully worked out.

In a report published in an advance online issue of *Development* in December 2005, Yukihiko Kubota, a research scientist, and colleagues in the Laboratory for Cell Migration (Kiyoji Nishiwaki; Team Leader) describe the involvement of the COG (Conserved Oligomeric Golgi) complex in DTC migration and its interaction with previously identified guidance molecules belonging to the ADAM family of proteases.

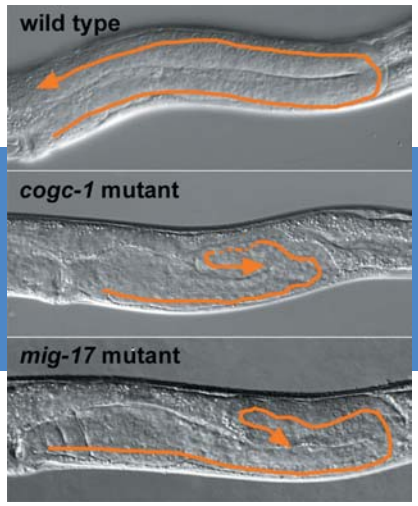
The Nishiwaki lab had previously shown that the ADAM protease MIG-17 is secreted from the body wall musculature in larvae and localized at the gonadal basement membrane, where it promotes the migration of distal tip cells. A pair of other genes, *mig-29* and *mig-30*, identified in the same mutation screen that

Kubota Y, Sano M, Goda S, Suzuki N and Nishiwaki K. The conserved oligomeric Golgi complex acts in organ morphogenesis via glycosylation of an ADAM protease in C. elegans.
Development 133:263-73 (2006).



yielded *mig-17* were found to code for homologs of components of the COG complex, which functions in many aspects of vesicle trafficking within the cell and has been identified in species from yeast to human. The genes were renamed *cogc-3* and *cogc-1* to reflect this homology, and Kubota and colleagues set out to analyze their role in DTC migration.

In other species, the COG complex comprises eight protein constituents structured as a pair of lobes, which was shown to be true in *C. elegans* as well. The Nishiwaki team then demonstrated the functional importance of each of the COG components by showing that interference by RNAi with the function of any of the eight COGs produced defective DTC migration or malformed gonad phenotypes, an effect that was found to be stronger for lobe A COGs (which includes the proteins encoded by both *cogc-1* and *cogc-3*) than for those in lobe B.



U-shaped migratory pathway of wildtype gonad (top) is disturbed in *cogc-1* and *mig-17* mutants, as shown by the meandering phenotype (route marked in orange).

This led Kubota to the possibility that the COG complex might interact with other factors known to be involved in DTC migration, specifically to MIG-23, a Golgi membrane protein the lab's previous work had identified as a regulator of MIG-17 via the addition of sugar residues, a process known as glycosylation. COGC-3 was observed to co-localize with MIG-23 in muscle cells of the body wall, and when the team looked at *cogc-3* and *cogc-1* mutants, they found that MIG-23 was destabilized and decreased in quantity. Following the evidence trail, they looked next to MIG-17 glycosylation in these mutants and again found that its glycosylation was incomplete, resulting in its failure to accumulate at the gonadal basement membrane.

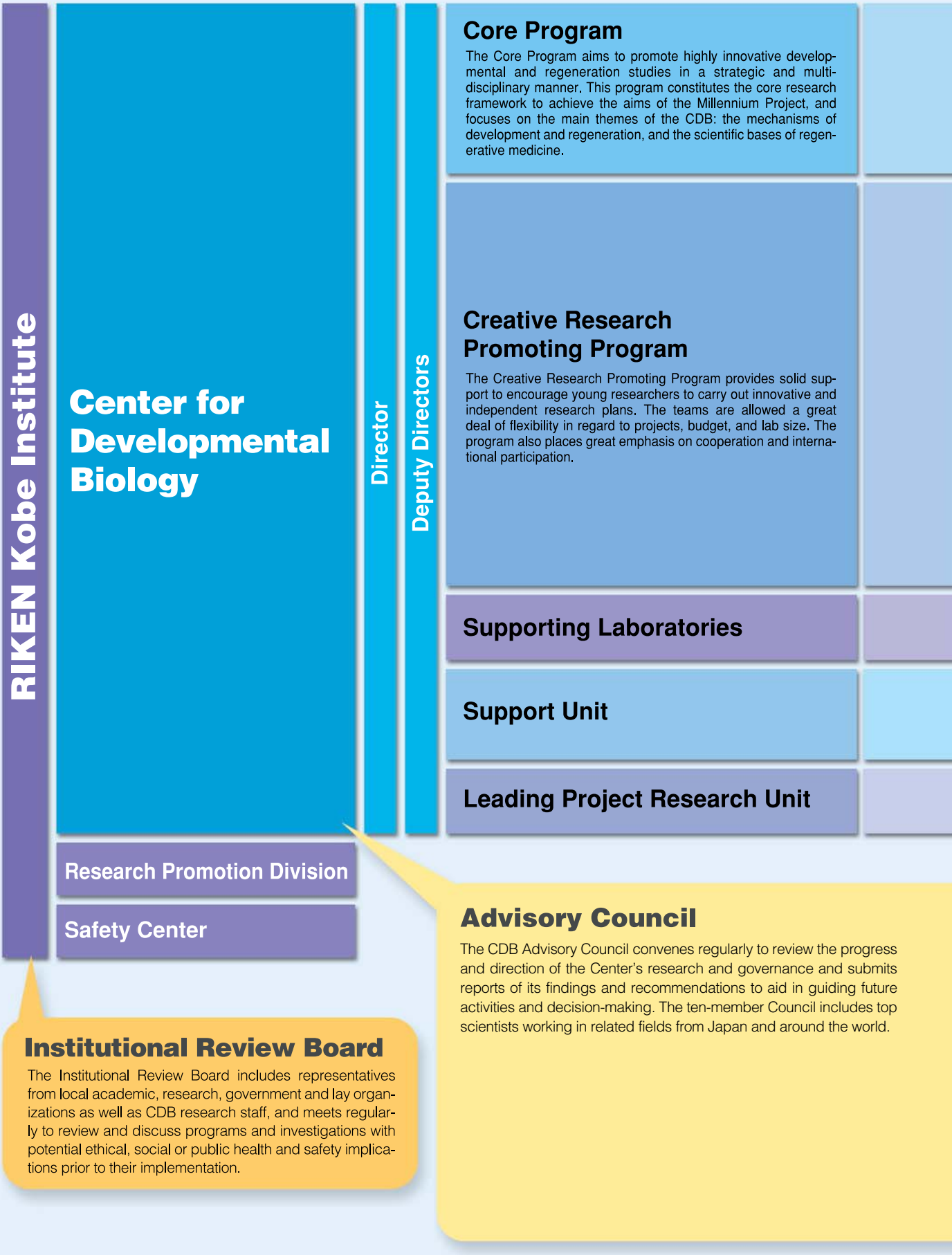
The body of findings from these studies prompted the Nishiwaki lab to develop a picture of the molecular mechanisms underlying DTC guidance in which the COG complex functions to stabilize MIG-23, thereby ensuring the glycosylation of MIG-17 in muscle cells, which then secrete it to remodel the adjacent basement membrane and so steer the distal tip cells on their parabolic pathways. Their work provides the first evidence of the function of the COG complex in an organ-forming process, which is of some biomedical interest as humans carrying a mutation for another COG component, COG-7, develop a glycosylation disorder characterized by multiple developmental defects. Here, as in many aspects of development, what is true in the worm may one day prove to be equally important in man.

October-December Seminars

date	title	speaker
2005-10-03	Maternal effects and the BMPs of vertebrate development	Mary MULLINS
2005-10-03	Motor axon guidance and motor behavior regulation in the zebrafish	Michael GRANATO
2005-10-05	What causes a nucleus to position itself at the cell center? Computer simulations and image processsing analyses in *C. elegans* embryos	Akatsuki KIMURA
2005-10-12	Global molecular mechanisms and evolution of early embryogenesis in *C. elegans* and its relatives	Fabio PIANO
2005-10-12	Annotating the phenome: Data mining and data integration	Kristin C. GUNSALUS
2005-10-20	Control of dynamic contractile arrays by local zones of active rho class GTPases	William M. BEMENT
2005-10-20	Dynamics of cell-matrix interactions	Kenneth M. YAMADA
2005-10-20	Regulation of actin dynamics during epithelial intercalation	Thomas LECUIT
2005-10-21	Oct-3/4 is a key player in early normal development and germ cell neoplasia	Yehudit BERGMAN
2005-10-25	Using proteomics and functional genomics to understand cytokinesis	Ahna R. SKOP
2005-10-27	Changes in the developmental system which have contributed to the evolution of beetle wings	Yoshinori TOMOYASU
2005-11-08	Detection and processing of color information in *Drosophila*	Claude DESPLAN
2005-11-11	Genetic regulatory circuits in development and disease: Tales of the Wnt-Axin network	Wei HSU
2005-11-14	Maternal and zygotic processes of cell fate specification during ascidian embryogenesis	Hiroki NISHIDA
2005-11-14	Reprogramming of a differentiated genome into a pluripotent genome	Jose SILVA
2005-11-18	Asymmetric cell division and neuroblast self-renewal in *Drosophila*	Chris Q. DOE
2005-11-22	Dynamic view of early mammalian development	Takashi HIIRAGI
2005-11-24	Genetic basis of cell polarity-insights from the retina	Jarema MALICKI
2005-12-01	Pax3 and Pax7 function in myogenic progenitor cells: From the embryo to the adult	Frederic RELAIX
2005-12-02	Differential segregation of mother and daughter centrosomes during asymmetric stem cell division in the *Drosophila* male germ line	Yukiko M. YAMASHITA
2005-12-06	Dopaminergic neuron differentiation from human embryonic stem cells	Mahendra RAO
2005-12-12	Neurogenesis and boundary formation in hindbrain segmentation	David WILKINSON
2005-12-13	From lens induction to functional genomics in *Xenopus*	Hajime OGINO
2005-12-14	Ear or skin? Wnt signaling mediates a placode-epidermis fate decision	Takahiro OHYAMA

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

RIKEN Kobe Institute



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
Mitinori SAITOU M.D., Ph.D.

Embryonic Induction
Hiroshi SASAKI Ph.D.

Cell Fate Decision
Hitoshi SAWA Ph.D.

Early Embryogenesis
Guojun SHENG Ph.D.

Developmental Genomics
Asako SUGIMOTO Ph.D.

Body Patterning
Yoshiko TAKAHASHI 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.

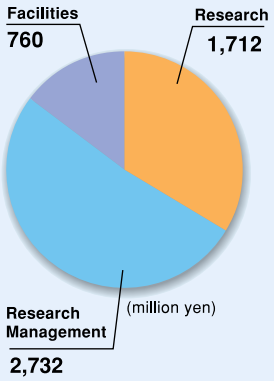
Genome Resource and Analysis Subunit Hiroshi TARUI Ph.D.

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

Research Unit for Cell Plasticity Mitsuko KOSAKA Ph.D.

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

2005 CDB Budget



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

2005 CDB Staff

Laboratory heads	31
Research scientists	122
Research associates	7
Technical staff	119
Assistants	27
Visiting Scientists	50
Student trainees	61
Part-time staff	33
Research Promotion Division	41
Other	48
Total	**539**

Academic Affiliations and Programs

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 itself 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:

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

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

Kwansei Gakuin University,
Bioscience Department

Osaka University, Graduate School of Science

Intensive Summer Lecture Program

Beginning in 2004, the CDB has held summer intensive lecture programs, in which students at its graduate school affiliates are invited to attend lectures on the latest in developmental biology and regeneration research, meet with research staff and tour the facilities. Students are eligible to earn course credits for their participation. These intensive lectures are also publicized over the CDB website and are open to participants other than students from graduate school affiliates as well. The 2005 program was a two-day event attended by more than 125 graduate students, postdocs and scientists from industry.

Focus on the future

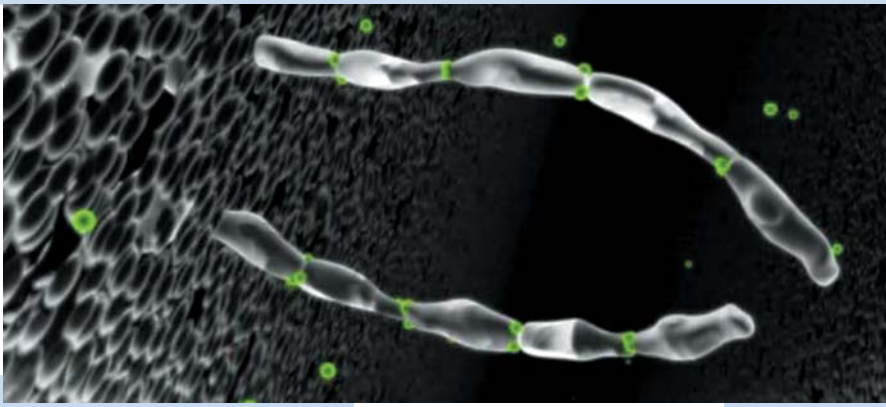
One of the core targets for our communications program is school-age children. The CDB has developed a software-based introduction to the Center in the form of a virtual lab tour, and a popular set of game cards featuring brief introductions to developmental biology themes, such as *Drosophila* mutants, stages of mammalian embryogenesis and various types of differentiated cells.

Science Communications and Outreach

Science does not take place in a vacuum, and the CDB has dedicated itself to engaging with the general public and the global scientific community through a diverse range of communications activities designed to enhance the awareness and understanding of the Center's research and the fields of development and regeneration in general.

Open House

This annual event features simple and fun explanations of topics in development, regeneration and regenerative medicine, from embryo-genesis and stem cells to model organisms used in research. All exhibits and demonstrations are staffed by scientists who explain their work and laboratory techniques to visitors of all ages and walks of life.



Multimedia

The SCIA made its first foray into scientific computer graphics in 2005 by co-producing an animation of cadherin structure and function in epithelial cells and the nervous system. The full animation can be downloaded from the CDB website or the CD-ROM version of this annual report, and is made freely available to the scientific community for presentations and classroom use.

Mock laboratory

The CDB enjoys high visibility and media coverage for a basic research center in Japan, and hosts more than 100 site visits every year. The Center maintains a permanent onsite exhibition gallery and mock laboratory equipped with actual lab equipment, including incubators, PCR tools, fly and worm stocks and optical and fluorescence microscopes that can be used for hands-on demonstrations and teaching exercises.

International Activities

Intraregional and International Meetings

The past year saw a number of labs within the CDB build and expand on the success of previous years' activities in organizing and hosting a range of regional and international meetings. With the fields of development biology and regenerative medicine attracting great interest in recent years, the CDB continues to increase the number of opportunities for researchers to meet and discuss their latest findings and exchange information. As well as continuing to be a hub of the rapidly expanding life sciences community in the Asia-Pacific region, 2005 also saw the CDB host a number of meetings and seminars with scientists from around the world, convened both within the Center and in other locations throughout the Kansai region of Japan.

Taiwan-Japan Bi-Lateral Symposium: Cellular and Developmental Biology
(January 13-14)

Organizers: Shigeo Hayashi (Laboratory for Morphogenetic Signaling) and Cheng-ting Chien (Academia Sinica)

This two-day meeting brought together researchers from the CDB and Taiwan active in the field of cell biology. The program provided an opportunity for leading researchers in this area to meet and discuss a range of topics concerning developmental processes at the cellular level.

Taiwan-Japan Bi-Lateral Symposium: Cellular and Developmental Biology

CDB Meeting: Diversity of Developmental Mechanisms in Invertebrates
(February 2-3)

Organizers: Shigeo Hayashi (Laboratory for Morphogenetic Signaling) and Sumihare Noji (University of Tokushima)

This conference focused on the diversity of development and pattern formation processes in arthropods and other invertebrates. With the participation of a number of distinguished researchers in the field, the meeting offered attendees the opportunity to grasp an overview of recent trends and research developments.

CDB Meeting: Diversity of Developmental Mechanisms in Invertebrates

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 take place primarily among individual researchers and laboratories. In 2005 the Center concluded a memorandum of understanding with the GSF National Research Center for Environment and Health, based in Neuherberg, Germany, bringing the total number of such international affiliations to six. 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

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

TEMASEK LIFE SCIENCES LABORATORY

Temasek Life Sciences Laboratory Limited, Singapore

Stem Cells in Reproduction and in the Brain (September 1-3)

Organizers: Shin-Ichi Nishikawa (Laboratory for Stem Cell Biology), John Morser (Nihon Schering K.K Research Center), Hans R. Schöler (Max Planck Institute for Molecular Biomedicine)

In conjunction with the Ernst Schering Research Foundation and Max Planck Society, CDB scientists organized a three-day meeting on the promises and challenges of regenerative medicine. Bringing together stem cell researchers from around the world, this workshop focused on the science underlying stem cell biology and regenerative processes, and the potential for their application in health problems from heart disease to cancer.

Stem Cells in Reproduction and in the Brain

Asian Reproductive Biotechnology Conference
(November 3-7)

Organizers: Nguyen Van Thuan (Laboratory for Genomic Reprogramming) and Rangsun Parnpai (Suranaree University of Technology)

Involving over 200 participants from across Asia, the second Asian Reproductive Biotechnology Conference was co-hosted by the CDB in Bangkok, Thailand. The three-day program sought to improve understanding of reproductive biotechnology in addition to strengthening co-operation between Asian scientists.

Asian Reproductive Biotechnology Conference

NAIST-CDB International Symposium: Frontiers in Development Biology
(December 1-2)

Organizer: Yoshiko Takahashi (Laboratory for Body Patterning; Nara Institute of Science and Technology)

Co-hosted by the CDB and the Nara Institute of Science and Technology, this symposium, held in the nearby city of Nara, featured pioneering talks on subjects ranging from neural development to somitogenesis.

NAIST-CDB International Symposium: Frontiers in Development Biology

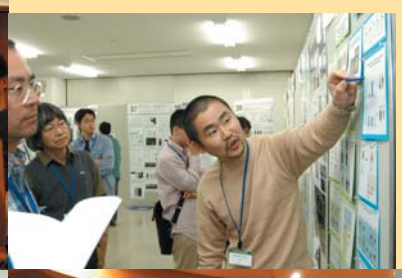
National Centre for Biological Sciences, TIFR, Bangalore, India

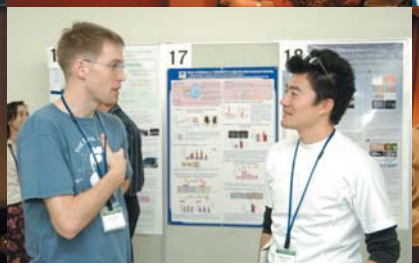
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

McGill University Faculty of Science, Montreal, Canada

National Research Center for Environment and Health, Neuherberg, Germany

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





2005 CDB Symposium

Origin and Development of the Vertebrate Traits

April 11 - 13 , 2005

The CDB's third annual symposium was held from April 11 to 13, 2005 in the Center's auditorium. An annual event aimed at promoting the free and timely exchange of ideas, this year's symposium focused on the origin and development of traits specific to vertebrate organisms. Approximately 150 research scientists came together to discuss and exchange views on a range of evolutionary topics in the field of evolutionary development, from bioinformatics studies of ascidians to the paleontology of fishes, in 25 oral presentations and additional poster sessions.

Session 1

Richard A. SCHNEIDER
(University of California at San Francisco, USA)
Shigeru KURATANI (RIKEN CDB, Japan)

Session 2

Michael J. DEPEW (King's College London, UK)
Martin SCAAL (University of Freiburg, Germany)

Session 3

Filippo M. RIJLI
(Institut de Génétique et de
Biologie Moléculaire et Cellulaire, France)
Per E. AHLBERG (Uppsala University, UK)
Ann Campbell BURKE (Wesleyan University, USA)

Session 4

Sayuri YONEI-TAMURA and Koji TAMURA
(Tohoku University, Japan)
Cheryll TICKLE (University of Dundee, USA)

Session 5

Hiroshi WADA (University of Tsukuba, Japan)
Patrick LEMAIRE (LGPD/IBDM, France)

Session 6

Marianne Bronner-FRASER
(California Institute of Technology, USA)
Thurston LACALLI (University of Victoria, Canada)

Session 7

Jo BEGBIE (University of Oxford, UK)
Yasunori MURAKAMI
(Institut de Génétique et de
Biologie Moléculaire et Cellulaire, France)
Clare V. H. BAKER (University of Cambridge, UK)

Session 8

Peter W. H. HOLLAND (University of Oxford, UK)
James HANKEN (Harvard University, USA)

Session 9

Nori SATOH (Kyoto University, Japan)
H. Joseph YOST (University of Utah, USA)

Session 10

Shin AIZAWA (RIKEN CDB, Japan)
Scott E. FRASER (Caltech, USA)

Session 11

Christine THISSE (IGBMC, France)
Yoshiko TAKAHASHI (RIKEN CDB, Japan)
Philip W. INGHAM (University of Sheffield, UK)

2006 CDB Symposium

Logic of Development: New Strategies and Concepts

April 10-12, 2006

The fourth annual CDB symposium will be centered on further understanding the fundamental basis of development through the logical use of novel biological phenomena such as functional genomics and molecular imaging technology. Particular attention will be paid to both novel biological phenomena, such as micro RNAs and the genetic bases of morphological variation, as well as novel strategies for systems level understanding of biological processes, including systems biology and functional genomics. Throughout the three days of the symposium it is hoped that the diverse range of presentations and caliber of participants will contribute to the further understanding of the fundamental issues underlying development through biological systems.

Victor AMBROS (Dartmouth Med. Sch., USA)
René KETTING (Hubrecht Lab., Netherlands)
Eric Alexander MISKA
(The Univ. of Cambridge/ The Wellcome Trust/CR UK Gurdon Institute, UK)
Detlef WEIGEL (Max-Planck Inst., Germany)
Tadashi UEMURA (Kyoto Univ., Japan)
Yasushi OKADA (The Univ. of Tokyo, Japan)
Hirokazu TSUKAYA (The Univ. of Tokyo, NIBB, Japan)
Shigeru KONDO (Nagoya Univ., Japan)
Atsushi MIYAWAKI (RIKEN BSI, Japan)
Shuichi ONAMI (Keio Univ., Japan)
Ron WEISS (Princeton Univ., USA)
Hiroki R. UEDA (RIKEN CDB, Japan)
Pernille RØRTH (EMBL, Germany)
Emmanuel FARGE (Inst. Curie, France)
Detlev ARENDT (EMBL, Germany)
Kiyokazu AGATA (Kyoto Univ., Japan)
Ralf J. SOMMER (Max-Planck Inst., Germany)
Haruhiko SIOMI (The Univ. of Tokushima, Japan)
Stephen COHEN (EMBL, Germany)
John B. HOGENESCH (The Scripps Research Inst., USA)
Norbert PERRIMON (Harvard Med. Sch., USA)
Asako SUGIMOTO (RKEN CDB, Japan)
Nicolas Bertin (Dana-Farber Cancer Inst., USA)
Duncan DAVIDSON (MRC, UK)

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RIKEN Activities

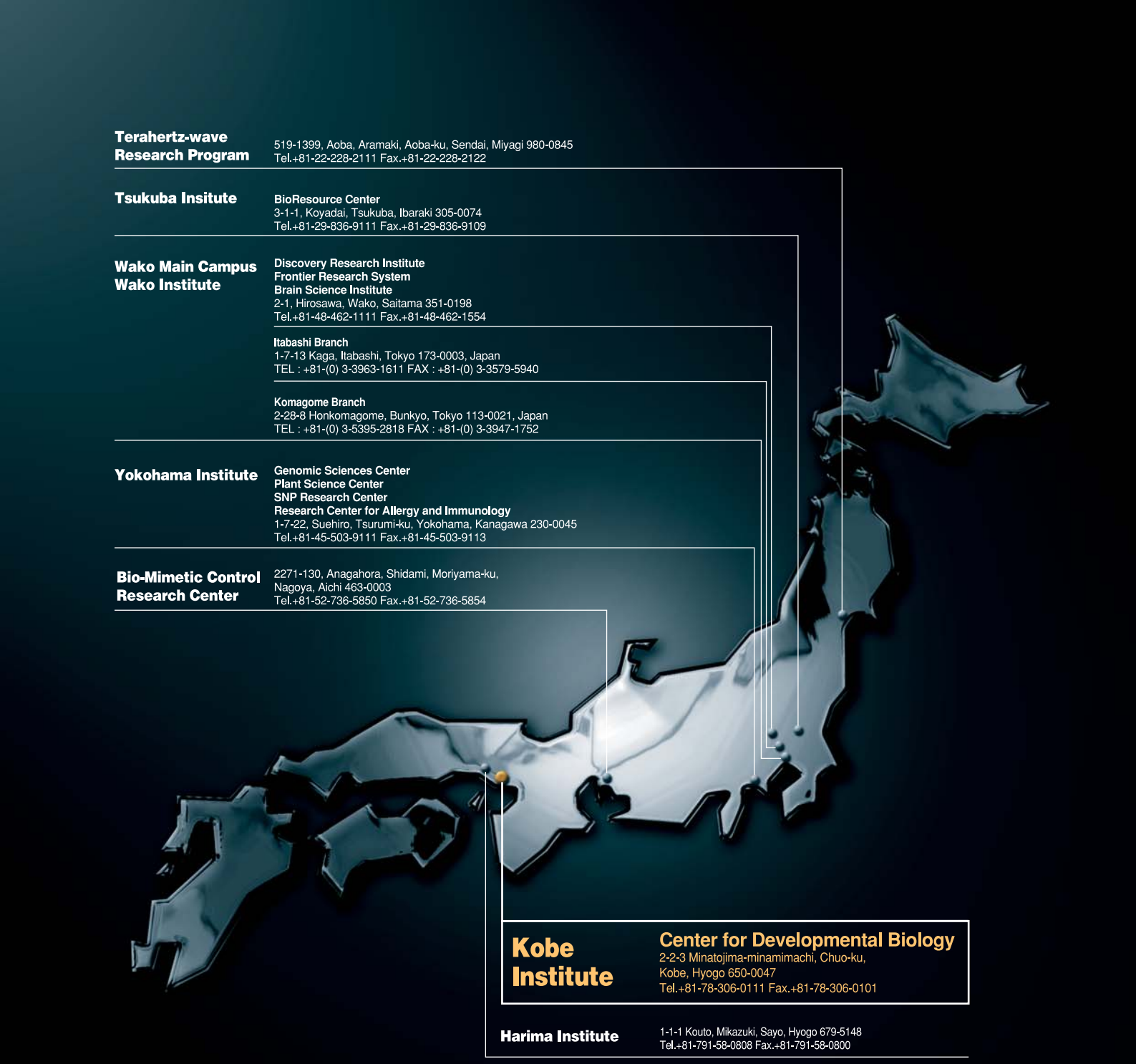
RIKEN is one of Japan's largest publicly-funded scientific research organizations, comprising a number of institutes and centers throughout the country. Seeking to capitalize on the strengths of the diversity of its research programs – which span physics, chemistry and the life sciences – RIKEN strongly 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.

RIKEN Joint Retreat

On May 9 and 10, 2005, RIKEN instituted its first joint retreat, with invited talks and posters by scientists working in four research centers located throughout Japan. The retreat, which was held in the spa town of Atagawa on the Izu peninsula, provided an environment in which researchers from diverse fields could share their findings with RIKEN scientists from other fields of specialty. This first joint retreat focused primarily on life sciences research, with participants from the Brain Science Institute (BSI) and Discovery Research Institute (DRI) in Wako, the Research Center for Allergy and Immunology (RCAI) in Yokohama and the CDB.

Intra-RIKEN Collaborations

CDB laboratories participate in a number of collaborative projects involving multiple RIKEN laboratories. These projects aim to take advantage of the excellence, breadth and diversity of RIKEN's nationwide network of laboratories to achieve synergies and new insights through interdisciplinary collaboration.



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RIKEN

RIKEN is a publicly funded research organization established to conduct comprehensive research in science and technology and to disseminate the results of its scientific research and technological developments. RIKEN carries out basic and applied research in a wide range of fields, including physics, chemistry, medical science, biology, and engineering.

RIKEN was first founded in 1917 as a private research foundation, *Rikagaku Kenkyuusho* (The Institute of Physical and Chemical Research). In 2003, the Institute was reorganized as an Independent Administrative Institution under the Ministry of Education, Culture, Sports, Science and Technology (MEXT), since which time it has engaged in wide-ranging research activities spanning the basic and applied sciences.

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On The Cover
Neural outgrowth as revealed by a neuronal-specific antibody occurs efficiently in the isolated nasal placode ectoderm taken from a 3-day old chick embryo

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