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An inward eye on the visual cortex

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Brain change

Cutting-edge imaging technology shows that monkey's brains grow as they learn to use tools

Many scientists once believed that the human brain doesn't change significantly after a person reaches maturity. This opinion has been overturned in the last few decades as studies have revealed that the brain has a very flexible structure, even in adulthood, and changes considerably according to use.

Now, Atsushi Iriki at the RIKEN Brain Science Institute (BSI) in Wako and colleagues have directly observed changes in the brain structure of macaque monkeys, while the monkeys were being taught to use tools[1]. The study, performed using a non-invasive imaging technique, is the first to reveal significant changes in the brain of an individual animal, and could provide insight into the evolution of human intelligence.

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Figure 1: Macaque monkeys rarely use tools in the wild, but they can be taught, and it appears that their brains change in response to these new tasks.

Growth through learning

Several research groups have measured changes in human brain structure by analyzing the brains of experts in a particular field and comparing them to non-experts. This work has revealed, for example, that London taxi drivers, who have to remember a huge network of streets, display enhanced growth in the hippocampus region associated with spatial memory, and expert musicians have larger auditory and motor cortices thanks to their years of practice.

Iriki was particularly inspired by a study conducted by German scientists in 2004, in which human volunteers were trained to juggle over a three-month period. After the training, the volunteers showed increased gray matter in regions of the brain associated with motor skills[2]. Iriki was keen to discover whether these effects could be observed in macaque monkeys (Fig. 1).

"In our previous work, we found that neurons in the intraparietal cortex of monkeys trained to use tools change their receptive field properties to represent tools as an extension of the body parts holding them," he explains. Iriki and his colleagues observed gene expression in the same areas, and the growth of new axons and synapses. This suggests that tool-use training might induce rapid structural changes in the brain, at least on a microscopic scale.

"So, when I read the paper describing expansion of cortical gray matter in jugglers, I decided to do a similar study in monkeys," says Iriki.

Scanning in stages

Macaque monkeys rarely use tools in the wild, but can master basic tools after a few weeks training. To gain insight into this learning process the researchers used magnetic resonance imaging (MRI) to examine the brains of three monkeys before, during, and after training them to use a rake to retrieve food that was just out of reach.

The researchers used a technique called voxel-based morphometry (VBM) to classify areas of brain tissue in their MRI images as grey matter, white matter, or cerebro-spinal fluid, and to compare the volume of each tissue at different stages of learning. The work was possible thanks to a fruitful collaboration between RIKEN BSI and the Institute of Neurology at University College London (UCL).

"Marsha Maria Quallo, a PhD student from UCL, came to BSI to train the monkeys and analyze behavioral data, during which time she acquired structural MRI images using our high resolution scanner," explains Iriki. "She took the MRI data back to London and analyzed them under supervision of VBM experts there."

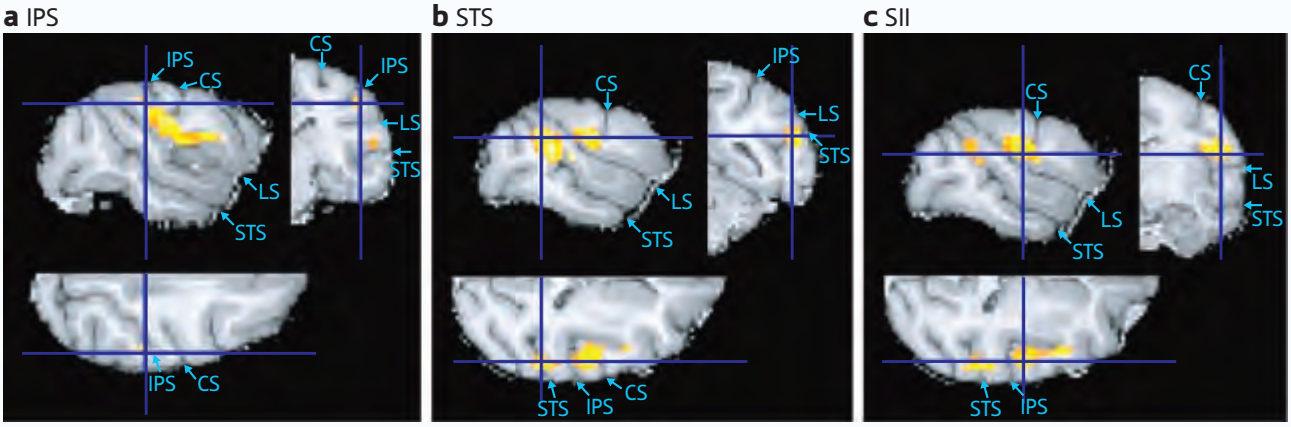


Figure 2: MRI images of the brain showing the areas around the (a) intraperietal sulcus (IPS), (b) superior temporal sulcus (STS) (c) and the secondary somatosensory area (SII) in which gray matter increased as monkeys learned to use a rake tool to retrieve food (CS, central sulcus; IPS, intraprietal sulcus; LS, lateral sulcus).

The analysis showed that the MRI signal from some areas of gray matter increased, suggesting their volume in the monkey's brains increased as the monkeys got better at using the tool. The growth was mainly in areas around the superior temporal sulcus (STS), intraparietal sulcus (IPS), and the secondary somatosensory area (SII) which all belong to a network previously associated with tool use (Fig. 2). The researchers also noticed an increase in signals, suggesting volume expansion, from white matter in the cerebellum, which is well known as having a role in motor control.

The benefit of monkey models

The study is important because it is the first to detect statistically significant brain structure changes in individual animals, compared to human studies that pooled data from several people.

"Although there have been some human VBM studies suggesting gray matter expansions in experts, they could only be detected in group analyses by comparing between groups of experts and non-experts," Iriki explains. "In contrast, we detected large changes in specific brain regions by using animals that were naïve to the task, and showed for the first time that these can be detected in individual animals."

For example, the juggling study reported only a 3% increase in signals averaged across twelve volunteers, whereas the individual macaques in Iriki's study showed up to 17% signal increase in some areas. The changes may be larger in the monkeys because they had never used tools, unlike humans who would have already performed many skilled motor tasks. This illustrates that monkeys are an ideal model for studying such brain changes.

Looking deeper

Iriki and colleagues now hope to discover exactly how different brain areas increase in volume when learning a task, on a cellular, genetic and molecular basis.

"Our study opens up the means to study concrete neurobiological mechanisms underlying gray matter expansion, which we have actually already started," says Iriki. "We are particularly keen, in collaboration with other groups in Japan, on using marmosets—smaller primates in which transgenic techniques are available."

Most interestingly, Iriki points out that the brain areas highlighted in his study correspond closely to the cortical areas that expanded most while primates were evolving into humans.

"I would hope this could give us some clues to understanding human intellectual evolution," he says. ■

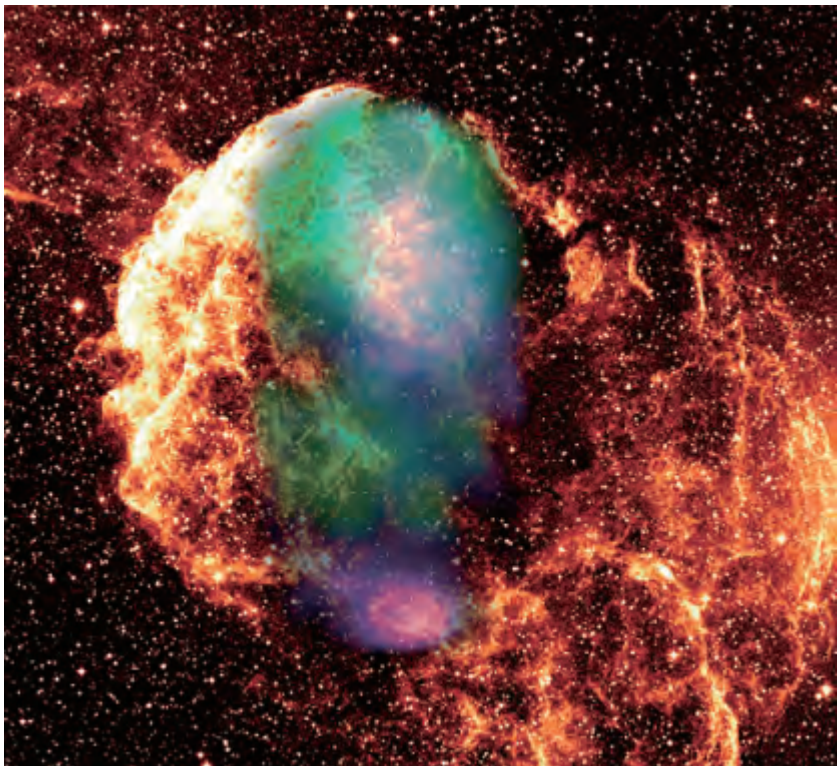
1. Quallo, M.M., Price, C.J., Ueno, K., Asamizuya, T., Cheng, K., Lemon, R.N. & Iriki, A. Gray and white matter changes associated with tool-use learning in macaque monkeys. *Proceedings of the National Academy of Sciences USA* **106,** 18379–18384 (2009).
2. Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U. & May, A. Neuroplasticity: Changes in grey matter induced by training. *Nature* **427,** 311–312 (2004).

About the researcher

Atsushi Iriki received his Ph.D. in Neurophysiology from Tokyo Medical and Dental University in 1986. He held research associate positions at the Tokyo Medical and Dental University and then at the Rockefeller University. He joined the faculty of Toho University Medical School as an assistant professor and as an associate professor in Physiology (1991–1999). In 1999, he returned to Tokyo Medical and Dental University as a full professor in Cognitive Neurobiology. Atsushi Iriki is now a Head of Laboratory for Symbolic Cognitive Development at RIKEN Brain Science Institute since 2004. He is an adjunct professor of Tokyo Medical and Dental University, The University of Tokyo, Keio University, and a visiting senior fellow of University College London. Based on behavioral and neurophysiological analyses on chronic macaque monkeys, which were trained to use tools and other high-tech apparatus, he tries to uncover evolutionary precursors of human higher cognitive functions grounded onto physical morphologies and patterns of structured bodily actions. He extrapolates these mechanisms to constitute bases of communicatory functions by sharing above machineries among individuals, and eventually understand neural mechanism of social behaviors. Further, he is aiming at extending these mechanisms onto evolutionary as well as developmental clues of symbolic cognitive functions to subserve inference, metaphysical thoughts etc. that characterise human intelligence.

Blast from the past

X-rays emitted from the remnant of a supernova provide clues to its explosive history



RIKEN/Suzaku/Digital Sky Survey (DSS)

Figure 1: X-rays emitted by fast-moving electrons in the Jellyfish Nebula shown here, the remnant of a supernova explosion, carry clues to how the remnant formed.

The supernova remnant known as the Jellyfish Nebula and IC 443 lies 5,000 light years away from Earth in the Gemini constellation (Fig. 1). Left after a stellar explosion, the remnant—hot plasma, surrounded by a cooler shell—is the first of its type to be observed by astronomers[1]. The finding is based on x-ray data collected aboard the *Suzaku* satellite.

"The satellite data have enabled us to investigate the explosion mechanisms that led to this supernova, as well as what was happening within the star before it exploded," explains Hiroya Yamaguchi from the RIKEN Advanced Science Institute, Wako, who led the multi-institutional study.

Light, from long radio waves to x-rays, carries information about the activity in a stellar explosion. Both hot ions and fast moving electrons radiate x-rays in IC 443, which at 4,000 years old is considered a middle-aged remnant. Astronomers estimate the temperature of the ions and electrons in the remnant plasma by measuring the spectrum of these x-rays—that is, how the x-ray intensity varies with energy. The electron and ion temperatures, and any difference between them, yield clues as to how the star exploded and progressed through time.

Yamaguchi and his team noticed a curious discrepancy by analyzing the x-ray spectrum of IC 443: the silicon and sulfur ions, which are estimated to be a searing 14 million degrees Celsius, are nearly twice as hot as the electrons. In fact, the silicon and sulfur ions are so hot that some of them are completely stripped of their electrons.

"This is the first discovery of such spectral features in the x-rays emitted by a supernova remnant," explains Yamaguchi.

This conclusive evidence for the process that astronomers call 'overionization' suggests that when the star that produced IC 443 exploded, a blast wave heated the dense gas around the star to the very high temperatures that stripped the electrons from the silicon and sulfur ions. This was followed by a shock wave that caused the gas to expand and allowed the electrons to cool, but rarefied the ions so much that they could not cool down again.

"Gamma-ray bursts and hypernova—which have energies more than 10 times that of supernova—are known to be some of the most energetic and explosive events in the universe, but the detailed explosion mechanism and nature of their progenitors are still unknown," says Yamaguchi. "The application of our method will play an important role to solve these issues." ■

1. Yamaguchi, H., Ozawa, M., Koyama, K., Masai, K., Hiraga, J.S., Ozaki, M. & Yonetoku, D. Discovery of strong radiative recombination continua from the supernova remnant IC 443 with *Suzaku*. *The Astrophysical Journal* **715**, L6–L9 (2009).

More bang for your bond

Size-controllable bulky ligands stabilize multiple bonds of heavy elements into photoactive materials

Organic compounds containing double or triple bonds can pack a powerful punch. By sharing electrons between atoms through a process called pi conjugation, unsaturated molecules often have exceptional photonic and electronic behavior, making them essential components in state-of-the-art products such as polymer light-emitting displays.

One way to boost the usefulness of multiply bonded materials is to add heavy elements other than carbon into organic frameworks. Now, Kohei Tamao and colleagues from the RIKEN Advanced Science Institute in Wako and Kyoto University have developed a size-controllable molecular ring system that enables double bonded silicon–phosphorus units (Si=P) to be securely incorporated into pi conjugated networks[1]—unlocking previously unseen photo-absorption and emission activity.

Because Si=P bonds are extremely reactive, chemists typically attach them to geometrically large molecules known as bulky ligands that protect the double bonded elements. Unfortunately, most bulky ligands cause the Si=P double bond to twist, disrupting the critical pi conjugation.

Tamao and his team designed a new type of bulky molecule—the so-called 'Rind' ligands—to address this rotational problem. Based on a rigid, symmetric skeleton of three fused rings known as s-hydrindacene, Rind groups also contain alkyl side chains that can be tailored in length to control ligand bulkiness.

Adding Rind ligands to Si- and P-based starting materials produced molecules with highly coplanar Si=P bond with aromatic groups on Si that maintained pi conjugation in the solid state and allowed a unique room temperature fluorescence to emerge (Fig. 1). According to Tsukasa Matsuo, a co-author of the study, the Rind ligands interlock with each other to enforce the favored planar geometry for pi conjugation.

"Rind groups can make planar arrangements out of a variety of conjugated systems," says Matsuo. "But the electronic effect of Rind itself is small, because they are perpendicular to the conjugated electron system."

The researchers also discovered that Rind ligands produced startling results when used to stabilize molecules containing copper atoms and organic groups[2]. While organocopper compounds are extremely useful in synthetic chemistry, their structures remain largely unknown because of continuous aggregation and dissociation processes in solution. By intoducing Rind ligands to copper bromide, the team isolated stable compounds containing remarkable internal architectures, such as four copper atoms arranged into a planar square (Fig. 2).

"We were surprised when we found the beautiful structures of the organocopper materials," says Matsuo. The four-copper framework also gave new luminescent qualities to these complexes—another reason why the researchers are continuing to explore ways to make functional materials using innovative bulky Rind ligands. ■

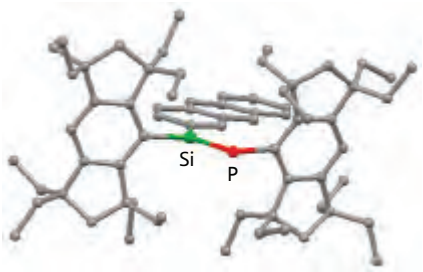


Figure 1: Molecular structure of the Si=P double-bond compound containing the anthryl aromatic group on the silicon atom (Si), and showing the high coplanarity of the pi-framework enforced by the two perpendicularly fixed bulky 'Rind' groups.

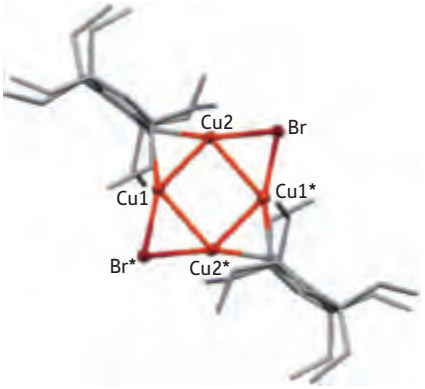


Figure 2: Schematic of the molecular structure of a new symmetric hybrid compound containing copper (Cu) and bromine (Br) atoms in a planar arrangement, stabilized by bulky 'Rind' ligands (grey frames).

1. Li, B., Matsuo, T., Hashizume, D., Fueno, H., Tanaka, K. & Tamao, K. π-Conjugated phosphasilenes stabilized by fused-ring bulky groups. *Journal of the American Chemical Society* **131**, 13222–13223 (2009).
2. Ito, M., Hashizume, D., Fukunaga, T., Matsuo, T. & Tamao, K. Isolated monomeric and dimeric mixed diorganocuprates based on the size-controllable bulky 'Rind' ligands. *Journal of the American Chemical Society* **131**, 18024–18025 (2009).

Precision molecular assembly

A finely tuned rare-earth metal catalyzes the exact interactions needed for site-selective molecular synthesis

Subtle electronic differences between metals in the periodic table can lead to radical changes in chemical reactivity. Now, a research team led by Zhaomin Hou from the RIKEN Advanced Science Institute, Wako, has found that scandium, a seldom-studied rare-earth metal, enables the catalytic addition of functional groups to unsaturated carbon bonds with better selectivity than other metals[1]—a boon to chemists seeking precise control over molecular assembly.

Hou and his team are experts in the field of rare-earth materials, and recently discovered that a so-called 'half-sandwich' complex, comprising a scandium cation and a pentagonal carbon ring, could efficiently catalyze production of long polymer chains[2].

"Our scandium complex acted as an excellent catalyst for olefin polymerization, with unprecedented activity and selectivity," says Hou. Because the scandium complex targeted unsaturated carbon bonds during the polymerization process, the researchers realized its enormous potential in other important synthetic reactions, such as carbometalation.

During carbometalation, a metal catalyst helps an organic unit, such as a methyl group, and a metal—commonly aluminum—to add to a carbon–carbon double or triple bond. Researchers can then replace the metal with another molecular group, making carbometalation an effective way to construct carbon-based frameworks containing multiple, branched functional units.

What is difficult, though, is controlling the regioselectivity of the catalytic addition—the precise positions where the organic and metal units add to the unsaturated carbon bonds. When the team first attempted carbometalation with the scandium catalyst and a typical triple-bonded carbon molecule, it achieved only moderate regioselectivity, similar to other transition metal catalysts.

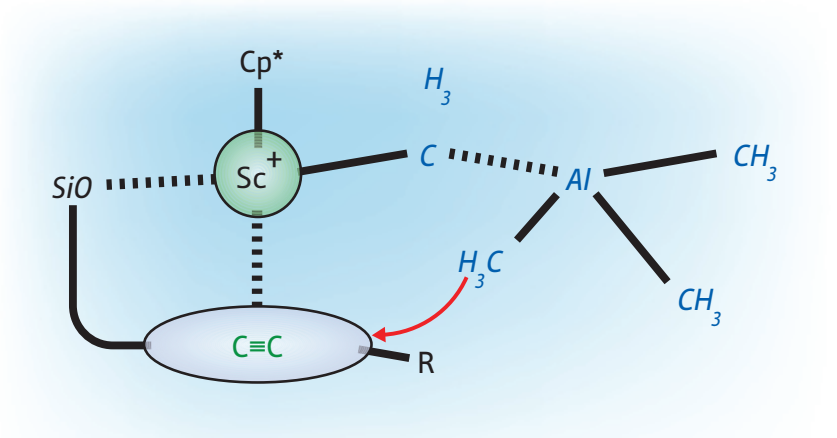


Figure 1: The interaction between a scandium-based catalyst (Sc+–Cp*) and a silyl ether group (SiO) allows the highly selective addition of methylaluminum (blue structure) to carbon–carbon triple bonds.

However, when the researchers tethered a silyl ether—a group containing silicon, oxygen, and hydrocarbon atoms—to the end of the triple-bonded carbon substrate, the carbometalation proceeded with extremely high regioselectivity; over 99% of the final product was isolated as a single chemical isomer. Further experiments revealed that the combination of a silyl ether tether group and a scandium-based catalyst enabled controllable carbometalation on numerous unsaturated organic molecules—in many cases, with higher regioselectivity than any other catalyst.

According to Hou, the unprecedented selectivity achievable through this method is due to a balanced interaction between the oxygen atom of the silyl ether adduct and the scandium cation (Fig. 1). "This interaction should not be too strong," he says, "otherwise coordination and insertion processes around unsaturated carbon–carbon triple and double bonds would be hampered."

The researchers are currently exploring new ways to utilize rare-earth complexes for chemical transformations involving carbon and other elemental bonds. ■

1. Takimoto, M., Usami, S. & Hou, Z. Scandium-catalyzed regio- and stereospecific methylalumination of silyloxy/alkoxy-substituted alkynes and alkenes. *Journal of the American Chemical Society* **131**, 18266–18268 (2009).
2. Li, X., Nishiura, M., Hu, L., Mori, K. & Hou, Z. Alternating and random copolymerization of isoprene and ethylene catalyzed by cationic half-sandwich scandium alkyls. *Journal of the American Chemical Society* **131**, 13870–13882 (2009).

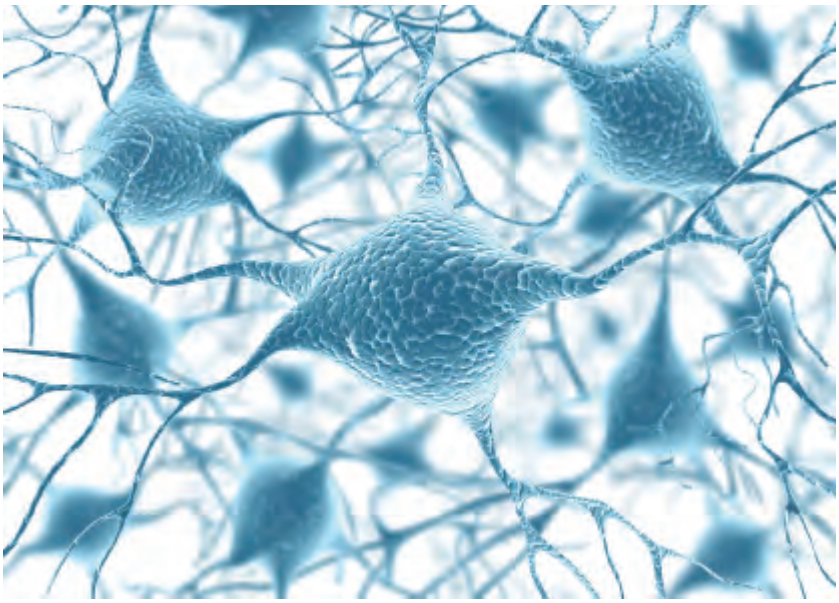
Switching neurons

Inhibitory neurons in the visual cortex of the brain exhibit a bidirectional form of plasticity after visual deprivation

If both eyes are open during mammalian development, pyramidal neurons in the visual cortex mature such that they 'preferentially' fire in response to the visual stimuli of one particular eye. If that eye is occluded during a critical period of development, the pyramidal neurons switch and fire in response to visual stimuli of the non-occluded eye. This leads to a loss of representation of the occluded eye in the visual cortex and the loss of visual acuity in that eye.

Pyramidal neurons receive inputs from so-called inhibitory interneurons within the visual cortex (Fig. 1), but it has been unclear how the interneurons respond to visual deprivation, and what their role is in inducing this plasticity of pyramidal neuron response. Now, a team led by Takao Hensch at the RIKEN Brain Science Institute (BSI) in Wako, has reported that inhibitory neurons also change their responsiveness to visual stimuli after one eye is occluded[1]. Surprisingly, unlike pyramidal neurons, which have a unidirectional change in responsiveness—only towards the open eye—the inhibitory neurons have a bidirectional change: initially, they respond preferentially to stimuli presented to the occluded eye, but later, they switch their responsiveness to the open eye.

In mice in which both eyes were open, Hensch and colleagues found that blocking the signals going from the inhibitory neurons to the pyramidal neurons caused the pyramidal neurons to lose their selective responsiveness to one eye. When they occluded one eye, blocking the inhibitory neuron signals caused the pyramidal neurons to flip their responsiveness from one eye to the other. This indicates that the signals sent from the inhibitory neurons to the pyramidal neurons help to control the response of pyramidal neurons to both normal vision and visual deprivation.



istockphoto/ktsimage

Figure 1: Artistic representation of inhibitory interneurons in the visual cortex of mammals, which affect the response of pyramidal neurons to visual stimuli.

After the researchers determined how the neurons would react to changes in visual stimulation, they developed a network model to understand how connectivity between neurons—and the plasticity of these connections—would explain the responses of the pyramidal neurons and the inhibitory interneurons. The model, developed in collaboration with Tomoki Fukai and team also at BSI, demonstrates how individual components of a neuronal cell circuit contribute to plasticity within the brain.

"We will now pursue the detailed mechanisms of this plasticity to determine sites for therapeutic interventions," says Hensch. Understanding how this cell type determines early brain plasticity offers the potential for cell-specific strategies to restore or reactivate proper brain function in several neurological disorders such as autism and schizophrenia. ■

1. Yazaki-Sugiyama, Y., Kang, S., Cateau, H., Fukai, T. Hensch, T.K. Bidirectional plasticity in fast-spiking GABA circuits by visual experience. *Nature* **462**, 218–221 (2009).

A window to embryonic development

Cell-cycle progression in fish embryos can now be tracked visually in three dimensions

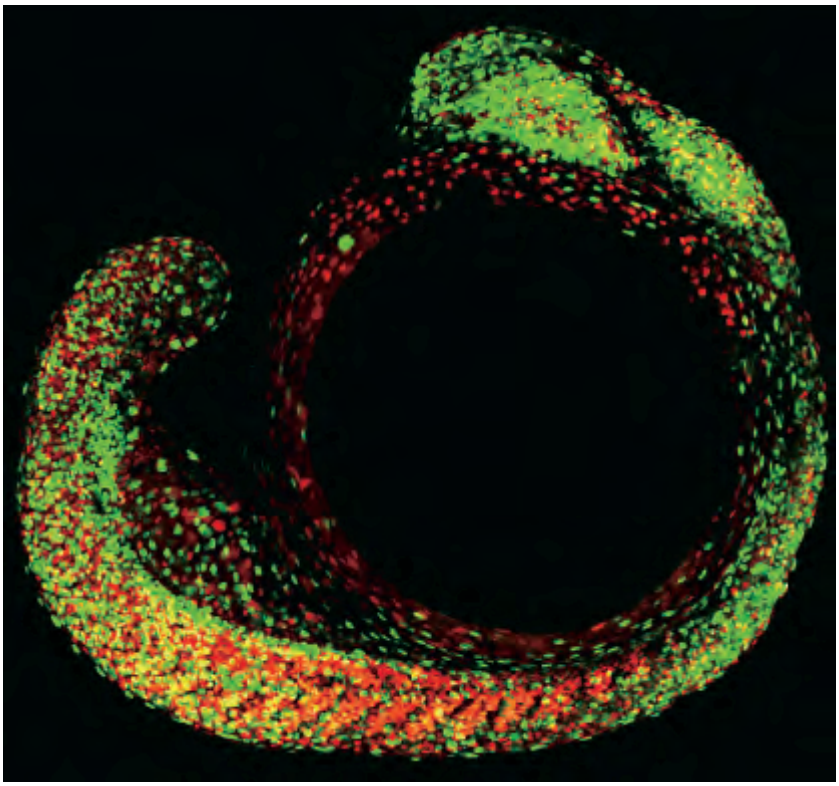


Figure 1: A view of cell proliferation versus differentiation, using Fucchi technology, in a whole zebrafish embryo during segmentation at 18 hours post-fertilization. The retina, full of green nuclei, is visible at the top of the image and the notochord is covered by skeletal muscles along the bottom.

A RIKEN-led team of molecular biologists has developed a visual means of tracking DNA replication as well as cell division and movement in transparent, whole zebrafish embryos. The technique could eventually lead to three-dimensional reconstructions of early development at a cellular level. The researchers already have used their technology to study the cellular development of the retina and of the primitive backbone or notochord, in which they found cell-cycle progression occurring in waves from head to tail.

The team, which is based at the RIKEN Brain Science Institute in Wako but includes researchers from other Japanese institutes and universities, had previously developed technology for tracing the cell cycle in mammalian cells. They called it Fucci—fluorescent ubiquitination-based cell cycle indicator.

Fucci technology utilizes reciprocal fluctuations in the levels of two proteins involved in regulating the cell cycle, Cdt1 and geminin. Cdt1 levels are highest just before DNA replication, whereas geminin levels build up during DNA synthesis and fall during the phase between divisions. The destruction of each of these proteins is preceded by tagging with the protein ubiquitin.

With the Fucci technology, the researchers attach a fluorescent marker to the part of the proteins to which ubiquitin links—orange for Cdt1 and green for geminin. Thus the cycle can be followed by visually tracking the fluctuation of orange and green in the cell. In the opaque mouse embryo, however, this can only be done in slices, not in the whole embryo in three dimensions. So they decided to transfer their technology to zebrafish whose early embryos are transparent. They found, however, that the original Cdt1Fucci tag did not work because of differences in the ubiquitin-mediated degradation system between mouse and zebrafish embryos.

To overcome this problem, the researchers designed and constructed a Cdt1 marker for the fish cells, and using that experience, also improved the geminin marker. After testing in cultured cells to determine that the tags worked and did not affect cell performance, they then created a zebrafish line, dubbed Cecyil, with the markers incorporated genetically.

Using their Fucci zebrafish, the researchers were able to observe development of the retina and differentiation of the notochord (Fig. 1). Their results are published in a recent paper in the *Proceedings of the National Academy of Sciences*[1].

"Remarkably, we discovered two waves of cell-cycle transitions traveling from anterior to posterior of the notochord," says project leader Atsushi Miyawaki. "In future we hope to develop indicators for different cell-cycle phases and other animals." ■

1. Sugiyama, M., Sakaue-Sawano, A., Iimura, T., Fukami, K., Kitaguchi, T., Kawakami, K., Okamoto, H., Higashijima, S. & Miyawaki, A. Illuminating cell-cycle progression in the developing zebrafish embryo. *Proceedings of the National Academy of Sciences USA* Published online before print, 18 November 2009 (doi: 10.1073/pnas.0906464106).

Helping cells reach out

An unexpected result leads to new insights about a poorly understood mode of communication between cells

Remarkably little is known about M cells, which reside in the intestine and participate in immune surveillance of potential pathogens. However, Hiroshi Ohno was taken aback when Koji Hase, a staff scientist in his laboratory at the RIKEN Center for Allergy and Immunology in Yokohama, identified a highly active M cell gene that triggered the formation of unusually long and thin membrane projections when expressed in other cell types.

"I thought it was an artifact," recalls Ohno, "but Koji insisted on its importance." Subsequent experiments proved Hase correct; a mammalian protein called M-Sec appears to drive formation of an enigmatic class of intercellular connections known as 'tunneling nanotubes' (TNT). TNTs are known to participate in the trafficking of a variety of cargoes, ranging from ions to proteins to cargo-laden membrane vesicles—and, less beneficially, viruses and prions. However, these findings offer the first real insights into the underlying mechanisms of TNT growth[1].

In a series of experiments using mouse immune cells known as macrophages that also express endogenous M-Sec, Ohno's group showed that formation of TNTs was inhibited upon knockdown of M-Sec expression (Fig. 1). These TNTs were also shown to be physiologically active, enabling signals to be transmitted from cell to cell via the movement of calcium ions.

Cells rely on a protein-based framework known as the cytoskeleton to provide infrastructure, and major morphological changes typically involve cytoskeletal rearrangements mediated by molecular switches known as small GTPases. Ohno and colleagues found that M-Sec associates with RalA, a small GTPase, and a membrane-bound protein complex known as the exocyst, and that these interactions are essential to the formation and growth of membrane protrusions that will ultimately form TNTs.

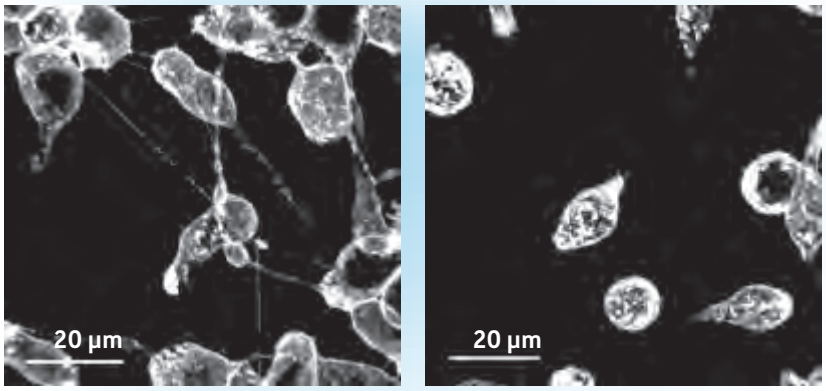


Figure 1: The formation of TNTs connecting mouse macrophage cells (left) is blocked when M-Sec is depleted by knockdown of gene expression (right). (Scale bar, 20 μm).

The researchers are now delving deeper into the bigger picture of M-Sec function by studying mice that have been genetically engineered to lack this protein, but are also exploring more direct ways to target M-Sec activity. "We have started screening M-Sec-binding small-molecular-weight chemicals that could inhibit M-Sec-mediated TNT formation," says Ohno.

He suggests that such drugs could ultimately be used *in vivo* to block the transmission of viruses via these intercellular conduits, but sees the most immediate benefits in simply understanding why these connections exist in the first place. "Virtually nothing is known about their *in vivo* relevance," he says. "In this regard, the discovery of M-Sec as a promoter of TNT formation will accelerate the understanding of their physiological role." ■

1. Hase, K., Kimura, S., Takatsu, H., Ohmae, M., Kawano, S., Kitamura, H., Ito, M., Watarai, H., Hazelett, C.C., Yeaman, C. & Ohno, H. M-Sec promotes membrane nanotube formation by interacting with Ral and the exocyst complex. *Nature Cell Biology* **11,** 1427–1432 (2009).

Discovery of a plant hormone that affects other organisms

Studies on mutant plants provide insights into the role of strigolactone, a plant hormone that inhibits shoot branching and attracts both beneficial symbionts and damaging root parasites

Shinjiro Yamaguchi

Team Leader
Cellular Growth and Development Research Team
RIKEN Plant Science Center

High-school biology textbooks describe auxin, cytokinin, gibberellin, abscisic acid and ethylene as plant hormones. Now, a new hormone, strigolactone, is joining the group. Strigolactone was discovered more than 40 years ago to be involved in promoting germination in root-parasitic plants. Shinjiro Yamaguchi, team leader of the Cellular Growth and Development Research Team at the RIKEN Plant Science Center, has recently clarified that strigolactone, which is also known for its role in attracting mycorrhizal fungi, is in fact a plant hormone that inhibits shoot branching. This is the first discovery of a plant hormone that affects external organisms. These findings are expected to encourage the development of new methods for controlling root-parasitic plants, which are responsible for serious damage to agricultural crops in Africa. Ultimately, the discovery is expected to lead to improved crop quality and yield.

What are plant hormones?

In August 2008, news agencies around the world reported the "Discovery of a Plant Hormone that Inhibits Shoot Branching". As Yamaguchi appreciates, "this was an important discovery that should be added to textbooks."

What are plant hormones? "Plant hormones are low molecular weight compounds produced by plants themselves. They control the germination, growth and response of plants to their environment in minute amounts. The same hormones are produced by many different types of plants," explains Yamaguchi. In contrast to the hormones found in animals, which have a specific function at a certain location and for a certain time, plant hormones have diverse functions at various times and locations.

Since receiving his PhD from the University of Tokyo, Yamaguchi has been heavily involved in research on plant hormones, particularly gibberellins. Gibberellins were discovered by Eiichi Kurosawa in 1926 and were further investigated by Teijiro Yabuta, a former chief scientist at RIKEN. Today, it is known that gibberellins promote plant growth and are related to the induction of seed germination and plant height. Yamaguchi has achieved many breakthroughs in his research on gibberellins, including identification of the genes of enzymes responsible for gibberellin production, elucidation of a gibberellin-induced control mechanism for the germination of seeds, and the discovery of a molecular mechanism that deactivates gibberellins. However, there remains a key question that he has been attempting to answer for a long time. "So far, we've discovered several plant hormones, but is that really all of them? Are there any other plant hormones that we don't know about yet?"

Focusing on mutant plants with excess shoot branching

Yamaguchi had one particular mutant plant in mind— a pea mutant with excess shoot branching. It was discovered by an Australian research

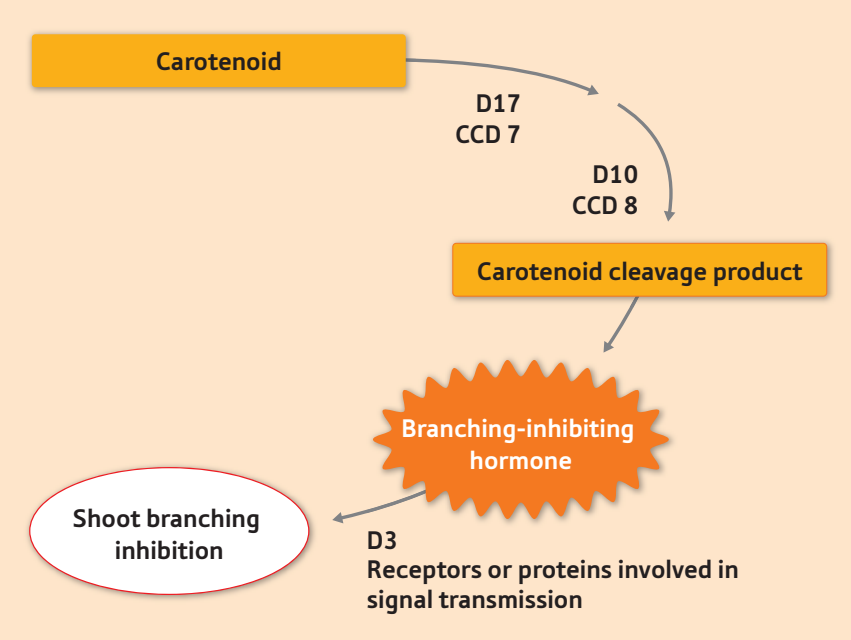


Figure 1: Estimated biosynthesis channel for branching inhibition hormones.
Deficiencies in genes *D17*, *D10* and *D3* in rice give rise to excess shoot branching. Genes *D17* and *D10* produce carotenoid cleavage dioxygenase (CCD) 7 and 8, respectively, and no shoot branching-inhibition hormone is produced in the absence of *D17* and *D10*. Thus, the shoot-branching inhibition hormone is thought to be produced when carotenoids are cleaved by the CCDs. Gene *D3* produces proteins related to receptors of the shoot-branching hormone or those involved in signal transmission. In the absence of this gene, no information is transmitted.

group in the mid-1990s.

"A branch is an adult form of axillary bud, which emerges laterally from the base of the leaf stem. However, not all axillary buds grow to become adult branches. Plants have a mechanism called apical dominance, by which axillary buds are prevented from growing at the same time as the terminal bud—the tip of the plant stem. Cytokinin and auxin are the plant hormones that control apical dominance; cytokinin promotes the growth of axillary buds, while auxin inhibits growth. It was believed that the growth and shoot branching depend on the balance between cytokinin and auxin."

In the mid-1990s, a group of Australian researchers investigated the amount of cytokinin and auxin contained in a mutant pea plant with excess shoot branching. They presumed that since the axillary buds in the dormant state started to grow with repeated branching, there must be an excess accumulation of cytokinin that promotes the growth of axillary buds. "Their results showed the opposite," says Yamaguchi. There were smaller amounts of cytokinin and larger amounts of auxin. "The functions of auxin and cytokinin could not always explain the excess shoot branching of the mutant plant. It was also known that shoot branching returned to the normal state when the mutant plant was grafted onto a normal pea plant. These facts made me think that there must be a plant hormone, other than auxin, that is capable of inhibiting shoot branching—a branching-inhibiting hormone that this did not function in the mutant, resulting in excess shoot branching."

Since then, mutants with excess shoot branching have been observed in other plants, including petunia (a popular garden plant), *Arabidopsis thaliana* (commonly used in experiments) and even rice plants. "It is highly possible that a plant hormone is involved in excess shoot branching because the same phenomenon has been observed in various mutant plants. I was convinced that studying these mutant plants would lead to the discovery of a previously unknown plant hormone," says Yamaguchi.

Discovery of a new plant hormone

To identify the new plant hormone, Yamaguchi began research on branching-inhibiting hormones in 2005 using mutant rice plants with excess shoot branching. Three mutant rice plants were known to exhibit excess shoot branching, each with respective deficiencies of *D17*, *D10* and *D3* genes.

Genes *D17* and *D10* were shown to produce carotenoid cleavage dioxygenase (CCD), which cleaves a group of pigments called carotenoids (Fig. 1). "This suggested that the branching-inhibiting hormone was produced when the carotenoids were cleaved. The *D17*- and *D10*-deficient mutant plants exhibited excess shoot branching because no branching-inhibiting hormone was being produced. Gene *D3* was considered to be a receptor for the shoot-branching hormone, or to be the gene responsible for the production of signal-transmitting proteins. It was concluded that the *D3*-deficient mutant plant produced branching-inhibiting hormones, but failed to respond to them, leading to excess shoot branching."

To identify the branching-inhibiting hormone, Yamaguchi conducted a series of bioassays to explore the response of the mutant plants to various compounds. "We wanted to find a compound that returns the *D17*- and *D10*-deficient mutant plants, which do not produce the branching-inhibiting hormone, to the normal branching state but does not affect the *D3*-deficient mutant, which produces the hormone but does not respond to it." Based on this idea, Yamaguchi administered the mutant plants with a liquid extracted from the tissue of plants that were believed to contain the branching-inhibiting hormone. If the extract elicited the expected response, the components of the extract were isolated and administered separately. This cycle of separation, administration and examination was repeated many times until the target compound was eventually identified.

The bioassay technique is a standard approach to identifying a target compound. However, Yamaguchi had a hard time narrowing down the target compound because of the large number of compounds produced by plants. The stalemate was finally broken in October 2005 when a paper on the root-parasitic plant *Striga* was published.

Striga is found in dry regions of Africa and South Asia, and is a parasite plant that attacks the roots of monocotyledonous plants, such as corn and sorghum, and absorbs nutrients and water from the host plant to grow. *Striga* attacks inhibit normal growth of the host plant, and are responsible for massive reductions in crop yields, posing a serious problem for agriculturalists.

The most striking feature of *Striga* is that its seeds germinate only when they recognize the presence of the compound called strigolactone, which is secreted from the roots of host plants. The secretion of strigolactone has been known for 40 years, but the question of how strigolactone is produced had remained a mystery. The 2005 paper showed that strigolactone is produced from carotenoids, much like the branching-inhibiting hormone that Yamaguchi was looking for, which is produced when carotenoids are cleaved by CCD. "There are many kinds of CCD. However, the investigation showed that there were only a few CCDs for which the functions had not been determined. Thus, I formed the hypothesis that strigolactone is the branching-inhibiting hormone we were looking for."

Yamaguchi then began conducting experiments to verify his hypothesis. He found that almost no strigolactone was produced in *D17*- or *D10*-deficient mutant plants, whereas large amounts of strigolactone were produced in *D3*-deficient mutant plants. "I felt sure that my hypothesis was correct. Based on previous studies, we knew that mutant plants that do not respond to gibberellin produce around 100 times the normal amount of gibberellin. Thus, the phenomenon could be reasonably explained if the *D3*-deficient mutant plants are plants that do not respond to strigolactone."

When strigolactone was administered to mutant plants, Yamaguchi was able to verify that the excess shoot branching observed in the *D17*- and *D10*-deficient mutant plants returned to normal, while the *D3*-deficient mutant plants were unaffected (Fig. 2). "There is now no doubt that strigolactone is a branching-inhibiting hormone." Strigolactone was thus confirmed to be a newly discovered plant hormone that inhibits shoot branching.

Yamaguchi attributes this textbook-revising breakthrough to two factors. "We had accumulated a great deal of know-how through our research into gibberellin. This is the first point. In addition, we were fortunate enough to be able to use the ultra-sensitive mass spectrometer at the RIKEN Plant Science Center. The amount of plant hormone contained in one gram of plant tissue is only one nanogram. Without the RIKEN mass spectrometer, we could not have achieved the structural analysis using such a small amount of fragile strigolactone."

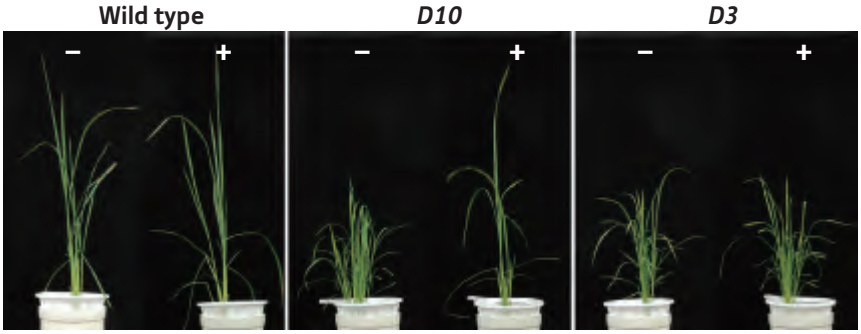


Figure 2: **Shoot branching inhibition by strigolactone administration.**
The *D10*-deficient mutant plant that does not produce the shoot branching inhibition hormone and the *D3*-deficient mutant that cannot respond to the hormone exhibit excess shoot branching and grow to a reduced height (–). When strigolactone is administered (+), the *D10*-deficient mutant plant grows taller and branching returns to the normal level, whereas the *D3*-deficient mutant shows no change.

Unexpected relationship between branching and mycorrhizal fungi

Despite his success, Yamaguchi felt that something still remained unclear. "Why does a plant secrete a germination-inducing substance that attracts root parasites? This is clearly contrary to the natural survival of the plant."

A research team at Osaka Prefecture University recently solved this mystery when they clarified that strigolactone attracts mycorrhizal fungi. Whereas *Striga* absorbs nutrients from the host plant without benefit for the host, mycorrhizal fungi perform a highly beneficial function: in return for invading the root cells of a plant and consuming some of the host plant's photosynthesized sugars, mycorrhizal fungi pass on water and nutrients, such as phosphorus, that the fungi absorb from the soil through their fibrillose hyphae (Fig. 3). "Mycorrhizal fungi develop a symbiotic relationship with the host plant, and the host plant actively secretes strigolactone to attract the beneficial mycorrhizal fungi."

Yamaguchi has yet more questions, such as why a single plant hormone is involved in both branching inhibition and mycorrhizal fungi attraction at the same time. "We think that this is the survival strategy of the host plant against a shortage of nutrients," says Yamaguchi. "In a nutrient-poor environment, host plants need to attract mycorrhizal fungi, which help the plants absorb nutrients. At the same time, the root needs to prevent the stem from unfruitful shoot branching in a nutrient-poor environment. This information can be transmitted more effectively if a

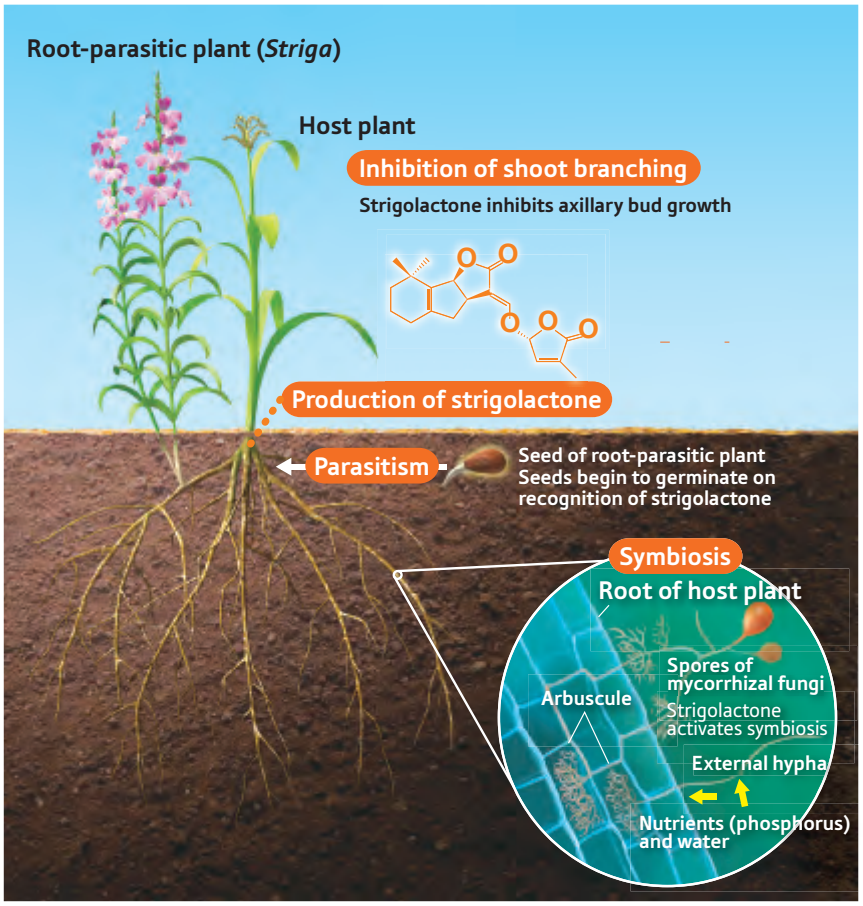


Figure 3: Relationships among strigolactone, root-parasitic plants and mycorrhizal fungi. Strigolactone is secreted from the roots of a plant. Strigolactone is a plant hormone that inhibits shoot branching and attracts mycorrhizal fungi, which help the roots absorb nutrients. In a nutrient-poor environment, the roots secrete increasing quantities of strigolactone to attract mycorrhizal fungi and also to suppress shoot branching. *Striga* uses strigolactone to find a host, which withers as a result of the parasite attack.

single plant hormone is used than if two separate signal molecules are used."

Protecting crops from root parasitic plants

Yamaguchi's findings are expected to lead to the development of a *Striga* control method. "Many researchers have been searching for plants like the *D10*-deficient mutants that do not produce strigolactone, because these plants cannot be parasitized by *Striga*."

With the cooperation of Ken Shirasu of the Plant Immunity Research Group at the RIKEN Plant Science Center, Yamaguchi has been able to examine whether *D10*-deficient mutant plants are resistant to *Striga* parasitism. When *Striga* seeds were placed around a normal rice plant, 20% of the seeds germinated, and 10% were found to be parasitic. However, when *Striga* seeds were placed around a *D10*-deficent mutant, almost no seeds germinated, and no parasitism was observed. "The results were what we had expected, but there are still problems," says Yamaguchi. "If no strigolactone is produced, more shoot branching will occur, but the plant will also be unable to attract mycorrhizal fungi, which will adversely affect plant growth. Thus we are currently conducting research to find compounds that attract mycorrhizal fungi but not root-parasitic plants. In cooperation with researchers in Africa, we hope to find a way of preventing crop damage caused by root-parasitic plants, thus contributing to solving the African food problem."

Developing a technique that can control shoot branching would greatly contribute to agriculture and horticulture. The number of branches ultimately affects the number and quality of flowers, fruit and seeds. For example, higher-quality tomatoes could be produced in lower yield. There are many other plants, including *Nicotiana tabacum* and chrysanthemum, for which crop yields and quality could be modified through branching control.

What obstacles are there to practical use of these findings in agriculture and horticulture? "It may be that strigolactone is converted into another active form when it functions. Thus, it is necessary to elucidate the main part that acts as a plant hormone. In addition, it is important to find strigolactone receptors; if we find differences between the receptors of plants, mycorrhizal fungi and root-parasitic plants, we may be able to attract mycorrhizal fungi without attracting root-parasitic plants," says Yamaguchi.

"Strigolactone can affect living things other than plants—no other plant hormone has been found to have this function. Although we have a long history of research in plant hormones, we are still ignorant about many things. I think there are other plant hormones that still remain undiscovered, and I really hope to find them."

About the researcher

Shinjiro Yamaguchi graduated from the Department of Agricultural Chemistry, Faculty of Agriculture, at the University of Tokyo in 1991. He obtained his PhD in 1996 from the same university, and subsequently joined the Frontier Research Program at RIKEN researching the identification of gibberellin biosynthesis genes in plants. He worked at Duke University in the United States as a postdoctoral fellow from 1997 to 2000, where he studied the regulation of gibberellin biosynthesis in *Arabidopsis*. He worked at the Laboratory for Cellular Growth and Development, RIKEN Plant Science Center from 2000 to 2005, where he studied regulatory mechanisms for seed germination and plant isoprenoid biosynthesis pathways. He has since been studying plant growth hormones as leader of the Cellular Growth and Development Research Team.

New cherry blossom tree blooms all seasons

A new breed of cherry blossom tree that blooms all year round has been created by RIKEN scientists using heavy ion beams at RIKEN Nishina Center for Accelerated-Based Science. The new breed blooms longer, produces more flowers and grows under a wider range of temperatures than existing cherry blossom trees, demonstrating the power of accelerator technology in horticulture.

Figure 1: Flowers of the Nishina Otome in the fall (left) and in the spring (right).

To create the new breed, researchers used beams of carbon ions from the RIKEN Ring Cyclotron at the RI Beam Factory to induce mutations in branches from the cherry blossom tree known as Keiou-Zakura No. 13. The branches were grafted and cultivated to create the new breed, which has been aptly named 'Nishina Otome'.

Unlike regular cherry blossom trees, Nishina Otome does not require a period of cold weather to trigger growth. As a result, the new tree is able to bloom all year round when cultivated indoors, and during autumn and spring when grown outdoors. Given sufficient exposure to low temperatures, it produces three times more flowers than the regular varieties, and its spring bloom lasts for twice as long.

The use of heavy ion beams to generate new breeds of plants by mutagenisis, an approach to horticulture unique to Japan, is drawing attention worldwide as a powerful alternative to conventional genetic engineering that is capable of shrinking breeding times to only a few years. The second breed of cherry blossom tree to be registered by RIKEN, the Nishina Otome hints at an exciting future for accelerator-based mutation breeding, one which opens the door to the design of plant varieties better able to cope with a changing environment. ■

Figure 2: Comparison of the mutant Nishina Otome (left) and the original breed (right).

KEIO-RIKEN centre hosts conference on sustainable social systems

Established in April 2009 through an agreement between RIKEN and Keio University, the Research Centre for Human Cognition (CHC) held its first international symposium on December 14 and 15, 2009. Under the title of 'Toward Sustainable Social Systems', scientists from around the world gathered to discuss a variety of issues under a common theme of complex systems.

Chris Wood, vice president of the Santa Fe Institute, a non-profit organization, kicked off the symposium with a discussion of research underway at his organization, which leads the world in complex systems research. Monique van Donzel from Nanyang Technological University outlined her institution's drive to foster research connections across diverse fields, a theme also expounded by Erling Norrby of the recently founded Institute Para Limes. Norrby, science board chairman and member of the Royal Swedish Academy of Sciences, stressed the importance of "vertical science"—new ways of thinking that break away from prevailing dogmas. All of these organizations maintain strong ties with the CHC centered around a common philosophy of encouraging vertical science and cross-disciplinary collaboration.

One field in which collaboration is common is brain science. Atsushi Iriki of the RIKEN Brain Science Institute demonstrated the reach of research in this area with his discussion of neuroscience experiments on macaque monkeys, which he showed can be trained to use sensory tools. Taking a step back, Michel Hofman of the Netherlands Royal Academy of Arts and Science drew a comparison between clustering of information in the brain and hubs in the World Wide Web.

Looking at evolution in a broader sense, John Holland of the University of Michigan, a pioneer in complexity research, emphasized the value of complex adaptive systems in tackling a range of problems. Hideyuki Okano of the Keio University Faculty of Medicine demonstrated the power of one such adaptive system, the marmoset, as a non-human model for neuroscience research that could reveal clues regarding the causes of neurological diseases and the basis of higher brain function. Other fields covered by the complex system approach included linguistics, discussed by Helena Hong Gao from Nanyang Technological University, intellectual property law, as discussed by Institute Para Limes President Jan Vasbinder, and even science itself, as shown by Rathenau Institute Director Peter van den Besselaar.

The challenges science confronts today are broad in scope and complex in nature. In offering a diversity of perspectives, CHC's first international symposium has set out a path toward a future science better able to understand these challenges, and a future society better able to overcome them. ■

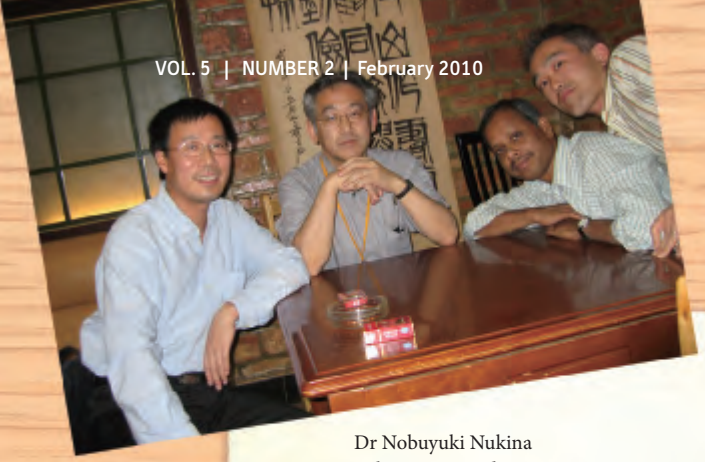
JAXA and RIKEN to share all-sky X-ray image data on the internet

Scientists, researchers and amateur astronomers alike will rejoice at the latest news from the Japan Aerospace Exploration Agency (JAXA) and RIKEN. In collaboration with six Japanese universities, JAXA and RIKEN, who have teamed up over recent years to develop the largest wide-field X-ray camera in the world, known as MAXI (short for "monitor of all-sky X-ray image"), have announced that they will release data acquired by the new camera onto the internet for use by the general public. Streamed from MAXI's perch atop the International Space Station's Japanese Experimental Module, the data will provide views of the universe that have never before been seen or analyzed.

The universe is full of fascinating astronomical phenomena, including quasars, black holes, neutron stars and relativistic jets. But absorbed by the Earth's atmosphere, X-rays emitted by these objects never reach ground level, making observation of these objects impossible by ground-based observatories. MAXI makes these observations from space at a level of sensitivity higher than any other all-sky camera to date, scanning the sky every 90 minutes and collecting observational data on changes in X-ray intensity.

Observations by MAXI are performed using multiple gas and solid-state slit cameras, and the data are processed, calibrated and transmitted immediately, providing updates within one day of actual observation. The data acquired so far cover roughly 100 astronomical objects, and JAXA and RIKEN plan to ultimately release data for a much larger number of phenomena and for time scales extending from days to months. And thanks to a notification system currently being established, any emergence or brightening of astronomical X-ray objects detected by MAXI will be shared immediately with the public through email alerts advising of the time of origin, position and intensity.

MAXI's high sensitivity means that its observations can go beyond our galaxy to monitor extra-galactic X-ray activity by objects such as quasars. It's all-sky coverage also complements the features of another Japanese X-ray camera known as Suzaku. Launched in 2005, Suzaku focuses on a very limited region of the sky at a sensitivity level several thousand times higher than that achieved by MAXI. By directing Suzaku's high-sensitivity telescopes toward interesting objects, MAXI promises to open a new window onto never-before-seen places in our universe for people from across the world to explore. ■



Dr Nobuyuki Nukina
Loboratory Head
Laboratory for Structural Neuropathology
RIKEN Brain Science Institute
Wako, Saitama
Japan

Dear Dr Nukina,

It is almost one year since I left your laboratory. I hope you are well. The four years that I stayed at RIKEN have had an important impact on my life, and I would like to express my thanks to you and all of my former RIKEN colleagues.

I joined your laboratory in October 2007 when it was first launched. I still remember well the days in that laboratory and our very kind colleagues. The scientific training I received at RIKEN was very valuable to me. RIKEN has a wonderful atmosphere for its researchers, and so many excellent scientists contribute to its culture and to science at an international level. It is a place where I received solid training and could develop my own scientific thinking. Everybody worked hard, and I could get help from anybody if I wanted to learn more. RIKEN and you gave me so much help in my research career, even after I came back to China to start my new laboratory at the University of Science and Technology of China.

In 2004, I secured a collaboration fund from RIKEN, and the grant helped me to run my new laboratory and further develop the collaboration between our two laboratories. RIKEN is a Japanese institute, but it has an international style. There, I met researchers from India, Russia and Korea, and our shared experience in your laboratory gave us a strong connection — some of us have even now established further collaborations. Dr Nihar Jana, an Indian scientist who worked in your lab at that time, is now running his own laboratory at the National Brain Research Centre in India, and is in regular communication and collaboration with us. RIKEN is a bridge that connects the scientists who once worked there.

Besides the research atmosphere, my life at RIKEN is also a very good memory for me. Two assistants, Miss Taniguchi and Miss Mizuno, gave me and other foreigner visitors a great deal of help. When I stayed at RIKEN, they helped both myself and my wife, who still tells me how much she likes Japan and the RIKEN staff. She too hopes very much to visit Japan again some day.

One of my fondest memories is when you and other members of our laboratory visited my wife in Narimasu hospital just after the birth of our child. The lovely toy you gave is still with us, passed on to our next child, even after moving several times and to several different countries. I hope one day that I will get the chance to take my family to visit Japan once again and to meet with you, Miss Taniguch, Miss Mizuno and our other friends at RIKEN.

My kind colleagues at RIKEN, the busy research days, the enjoyable RIKEN life and the beautiful cities in Japan, all gave me unforgettable memories. I hope I will one day return to visit you in the near future.

Best regards to you and our colleagues,

Guanghui Wang
Laboratory of Neuropathology
School of Life Sciences
University of Science and Technology of China,
Hefei, Anhui, China

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For further information on the research presented in this publication or to arrange an interview with a researcher, please contact
RIKEN Global Relations Office
2-1, Hirosawa, Wako, Saitama, 351-0198, Japan
TEL: +81 48 462 1225
FAX: +81 48 462 4713
E-Mail: rikenresearch@riken.jp

www.rikenresearch.riken.jp