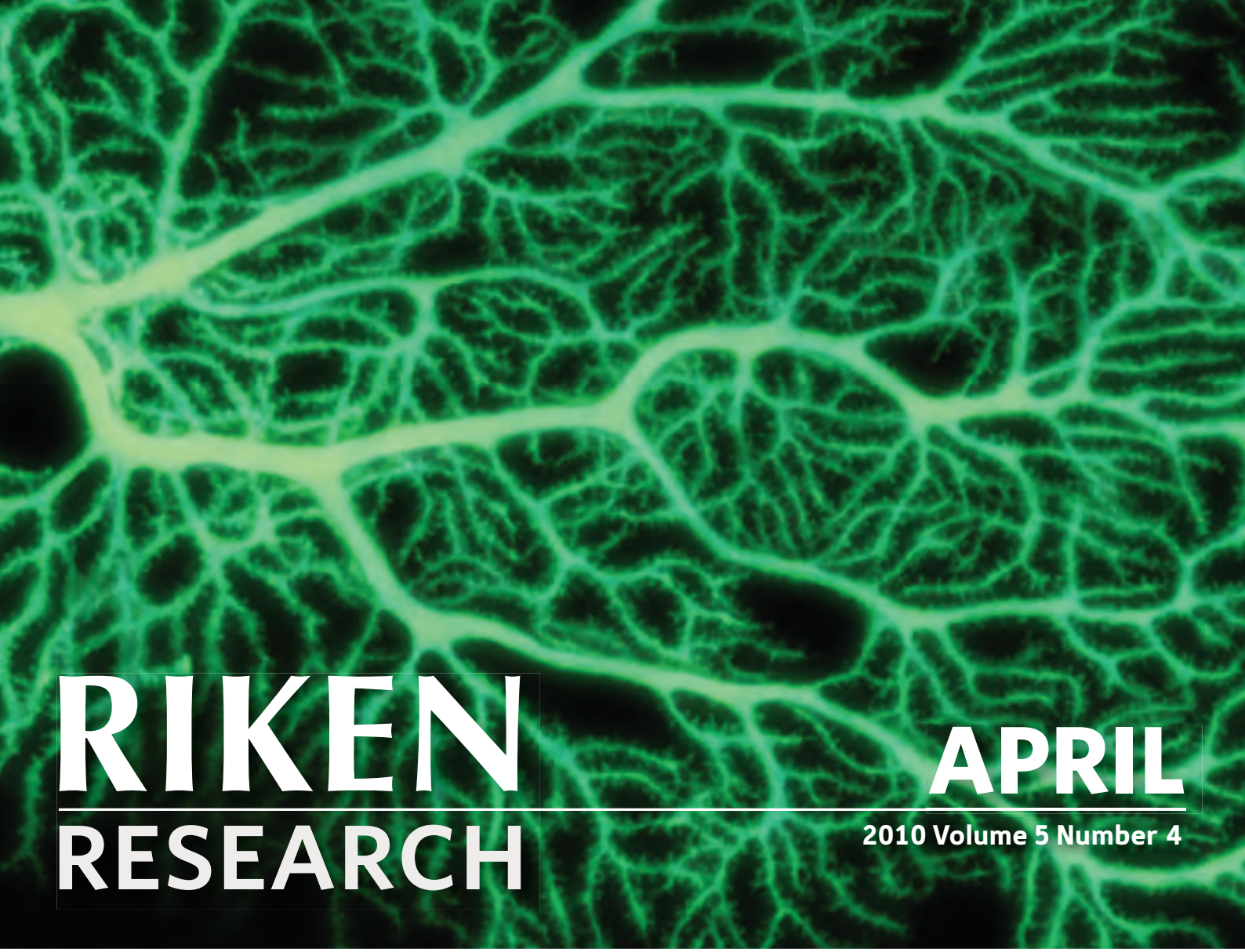

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Unearthing the pathways of plasticity

Lipid signaling at the synapse modulates the strength of neuronal communication in the brain

Changes in synaptic strength after repeated communication between neurons is a key mechanism for induction of learning and memory in the brain. Identifying molecules involved in this 'synaptic plasticity'—and targeting these signaling pathways with drugs—could pave the way to augmenting learning and memory in humans. This is particularly necessary for diseases that have been linked to deficits in synaptic plasticity and memory, such as Alzheimer's disease. Now, Masao Ito and colleagues at the RIKEN Brain Science Institute in Wako and at The University of Tokyo have shown that lipid signals regulate synaptic plasticity in the cerebellum, a structure at the back of the brain that is involved in motor learning[1].

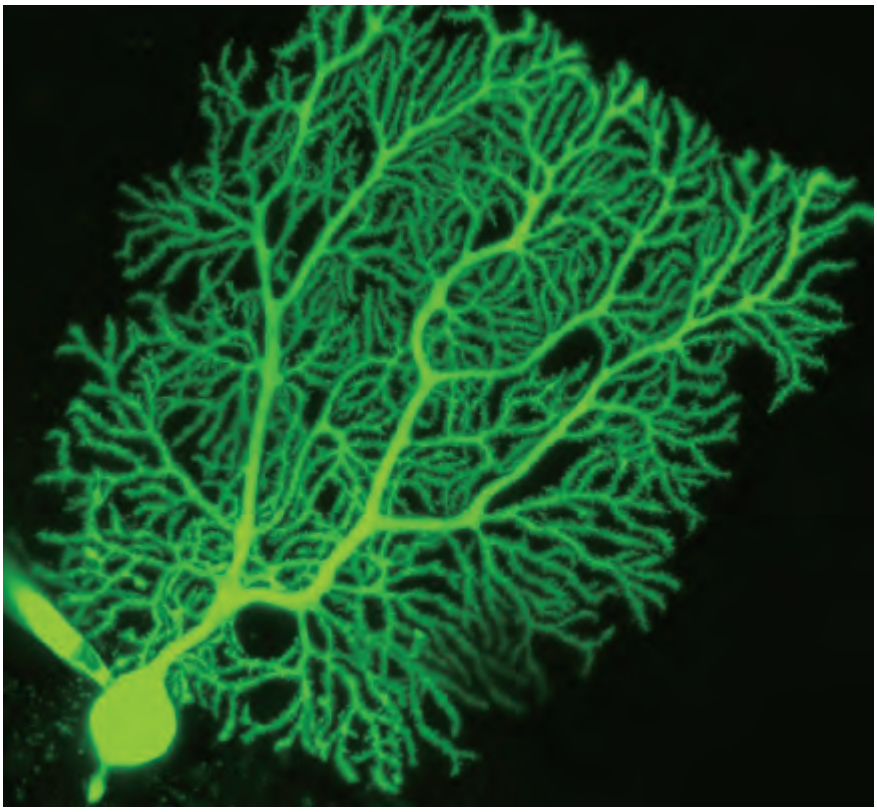

Figure 1: A rat Purkinje neuron injected with a fluorescent dye. Purkinje neurons are the major output neurons of the cerebellum.

Driving depression

The major output neurons of the cerebellum are called Purkinje neurons (Fig. 1). Repeated electrical stimulation of two separate neuronal inputs onto a Purkinje neuron—called conjunctive stimulation—leads to a reduction in this neuron's response to subsequent stimulation of one of these inputs. This kind of synaptic plasticity is called long-term depression (LTD) of the neuron's response. Purkinje neuron LTD has been linked to motor learning, an example of which would be learning to walk and run during early childhood in humans.

During repeated conjunctive stimulation of Purkinje neurons, calcium ions flow into the cell (Fig. 2). These ions activate a cascade of signaling molecules, including phospholipase A_2 (PLA_2), an enzyme that cleaves lipids in the membrane to release a compound called arachidonic acid (AA). Three subtypes of PLA_2 are present in Purkinje neurons.

While two of these isoforms seemed to play no role in LTD, the researchers observed that mice lacking the gene for the third isoform, called $cPLA_2\alpha$, exhibited no LTD in cerebellar brain slices after repeated electrical stimulation. Consistent with this finding, a drug called pyrrolidine-1, which blocks $cPLA_2\alpha$, also inhibited LTD. Because pyrrolidine-1 only inhibited LTD soon after conjunctive stimulation of the Purkinje neurons, the researchers realized that $cPLA_2\alpha$ activity was required—but just at that time—for induction of LTD.

Plasticity restored

Next, Ito and colleagues determined the role of AA in LTD because it is produced by PLA_2 along with many other lipid signaling molecules. When they infused AA during conjunctive stimulation of the cerebellar slices, they found it rescued LTD in mice lacking the gene for $cPLA_2\alpha$. However, adding AA onto cerebellar slices that had not been conjunctively stimulated failed to induce LTD on its own. This suggests that LTD also requires other signaling pathways induced by conjunctive stimulation.

Delving deeper into the machinations

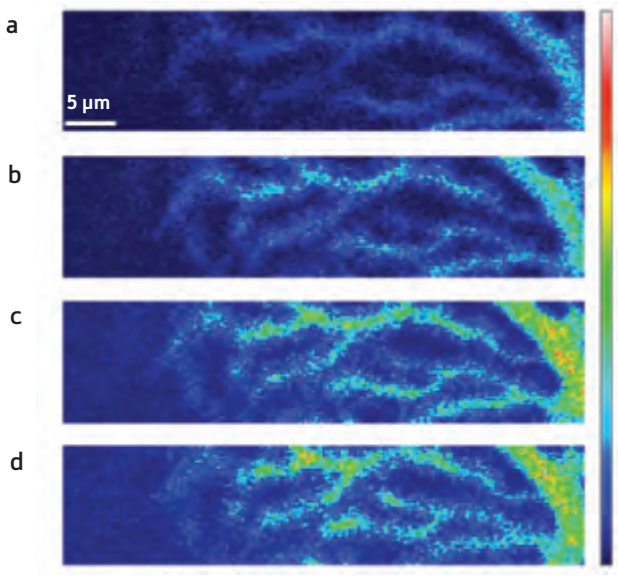

Figure 2: Images showing increasing calcium ion influx into the same area of a Purkinje neuron after conjunctive simulation. Image a was taken 0.5 minutes before simulation, and images b–d were taken at 0.5, 2.5 and 4.5 minutes after the start of simulation, respectively (scale bar, 5 um).

of the pathway, the researchers then examined the role of cyclooxygenase-2 (COX-2), which is an enzyme that acts on AA to produce additional lipid signaling molecules. They observed that, as with cPLA$_2$α inhibitors, COX-2 inhibitors blocked induction of LTD by repeated conjunctive stimulation of Purkinje neurons. Because the time during which COX-2 inhibitors could block LTD—right after conjunctive stimulation, but not later—was equivalent to the time during which the cPLA$_2$α inhibitors were able to prevent LTD, the researchers concluded that both enzymes, COX-2 and cPLA$_2$α, participate in the same signaling pathway to drive LTD induction.

Investigating further, Ito and colleagues then focused on prostaglandin (PG), which COX-2 produces from AA. They observed that PG rescued LTD not only in the presence of COX-2 inhibitors, but also in brain slices lacking the *cPLA$_2$α* gene. This suggests that this lipid signaling pathway induces LTD owing to its eventual production of PG. However, when the researchers treated conjunctively stimulated cerebellar slices from normal mice with PG, LTD was not enhanced any further. The team postulates that the PG that is created within the cerebellum during conjunctive stimulation—the 'endogenous' PG—induces as much LTD as is possible, and this is why adding extra PG will have no additional effect on further augmenting LTD.

PG binds to and activates various types of prostanoid receptors on the surface of cells. Interestingly, though, when the researchers treated conjunctively stimulated cerebellar slices with presently available inhibitors of these receptors, none of them affected LTD. Ito suspects "that the LTD induction is mediated by an as-yet-unidentified prostanoid receptor or by a direct interaction of PG with receptors for the excitatory neurotransmitter glutamate, which mediate the response of Purkinje neurons to stimulation of one of their inputs."

The eyes have it

Ito and colleagues next tested whether this lipid signaling pathway, which plays a key role in LTD induction, is also involved in the optokinetic eye movement response (OKR), a type of motor learning of the eye.

When observing an oscillating screen, mice learn to increase their eye movements over time as they follow the motion of the screen. This is called OKR adaptation. Scientists can measure these eye movements and then calculate the rate of OKR adaptation. In mice treated with COX-2 inhibitors, Ito and colleagues observed very low adaptation rates, suggesting that their motor learning of OKR was less robust than in untreated animals. Because COX-2 inhibitors also blocked LTD, these findings provide a strong link between defects in LTD and motor learning dysfunction.

If this signaling pathway is also involved in other types of learning and memory, targeting the pathway with drugs that activate it could be a way to drive learning and memory during disease. "Although it is still unclear how LTD is converted to a permanent memory," says Ito, "our findings add a novel element to the intricate signal transduction pathways for LTD induction." ■

1. Le T.D., Shirai, Y., Okamoto, T., Tatsukawa, T., Nagao, S., Shimizu, T. & Ito, M. Lipid signaling in cytosolic phospholipase A$_2$ α – cyclooxygenase-2 cascade mediates cerebellar long-term depression and motor learning. *Proceedings of the National Academy of Sciences USA* **107**, 3198–3203 (2010).

About the researcher

Masao Ito was born in Nagoya, Japan, in 1928. He graduated from The University of Tokyo with an MD in 1953, and received his PhD in 1959 from the same institution. Until 1962, he worked in the John C. Eccles laboratory at the Australian National University. He then returned to The University of Tokyo as an associate professor, became professor in 1970, and later served as medical dean from 1986 to 1988. He joined RIKEN in 1989, and became the founding director of the Brain Science Institute in 1997. After leaving the post in 2003, he has acted as a senior advisor. His achievements include discovery of the exclusive inhibitory nature of cerebellar Purkinje cells, establishment of the vestibulooocular reflex as a model system of cerebellar motor adaptation, and experimental verification of long-term depression in Purkinje cells and analyses of its signal transduction mechanism.

The geometry of randomness

Geometric factors can have a strong influence on the seemingly random walk of objects across pores in a thin membrane

When a butterfly flaps its wings in Europe, a storm may be caused in Asia. This often-used metaphor illustrates the strong resonances that may occur in random physical systems. Indeed, in systems affected by random effects, the influence of stimuli, such as external forces, on the eventual outcome is often poorly understood. An international team of researchers working at the RIKEN Advanced Science Institute in Wako has now discovered that geometric factors can play an important role in random processes such as the movement of a particle through a cell membrane[1].

One particular consequence of random effects is stochastic resonance, where the movement of an object between two states—for example, across a membrane partitioning a box—depends on the external force, or 'noise' applied to the system. Certain levels of noise may even amplify the response of the system, so that the particle in the box travels faster from left to right. "Stochastic resonance is a common effect in electrical circuits or in biological sensory organs, where it is used to increase the system's response," says Franco Nori, who led the research team.

Stochastic resonance is a purely random effect, although the size and shape of a variety of constrained biological systems, such as pores or channels, can influence the response of the system, according to team member Pulak Kumar Ghosh.

The researchers therefore studied the influence of geometrical effects on stochastic resonance (Fig. 1). "We considered systems where the membrane has different pore geometries," says Fabio Marchesoni, also from the team. In order to stimulate a geometric response, the researchers simulated the effect of an oscillating force perpendicular to the membrane that shakes the particles back and forth. Previous studies of stochastic resonance effects showed that a force that produces no net movement should have no influence on the particle transport. Yet, Nori and colleagues observed a strong influence on the frequency of the driving force as well as its amplitude. In addition, the shape of the membrane and that of the surrounding cavities plays a role in the efficiency of the particle transfer.

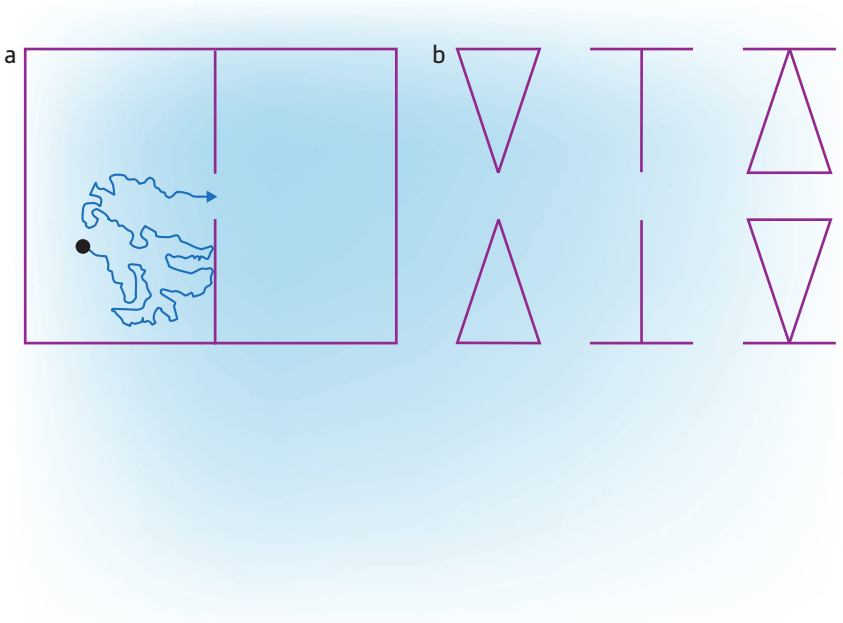

Figure 1: An illustration of stochastic resonance. (a) The path of the 'random walk' of a particle in a box with a partition that has one pore. (b) Three different geometries of the pore will have three different influences on the transfer of the particle across the membrane when an oscillating external field is applied to the system.

Owing to the geometric dependence of this effect, the researchers have coined it 'geometric stochastic resonance', and expect to find it in certain physical systems. Team member Sergey Savel'ev suggests that, "the transport of magnetic fields across superconducting samples with thin barriers may be a good first experimental example that demonstrates geometric stochastic resonance." ■

1. Ghosh, P.K., Marchesoni, F., Savel' ev, S.E. & Nori, F. Geometric stochastic resonance. *Physical Review Letters* **104,** 020601 (2010).

X-rays in a new light

The SPring-8 synchrotron opens the door to study the nonlinear interaction of high-intensity x-rays with matter

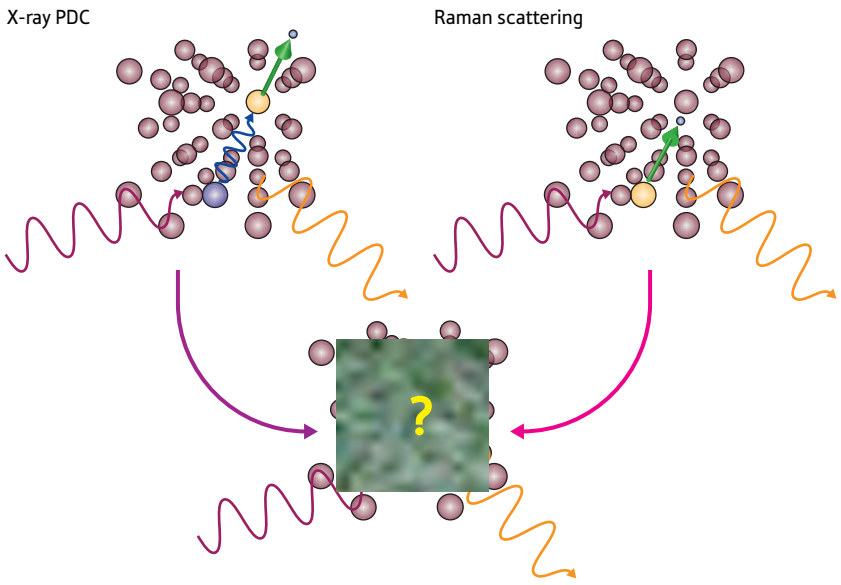

Figure 1: A schematic showing two competing processes to produce photons. The scattering of x-rays in diamond can either occur through a nonlinear optical parametric down-conversion (PDC) effect (left), or through a scattering effect of x-rays from electrons (right). Both processes can produce the same x-ray signal so an observer cannot distinguish which path was followed by the x-rays (lower part).

Visible light and x-rays are different types of radiation. Visible light, for example, doesn't penetrate the human body, whereas x-rays are absorbed weakly and can be used in medical imaging. Similar differences exist at very high light intensities, which make x-rays potentially useful in materials science, but this area—referred to as 'nonlinear optics'—remains largely unexplored. Now, researchers from the RIKEN SPring-8 Center in Harima have taken the first step in establishing a more systematic approach to studying nonlinear x-ray effects[1].

The team investigated the so-called parametric down-conversion of a single x-ray photon that splits into two separate photons, whose combined energy equals the original photon's energy. This effect was studied in a diamond crystal, which provided the medium for this process to occur. The necessary high intensity x-ray radiation came from the SPring-8 synchrotron, which is ideally suited for the task, according to Kenji Tamasaku from the research team. "It delivers some of the world's brightest x-rays."

However, a competing process can occur in addition to the down-conversion: the creation of only one x-ray photon and the simultaneous excitation of one of the material's electron to another state from the remainder of the original energy. An observer cannot distinguish which of these processes actually occurred in the material to produce outcoming photons of the same energy, which means that there is a quantum mechanical interference between both processes (Fig. 1). This is known as the Fano effect.

Tamasaku and colleagues studied the Fano effect for a range of parameters including x-ray photon energy. Based on theoretical modeling of a large dataset available from their experiments, they quantified efficiency of the nonlinear down-conversion process of x-rays for the first time. The possibility of this achievement had long been doubtful, as it requires not only a careful experimental calibration, but also very high x-ray intensities that are available at SPring-8.

The quantitative results for the nonlinear optical parameters of the down-conversion process are convincing and largely in line with theoretical expectations, even though some of the features observed remain poorly understood.

Nevertheless, Tamasaku is confident that "these results represent the first firm base from which to venture into the frontier of x-ray nonlinear optics." In particular, he is hopeful that the completion of a new x-ray laser called X-ray Free Electron Laser (XFEL) at SPring-8 next year will significantly expand the potential for the study of these non-linear optical effects. ■

1. Tamasaku, K., Sawada, K. & Ishikawa, T. Determining x-ray nonlinear susceptibility of diamond by the optical Fano effect. *Physical Review Letters* **103**, 254801 (2009).

Insights to air supply

The structure of a key membrane protein expressed in red blood cells could reveal how oxygen supply to tissues is regulated

Teruhisa Hirai of the RIKEN SPring-8 Center, Harima, and colleagues have obtained structural information about the physiologically important protein called erythrocyte anion exchanger 1 (AE1)[1].

As well as being expressed in the kidney, AE1 is the most abundant protein in the membrane of red blood cells, or erythrocytes, and helps prevent tissue damage by regulating oxygen supply.

Carbon dioxide, produced when food is burnt for energy, is released into the bloodstream and, on encountering erythrocytes, is converted into bicarbonate (HCO_3^-) by the enzyme carbonic anhydrase bound to AE1. In a crucial step—the 'chloride shift'—chloride is exchanged for HCO_3^- across the erythrocyte membrane via AE1. This lowers erythrocyte pH, resulting in the regulated release of oxygen from hemoglobin (Fig. 1).

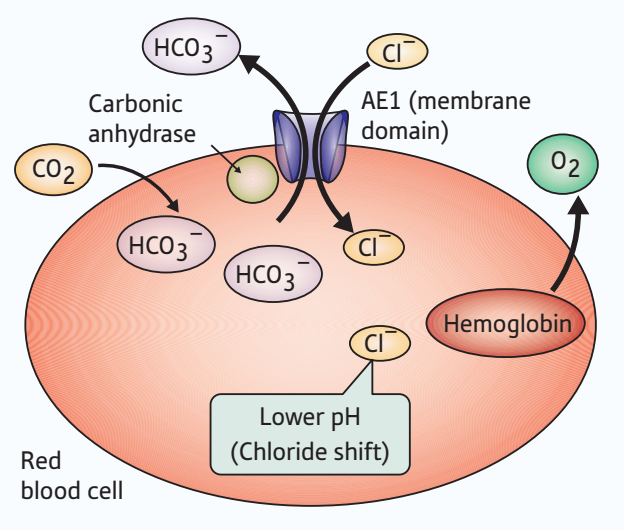

Figure 1: Schematic showing the exchange of bicarbonate (HCO_3^-) and chloride (Cl^-) ions via AE1, which promotes the release of oxygen from hemoglobin by lowering the pH within the red blood cell.

Despite its importance, there is limited structural information about AE1, particularly the membrane-spanning domain. "Like many membrane proteins, AE1 is very fragile, making structural information difficult to obtain," says Hirai.

Naotaka Hamasaki and colleagues at Nagasaki International University and Kyushu University purified AE1 stably with the membrane domain fixed in an outward-open conformation, and good two-dimensional crystals were prepared at RIKEN by Tomohiro Yamaguchi. "These were critical steps for structural analysis," explains Hirai.

The researchers merged 31 images, each taken from a different angle, to produce a three-dimensional image. Interestingly, they found structural similarities between AE1 and a bacterial transporter protein related to a class of chloride channels called 'ClC channels' found in animals, including humans.

DNA sequence information is available for the anion exchanger and ClC gene families, but the researchers are the first to uncover structural similarities between the encoded proteins.

ClC channels of mammals conduct chloride ions and are involved in regulating the electrical excitation of skeletal muscles. The bacterial protein, however, functions as a transporter, exchanging chloride ions and protons across the bacterial membrane.

"AE1 has a putative chloride binding site similar to that of the bacterial ClC protein, although this is yet to be proven biochemically," explains Hirai.

The observed resemblance between AE1 and ClC should help address the chloride transport mechanism, which is not well understood for either family.

"We need to improve the resolution of the current outward-open conformation structure of AE1 and solve the structure of the inward-open conformation to understand the conformational change during transport," Hirai notes.

AE1 mutations are associated with the human genetic disorders Southeast Asian ovalocytosis and distal renal tubular acidosis, so structural knowledge of AE1 might eventually lead to treatments. ■

1. Yamaguchi, T., Ikeda, Y., Abe, Y., Kuma, H., Kang, D., Hamasaki, N. & Hirai, T. Structure of the membrane domain of human erythrocyte anion exchanger 1 revealed by electron crystallography. *Journal of Molecular Biology* **397**, 179–189 (2010).

Unlocking the power of wood

A 'family' tree of enzymes from protists in the termite gut may help boost biofuels research

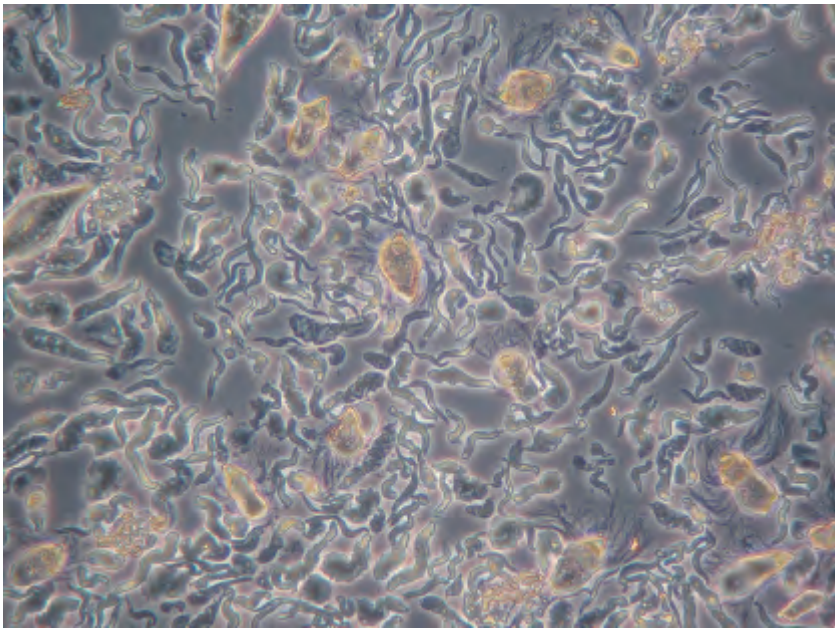

Figure 1: Micrograph of a diverse community of gut protists and bacteria of termites, which help them deal with the challenges of extracting nutrition from wood.

Wood-derived biomass offers a promising source for cellulose-based fuels, but efforts to exploit this energy have been thwarted by the need for methods to deal with a component of the plant cell wall that binds cellulose and interferes with enzymatic processing.

Termites have developed a natural workaround for this problem. Over the course of evolution, the various 'lower termite' species have formed an essential partnership with bacteria and protists dwelling within their gut (Fig. 1); these derive support from their termite hosts, and in turn facilitate the digestion of the insects' woody diet.

Glycosyl hydrolase family (GHF) enzymes produced by these symbionts are a key component in the cellulose digestion process, enabling efficient cellulose processing without the need for lignin breakdown. "Some of the enzymes that we have found have more than 10-fold higher activity than current industrial enzymes," says Shigeharu Moriya of the RIKEN Advanced Science Institute in Wako. Since 2001, Moriya and colleagues have been working to characterize these enzymes, and they have now published their analysis of the various GHFs expressed within the gut protist communities of four lower termite species as well as a related wood-eating cockroach[1].

These protists are exceptionally challenging to culture and analyze individually, but can be characterized collectively via 'metatranscriptome' techniques that make it possible to assemble massive gene catalogues from a diverse mixture of cell types. This approach revealed a total of 154 clones representing variants of five different GHFs, and the researchers used this sequence data to assemble a phylogenetic tree—essentially a detailed timeline of the evolutionary history of these genes.

GHF5 and 7 were represented in every termite symbiont community investigated, suggesting that their evolution either precedes or coincides with the emergence of termite–protist symbiosis. Interestingly, the data suggest that GHF5 may have been initially acquired by protists from bacteria over the course of one or more ancient gene transfer events. GHF7, on the other hand, appears to have evolved specifically within protists.

The other three enzyme classes—GHF10, 11 and 45—are less broadly conserved, and the author speculate that they provide support for the core GHF5–GHF7 cellulose degradation machinery. "This system is well conserved among various termites, and it may be composed of high-performance enzymes," says Moriya. His team is now partnering with with other RIKEN teams and teams outside RIKEN to develop novel techniques for characterizing the metabolic pathways of these protist communities in an effort to identify additional factors that expedite biomass processing. ■

1. Todaka, N., Inoue, T., Saita, K., Ohkuma, M., Nalepa, C.A., Lenz, M., Kudo, T. & Moriya, S. Phylogenetic analysis of cellulolytic enzyme genes from representative lineages of termites and a related cockroach. *PLoS ONE* **5**, e8636 (2010).

All natural ingredients

A catalog of the chemicals produced within a plant's tissues yields fresh insights into its metabolic pathways and gene function

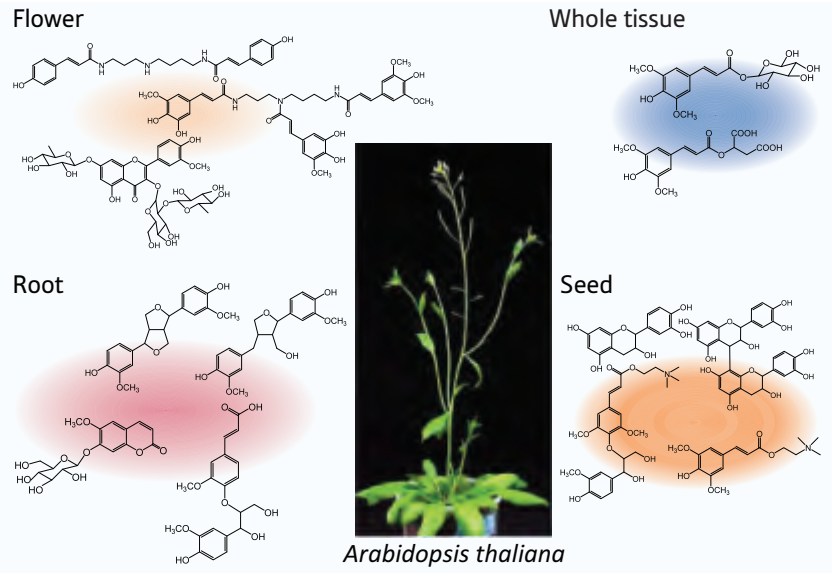

Figure 1: As revealed by the AtMetExpress project, various tissues of *Arabidopsis thaliana* contain compounds associated with metabolism that are diverse in structure.

The various metabolic pathways in a given plant generate a staggering array of molecules that enable growth and survival under diverse conditions—and in some cases, hold value for scientific applications ranging from pharmaceutical research to the development of new materials. "The huge chemical diversity of plants exceeds that of most animals and microorganisms," says Kazuki Saito of the RIKEN Plant Science Center in Yokohama.

The thale cress, *Arabidopsis thaliana*, is a widely used model for genetic and developmental research and possibly the best characterized of all plant species; nevertheless, scientists remain far from completing a comprehensive 'metabolome', or atlas of metabolites, for this organism. The AtMetExpress project, launched by Saito and colleagues, seeks to rectify this situation by assembling a massive, annotated roster of molecules gathered from 36 different *Arabidopsis* tissue samples[1].

"What we needed was the pattern of metabolite accumulation during plant development to understand cell function more precisely," explains Saito. To start with, his team used a method called liquid chromatography-mass spectrometry (LC-MS) to derive information about the chemical content of a variety of plant organs collected at different developmental stages. The researchers subsequently cross-referenced these against a library of tandem mass spectrometry spectral tags (MS2Ts)—essentially an index of the individual compounds that can be detected in *Arabidopsis*. By this process, they were able to assign unique MS2Ts to approximately 95% of the molecules detected via LC-MS and subsequently managed to derive structural information for a total of 167 metabolites (Fig. 1).

A comparison of the spatial and temporal distributions for these various metabolites with detailed datasets describing gene expression in *Arabidopsis* allowed Saito and colleagues to obtain new insights into metabolic regulation, revealing several especially complex pathways where levels of a given metabolite were seemingly decoupled from expression levels of the genes involved in its synthesis, suggesting the existence of potentially diverse additional modes of control.

Saito and colleagues were also able to assign roles to previously unknown putative components of biosynthetic pathways. "We found a number of correlations between metabolite peaks and uncharacterized genes," says Saito. "These are potential targets for discovery of new genes involved in metabolite production."

This first iteration of AtMetExpress will serve as a foundation for more targeted future investigations, including the exploration of metabolic pathways triggered by the activity of specific plant hormones, and ultimately may lead the way for similar analyses of other plant species with important agricultural or medicinal applications. ■

1. Matsuda, F., Hirai, M.Y., Sasaki, E., Akiyama, K., Yonekura-Sakakibara, K., Provart, N.J., Sakurai, T., Shimada, Y. & Saito, K. AtMetExpress development: A phytochemical atlas of *Arabidopsis thaliana* development. *Plant Physiology* **152**, 566–578 (2010).

The ABC of a stress response

The identification of a gene involved in the response of plants to water stress should help breed better crop varieties

When plants become desiccated, tiny leaf pores collectively called stomata close to conserve water. Each stoma is flanked by a pair of 'guard cells', which change shape to open or close the central pore.

This stress response involves the plant hormone abscisic acid (ABA). When produced in vascular tissues, ABA can act locally within cells via several known ABA receptors. However, to act on distant targets such as guard cells, this hormone must first be released from ABA-producing cells, raising the question of how it crosses the outer cell membrane.

To address this, Takashi Kuromori and colleagues of RIKEN Plant Science Center, Yokohama, screened over 12,000 lines of *Arabidopsis*—a commonly used plant model—for ABA-related mutants. Their identification of a mutant hypersensitive to ABA at the germination and seedling stages has led to the isolation of a gene encoding a type of 'ATP-binding cassette (ABC) transporter'[1].

ABC transporters use chemical energy stored in the biological molecule ATP to transport molecules across cell membranes and are present in organisms from bacteria to animals, including humans, but are especially prevalent in plants.

"*Arabidopsis* has 130 ABC transporter genes, while rice and beans each have more than 100, which means plants have 2–3 times more than other species," says Kuromori."We believe that some kinds of ABC transporters evolved to have important plant-specific functions in plant development and physiological regulation."

The newly identified transporter, AtABCG25, is expressed mainly in vascular tissues, such as roots and leaf veins. Importantly, this transporter localizes to the outer cell membrane. Some types of ABC transporter localize to membranes surrounding structures within cells which means that they could not transport ABA out of ABA-producing cells to be released into the spaces between cells.

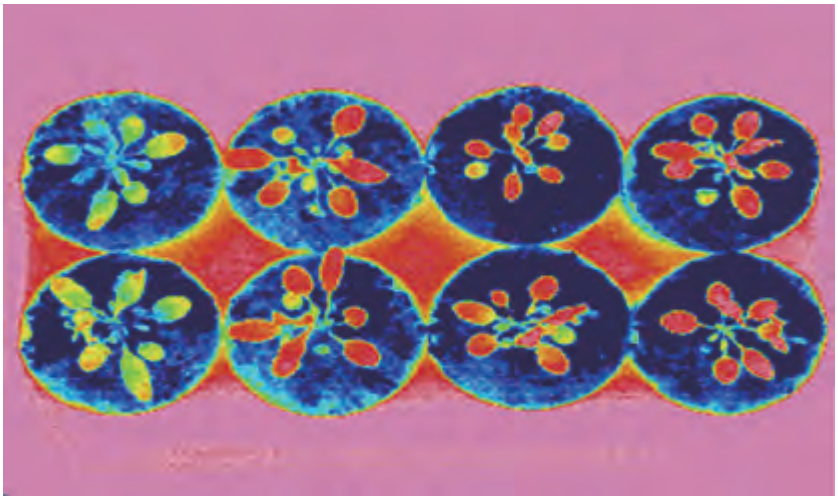

Figure 1: Thermal images of two control plants (left) and six 4-week-old AtABCG25-overexpressing plants (right), captured by an infrared thermography device.

Plants genetically engineered to over express AtABCG25 had higher leaf temperature compared to normal plants (Fig. 1), and decreased water loss from isolated leaves. The researchers believe that ABA built up in the guard cells of the engineered plants, causing enhanced stomatal closure.

Although it remains unclear how ABA reached the guard cells from vascular tissues, they hope that their findings will lead to the breeding of stress-tolerant crops.

"To date, research on plant stress tolerance has focused on ABA synthesis and/or the expression of ABA target genes," says Kuromori. "However, our results suggest the possibility of establishing methods to control ABA transport and migration, which could lead to new techniques for breeding stress-tolerant plants." ■

1. Kuromori, T., Miyaji, T., Yabuuchi, H., Shimizu, H., Sugimoto, E., Kamiya, A., Moriyama, Y. & Shinozaki, K. ABC transporter AtABCG25 is involved in abscisic acid transport and responses. *Proceedings of the National Academy of Sciences USA* **107**, 2361–2366 (2010).

Nano-aquarium opens up a new realm of research into microorganisms

A microchip fabricated with femtosecond lasers at RIKEN allows the rare observation of microalgae behavior

Microalgae are photosynthetic organisms that appeared on Earth more than one billion years ago—the most primitive living things known. Cyanobacteria, Euglena and other members of this family typically inhabit in the sea or fresh water, and possess a very simple, unicellular form. Some of them are even able to move or glide using tiny appendages known as flagella. Perhaps the easiest and simplest example for use in scientific experiments for school children, algae are also in high demand for next-generation industrial research and development as a raw material for the production of biofuel.

At RIKEN, researchers are unraveling a variety of hidden functions of single-cell, flagellated algae that swim in fresh water using a newly developed 'nano-aquarium' (Fig. 1). Far from an ordinary fish tank for ornamental purposes, the nano-aquarium is actually a tiny microchip; a glass plate just five square millimeters in size embedded with flow channels and micro-devices. The algae, which normally jump around at lightning speed, move within the tightly controlled channels, and sometimes are given physical stimuli using a movable micro-needle in order to observe their response. Such nano-manipulation techniques provide great assistance for analyzing the detailed mechanisms of algae using RIKEN's cutting-edge optical microscopes, opening up a new realm of biological and evolutional research on these ancient microorganisms.

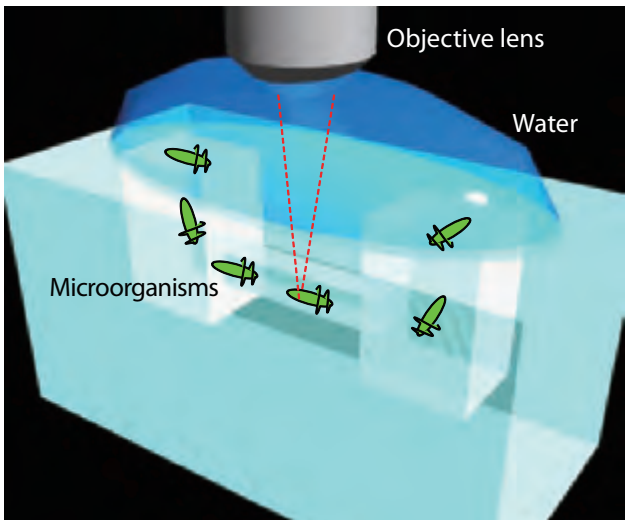

Figure 1: The nano-aquarium glass microchip confines microorganisms within an embedded channel, allowing their behavior to be captured easily.

The great potential of algae

The nano-aquarium project was initiated by Hiroyuki Kawano, a research scientist specialized in laser physics in the Laboratory for Cell Function Dynamics of the RIKEN Brain Science Institute in Wako, Saitama. Kawano joined the laboratory in 2003 to take advantage of his expertise gained at the Laser Technology Laboratory (headed by Katsumi Midorikawa) of the RIKEN Advanced Science Institute, also in Wako. The Laboratory for Cell Function Dynamics, headed by Atsushi Miyawaki, is at the world's forefront in the development of fluorescence proteins and related optical technologies.

In 2005, Miyawaki's laboratory created a high-performance video microscope for in vivo observation of neurons in collaboration with Olympus and Kinki University. Ikuko Ishikawa, a former professor of Tokyo Gakugei University and specialist in algal physiology, was invited to act as a research scientist on the project with the aim of utilizing micro-organisms as a sample to maximize the performance of the new device. However, "soon after we started experiments we found it very difficult to capture clear images of microalgae even with our cutting-edge microscope because some algae move around too fast to be observed, and others make unexpected movements," Ishikawa says. "So, I consulted with my colleagues to see whether we can control their behavior." Kawano adds.

The result of those consultations was the nano-aquarium project, which was awarded funding under the category of 'challenging research' from the President's Fund. The project, which kicked off in 2006, was initiated by four researchers—Kawano and Ishikawa from the Laboratory for Cell Function Dynamics, and Koji Sugioka, a senior research scientist, and Yasutaka Hanada, a special postdoctoral researcher, from the Laser Technology Laboratory (Fig. 2).

Direct fabrication of three-dimensional voids using femtosecond lasers

The development of the nano-aquarium microchip would not have been achieved without Sugioka's femtosecond laser expertise. Ultrashort light pulses can be focused into a spot with a diameter of just 0.3 micrometers, equivalent to the length of a virus. These ultrashort pulses of light make it possible to achieve the processing precision necessary to fabricate the fine channels and voids that make up the nano-aquarium. Longer pulses of light, such as those produced by conventional lasers, cause heating in the materials leading to thermal expansion and cracking. Femtosecond lasers are therefore becoming known as the next-generation technology for shaping glass, semiconductor and even diamond with nanoscale precision.

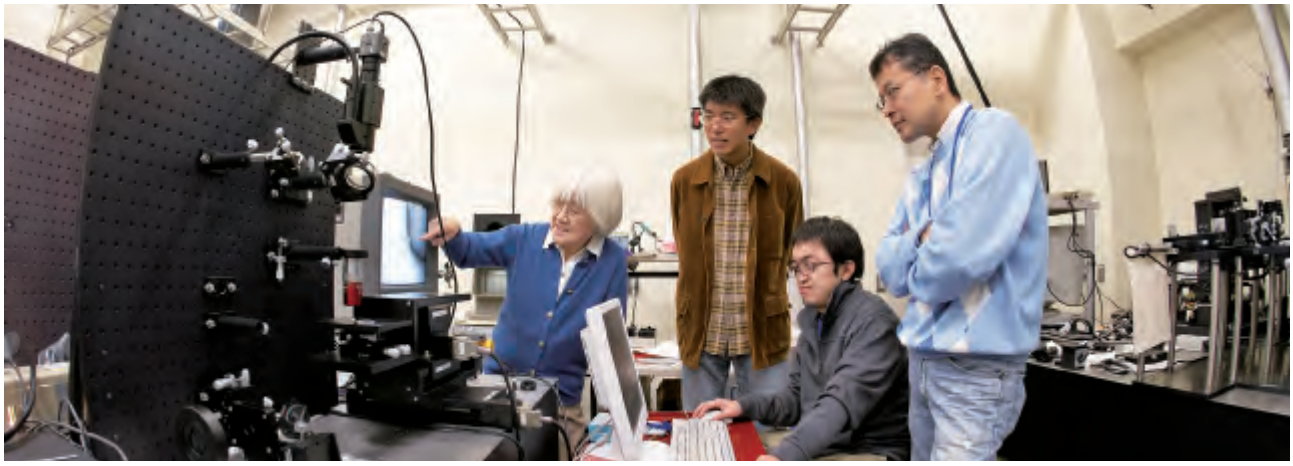

Figure 2: RIKEN's laser physicists (from right, Koji Sugioka, Yasutaka Hanada and Hiroyuki Kawano) and Ikuko Ishikawa (left) continue to uncover interesting behaviors of the most ancient of Earth's organisms.

Around 2002, Sugioka started creating three-dimensional tunnels inside a glass plate by shifting the focal point of laser beam a fraction at a time. The fabrication of voids is completed by immersing the patterned glass plate in acid solvent, which etches away the glass around the tunnels. The manufacturing process for the nano-aquarium differs from the process used to fabricate common microfluidic chips, which are fabricated using semiconductor-etching technology and are used to control and analyze the biological and chemical properties of a tiny amount of fluid sample.

Based on his laser-processing expertise, Sugioka joined hands with Hanada to develop the nano-aquarium. Sugioka recalls that one of the greatest technical challenges was to create channels with a square cross-section in order to give the observer a clearer view of a sample movement under the microscope. Hanada also developed a built-in, movable needle that researchers can use to stimulate the algae samples. "By trial and error I adjusted the beam strengths, exposure time and pulse length to process the nano-device in just the right way," he says. Sugioka adds that they can even make a micro-wheel to generate water flow in the channel.

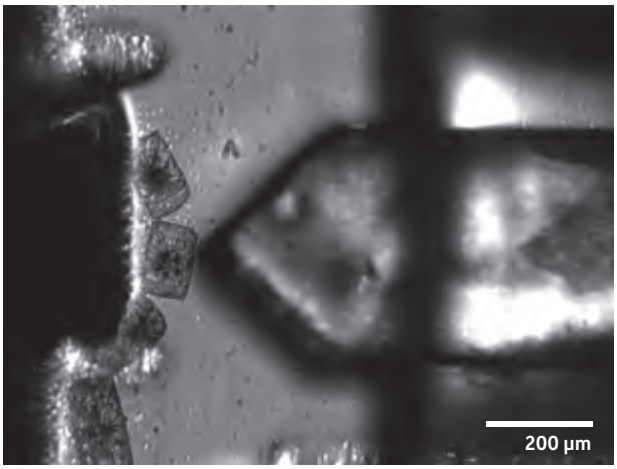

Figure 3: When stimulated by a micro-needle, a unicellular alga responds by assembling chloroplasts, spurring the same reaction in one cell after another.

Unraveling the secrets of algae

Kawano's team has been developing a number of different microchips, each tailored to a different sample, because the design of channels and microdevices determines the angle of observation. "It was fascinating to see directly and clearly how chloroplasts get assembled in the middle of algae when stimulated with a micro-needle," Ishikawa says (Fig. 3). Kawano has recently discovered that another type of algae uses its flagella in a different manner to what had been long believed. "We could confirm the finding because we made it swim vertically in the H-shaped channel. Other researchers would have no choice but to observe it two-dimensionally in a petri dish," Kawano notes.

The team published a joint paper[1] in 2008, and although the support of the President's Fund ended in March 2009, the researchers continue to work together to polish the quality of the microchips and uncover more truths about algae using their own research funding. "I am grateful for our team's enthusiasm for collaboration," Ishikawa says. "Thanks to them, we have now entered a new stage in the dynamic and beautiful world of colorful microorganisms." ■

1. Hanada, Y., Sugioka, K., Kawano, H., Ishikawa, I. S., Miyawaki, A. & Midorikawa, K. Nano-aquarium for dynamic observation of living cells fabricated by femtosecond laser direct writing of photostructurable glass. *Biomedical Microdivice* **10,** 403–410 (2008).

Molecular imaging opens up a vast new world for neuroscience

Advances in molecular imaging reveal startling insights into the workings of the brain and could make it possible to diagnose migraine definitively for the first time

Yosky Kataoka

Team Leader
Cellular Function Imaging Laboratory
RIKEN Center for Molecular Imaging Science

Molecular imaging allows molecules in a living organism to be visualized, and provides a means of observing the distribution and behavior of molecules. One of the most exciting applications of this technology is the ability to examine a patient internally without affecting the subject. "The biggest advantage of molecular imaging using positron emission tomography lies in its applicability to humans." says Yosky Kataoka, team leader of the Cellular Function Imaging Laboratory at the RIKEN Center for Molecular Imaging Science. Molecular imaging using positron emission tomography (PET) is expected to contribute to the diagnosis of disease, as well as to our understanding of pathologic conditions and therapeutic effects, and to the development of new drugs. It is already being used to diagnose Alzheimer's disease and cancer. In October 2009, Kataoka's laboratory announced a groundbreaking achievement that could lead to the development of a diagnostic method for migraine. While many people suffer from migraine, no objective method for diagnosis or treatment has been found so far. Their achievement is attracting attention as a discovery that should dramatically change this situation.

Origins in an experiment conducted ten years ago

Severe pulsing pain on one side of the head. This is the most obvious symptom of a migraine, but it can also be accompanied by vertigo and nausea. If aggravated, this complex of symptoms interferes with daily activities, as any chronic sufferer will attest. "The pain of a migraine is thought to be triggered by repeated contraction and dilation of the blood vessels in the brain. However, the pathology remains unclear, so there is still no objective method for migraine diagnosis," says Yosky Kataoka. "We have made a discovery that will dramatically change the situation."

In October 2009, Kataoka and his colleagues made the headlines with their achievement in 'Unlocking the Mysteries of the Brain with PET'. Their research was published in the November issue of the Journal of Nuclear Medicine in the US. "PET performed on rats enabled us to confirm that a phenomenon known as spreading depression is followed by activation of the microglia, which mediate inflammatory reactions in neural tissue. Our achievement is attracting attention because it is expected to give us an understanding of the pathology of migraines, and their diagnosis and treatment."

Kataoka's link between spreading depression and migraine emerged from an experiment he conducted while at the Osaka Bioscience Institute. "Everything traces back to an experiment I conducted ten years ago," he says.

First encounters with spreading depression

In 2000, while investigating how light could be used to control central nervous activities, Kataoka developed a photo-oxidation method to suppress the signaling function of synapses. Neurons in the brain release neurotransmitters from the axon termini, which extend from the neuron body and transmit information to adjacent neurons. In the photo-oxidation method, a photosensitizing dye is administered to the brain, which causes oxidative stress when exposed to light through the generation of reactive oxygen species. This oxidative stress suppresses the signaling function of synapses, but only at the site exposed to light, and the site gradually returns to its original state when the light is removed. This method attracted attention as a groundbreaking approach for examining localized

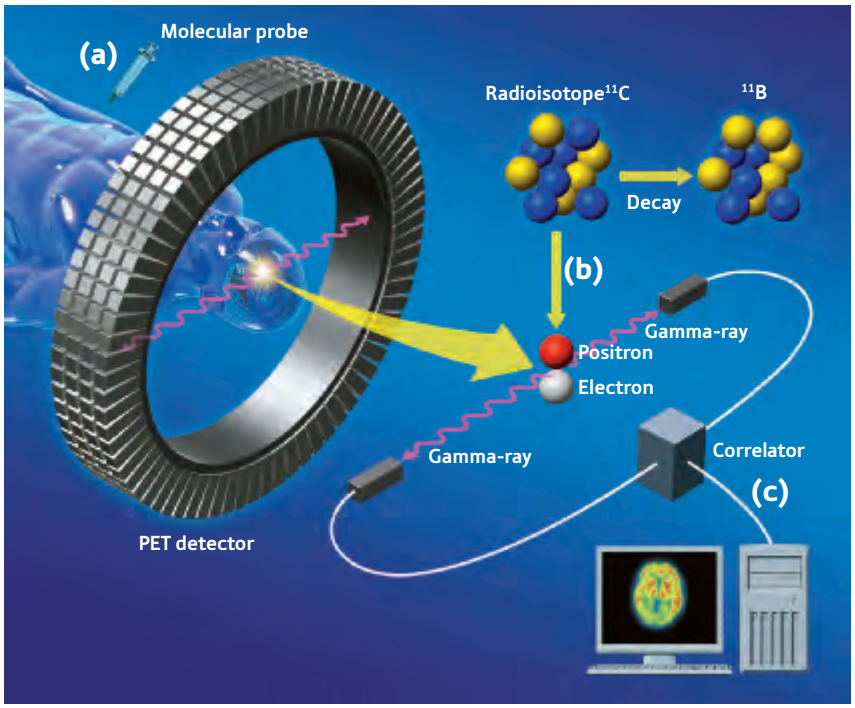

Figure 2: Molecular imaging by PET.
(a) When a specific molecule in a living organism is to be examined, a molecular probe is prepared by attaching a radioisotope to a molecule that binds only to the target molecule, and the probe is administered to the organism. (b) As the atomic nucleus of the radioisotope decays, positrons are emitted. (c) The positrons collide with ambient electrons to produce gamma-rays, which are counted and visualized, showing where and how abundantly the target molecule is present in the organism.

functions of the neural network.

Kataoka later used the photo-oxidation method in a PET study of how the neural function of the monkey cerebral cortex could be suppressed topically. PET is one of the most powerful tools for monitoring the distributions and functions of molecules in living organisms, and involves administering a molecular probe—a site-targeting molecule bearing a radioisotope—to the subject organism. The positrons, or 'positive electrons', emitted by the radioisotope collide with ambient electrons to generate gamma rays, which are then counted and visualized (Fig. 1). The molecular probe used by Kataoka was fluorodeoxyglucose (FDG), which is prepared by attaching fluorine-18 (^{18}F) to deoxyglucose. This probe allows the metabolism of glucose to be examined, and is also used in the PET diagnosis of cancer.

"Neurons in action exhibit high levels of glucose metabolism and take up large amounts of FDG. PET imaging revealed a major reduction in FDG uptake at the photo-oxidized sites because the synaptic function had been suppressed. I was planning to announce the findings with the headline: 'Suppression of central nervous function by photo-oxidation confirmed by PET,' but I encountered an unexpected and mysterious phenomenon." PET images taken just after photo-oxidation revealed suppressed neural metabolism only at the photo-oxidized sites. One day later, however, FDG uptake decreased and neural metabolism was suppressed over the entire hemisphere of the cerebral cortex on the photo-oxidized side. At the same time, the opposite hemisphere remained normal. "I could not understand why that happened, and remained puzzled for the next two years."

It turned out the suppression of neural metabolism over the entire hemisphere cortex was caused by spreading depression. "When neural tissue is exposed to severe oxidative stress, cations flow into the cells, and the potential in the cells rises for a short time; this phenomenon is called depolarization. When a population of cells undergoes depolarization, the phenomenon propagates like waves at a speed of two to three millimeters per minute, causing metabolic suppression in the path of the depolarizing waves. The depolarization waves spread over the entire hemisphere of the cortex, but are never transmitted to the opposite hemisphere. Although spreading depression was discovered back in 1944, it had not attracted the attention of researchers in brain science until only recently." Kataoka attempted to induce spreading depression artificially using his photo-oxidation method and to examine what occurs in detail (Fig. 2).

Deep sleep induced by over-excitation

Rats affected by spreading depression quickly become drowsy and fall asleep. Puzzled by this finding, Kataoka and others examined rats with spreading depression and found that levels of the enzyme cyclooxygenase 2 (COX2) increased after a spreading depression event. COX2 produces large amounts of prostaglandin, a hormone-like substance that induces deep sleep. Kataoka explains, "I suppose that having a deep sleep allows the rat to rest its over-stimulated brain and restore its functions."

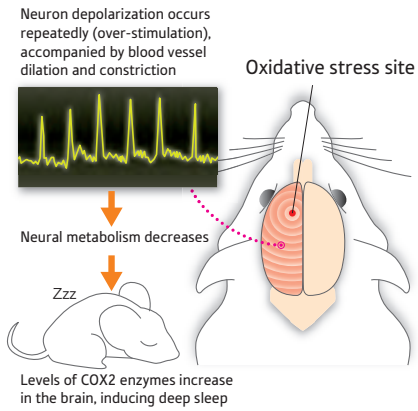

Figure 2: Spreading depression.
Starting at the site exposed to oxidative stress (red circle), depolarization propagates in waves at speeds of 2–3 mm per minute. The waves of depolarization spread over the entire hemispherical cortex, but are never transmitted to the opposite hemisphere. Neurons are over-stimulated by the depolarization, and the episode is followed by a reduction in neural metabolism.

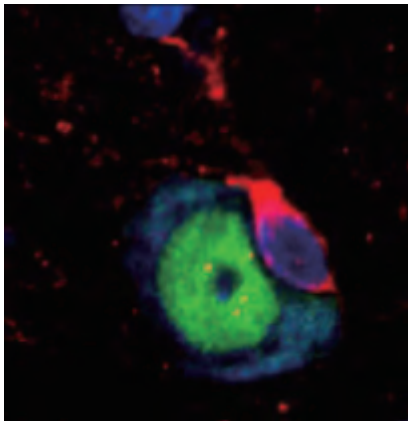

Figure 3: A perineuronal progenitor cell. Photomicrograph of a perineuronal progenitor cell (red) obtained using a confocal laser microscope. The nucleus of a mature neuron is shown in green. The perineuronal progenitor cell divides and propagates by spreading depression. Perineuronal progenitor cells are not only capable of self-replication, but also have pluripotency for differentiation into glial cells and neurons.

The same effect, drowsiness after tackling something difficult, is also commonly felt by humans, and occurs because prostaglandin is produced as a result of over-stimulation of neurons. However, being busy or being absorbed in something bypasses the sleep impulse. "Going without sleep for a long time is bad for brain functioning," warns Kataoka. "The brain becomes fatigued, and the nerves are impaired functionally. One human study shows that learning is more effective if you have a sleep after the learning process. 'Before an exam, don't study through the night; instead get a good night's sleep' is good advice from the viewpoint of brain science."

Discovering the dual differentiation of perineuronal progenitor cells

Deep sleep was not the only state found to be caused by spreading depression. "Newborn cells are produced in the brain following spreading depression," says Kataoka. "We speculate that when the brain takes a rest, it proactively rebuilds neural tissue to increase its resistance to stress."

In the brain, neurons are responsible for information transmission, and glial cells act to deliver nutrients to the neurons and in their way support information transmission. It had been thought that neurons are not regenerated, except in the hippocampus and olfactory bulb, whereas glial cells are regenerated in all portions of the brain and are renewed in entirety over a year or so.

However, this consensus was upset by a study reported by Kataoka and his colleagues. They examined the brain to determine which cells divide and what kinds of cells they differentiate into, in the context of spreading depression, using a confocal laser microscope (Fig. 3). "We found that dividing cells were present in the vicinity of neurons. But rather than merely occurring in the vicinity of the neurons, the cells actually invaded the neurons. It was commonly believed that the dividing cells are the progenitors of glial cells, and that all progenitor cells differentiate into glial cells. The reality, however, was not like that." Monitoring dividing cells revealed that most of the progenitor cells underwent self-replication, some became glial cells, and a small number, less than 1%, became juvenile neurons. Surprisingly, the progenitor cells proved to be capable of differentiating into both neurons and glial cells.

"Cells capable of self-replication and differentiation into both glial cells and neurons seem to be the tissue stem cells of the nervous system. We call the cells 'perineuronal progenitor cells', and they are found throughout the entire brain and spinal cord, including the cerebral cortex. Neurons are constantly produced in areas other than the hippocampus and olfactory bulb. This is a major discovery that upsets conventional knowledge."

Although research into perineuronal progenitor cells has only just begun, some interesting results have already been reported. One example is the effect of aging. There are two types of cerebral cortex: the limbic cortex involved in the control of instincts and emotions, and the neocortex involved in higher attributes such as information processing and cognition. Examination of rats bred until old age has shown that perineuronal progenitor cells in the neocortex—the part of the brain involved in higher attributes such as information processing and cognition—decreases significantly with age. This is not the case for the limbic cortex—the part of the brain associated with instincts and emotions. The number of neurons, however, was found to remain unchanged, departing from the general opinion that the number of neurons decreases with age. It has also been found that the brain begins to atrophy when the number of perineuronal progenitor cells starts to decline. "Unlike in the neocortex, the perineuronal progenitor cells in the limbic cortex decrease very little in number with age. I can't help thinking that this may be related to the fact that grandparents continue to feel love towards their grandchildren, while at the same time their capabilities for calculation and memory diminish with age. I suspect that perineuronal progenitor cells are one of the keys to brain senescence. By stopping the reduction in their number, functional impairments that occur with aging may be prevented. With this thought, I am continuing my research."

Toward PET diagnosis for migraine

Spreading depression was not well-known ten years ago, when Kataoka encountered the phenomenon. However, the situation has changed recently. "The involvement of spreading depression in the onset of migraine has been confirmed in humans, and the phenomenon is attracting attention."

Scintillating scotoma is one of the symptoms that can affect migraine sufferers at the onset of a migraine attack. This symptom appears to the sufferer as a field of shimmering lights that expands to create what is commonly known as the migraine aura—localized temporary blindness. When converted to the speed of transmission of nerve excitation in the visual field of the brain, the speed at which the light field expands turns out

to be the same as the transmission speed of spreading depression. The feeling of pain only on one side of the head is also consistent with the fact that spreading depression is not transmitted from one cerebral hemisphere to the other. "When spreading depression is transmitted, the blood vessels in the brain contract and dilate repeatedly. During this time, plasma proteins leak from the vessels, causing immune responses in the neural tissue similar to those during inflammation, and these responses are transmitted to the sensory centers to produce pain. This is the pathologic mechanism of migraine suggested by current research."

Kataoka and his colleagues decided to use PET to confirm that immune responses are actually caused by spreading depression in neural tissue in rats. "Spreading depression is a good target for molecular imaging." In such experiments, it is important to compare treated animal subjects with their non-treated counterparts. It is difficult, however, to make an individual-based comparison of constantly changing brain activity. Fortunately, in the case of spreading depression, responses are not transmitted to the opposite hemisphere, so that a simultaneous comparison can be made between the left and right hemispheres of the brain in the same individual.

Immune responses in neural tissue are mainly associated with microglia, a type of glial cell. With this in mind, Kataoka and his colleagues constructed a molecular probe by attaching the radioisotope carbon-11 (^{11}C) to PK11195, a molecule that binds only to activated microglia. In the experiments, they induced spreading depression in the left hemisphere of the cerebral cortex, and visualized the distribution of the ^{11}C-PK11195 molecular probe. They found that the molecular probe accumulated in part of the left cerebral cortex. "This demonstrates that microglia activation, or brain immune responses, were caused by spreading depression."

These are Kataoka's latest findings, and he is already moving on to the next stage. "In collaboration with universities, we are planning to use PET as a diagnostic tool for patients where excess immune responses in the brain are suspected of being involved in nervous diseases. Because ^{11}C-PK11195 is a molecular probe for which safety in humans has already been established, our plan can be carried out after testing has been approved. Provided that microglia activation is confirmed in humans as well, a drug that suppresses immune responses can be used therapeutically. If testing is possible on patients in the future, they will be able to receive the appropriate treatment that distinguishes between migraines and other types of headache."

The idea is emerging that immune responses in the brain are involved in many diseases, including depression, schizophrenia and chronic fatigue syndrome. Additionally, Kataoka's most recent study has yielded results suggesting the possible involvement of perineuronal progenitor cells in immune responses in neural tissue. This finding is attracting attention that will encourage further research into spreading depression and perineuronal progenitor cells, and related medical applications.

Linking perineuronal progenitor cells to regenerative medicine

Kataoka says with a laugh, "I am now going in a totally different direction from what I wanted to do initially. I encountered spreading depression, a phenomenon that was initially difficult to explain, and it has led to investigations into sleep induction, perineuronal progenitor cells and migraine. How can I correlate these different pieces of information? I would like to emphasize this. Realizing that things that have happened unexpectedly are linked and working under the same single principle makes me very happy. I want to use PET to examine perineuronal progenitor cells and understand their behavior and function. In the future, molecular imaging may help us to understand the mechanisms behind aging, and lead to the discovery of a method to prevent aging-related functional deterioration. Any method that increases the number of perineuronal progenitor cells or boosts their differentiation into neurons will lead to regenerative medicine." However, there is as yet no molecular probe that can be used to monitor perineuronal progenitor cells. "Although it is quite difficult to prepare a new molecular probe and examine molecules using it, there is a good chance that we can do it. Here at the RIKEN Center for Molecular Imaging Science, researchers working in a broad range of fields, including biologists who search for molecules that bind to specific targets, chemists who prepare molecular probes by attaching a radioisotope to molecules, and physicists and technologists who develop equipment and instruments, are readily at hand. This means that we have the chance to do unique work on new and challenging issues." ■

About the researcher

Yosky Kataoka was born in Kyoto, Japan, in 1965. He graduated from the Faculty of Medicine, Shiga University of Medical Science, with an MD degree in 1992, and proceeded to the Kyoto University Graduate School of Medicine in the same year. After obtaining his PhD in 1996, he was awarded the position of research scientist in the Department of Neuroscience of the Osaka Bioscience Institute, where he developed photo-dynamic techniques for controlling the central nervous system. In 2001, he joined the Department of Anatomy and Cell Science of Kansai Medical University as assistant professor, and in 2005, moved to the Department of Physiology at the Osaka City University Graduate School of Medicine. In April 2009, he began as head of his own research team at the RIKEN Center for Molecular Imaging Science, and is now investigating new strategies for treating neurological disorders and malignancy using molecular imaging techniques. His interests focus on understanding the integrated systems of the brain through multi-modal analyses of functioning molecules, neurotransmission and behavior based on a combination of molecular imaging and other experimental techniques including electrophysiology.

Toward a more responsible approach to research

In April 2005, as part of efforts to combat all types of misconduct, particularly as it occurs in research, RIKEN established the Auditing and Compliance Office, a center tasked with the duty of coordinating a centralized response to suspicions of misconduct when they first arise. RIKEN's Board of Executive Directors adopted a set of basic policies regarding research misconduct, defined as the 'fabrication', 'falsification' and 'plagiarism' of research papers and data, and proposed a plan for how RIKEN should respond to suspicions of such misconduct. To support these policies, RIKEN holds annual lectures for RIKEN employees to raise awareness regarding research misconduct and the ethics of scientific research. The seventh lecture in the annual series, held at the Wako Institute in Tokyo in December 2009, was given by Nicholas H. Steneck, professor emeritus and director of the Research Ethics and Integrity Program at the Michigan Institute for Clinical and Health Research in the US and a world leader in efforts to combat research misconduct. The lecture, entitled 'Good Research Practice', provided valuable insight into the issues surrounding research misconduct and the factors that contribute to its occurrence.

'Questionable' research practices can lead to research misconduct

Steneck's lecture began by drawing the audience's attention to the existence of a behavioral gray zone between 'responsible' research activities and outright research misconduct, an area he calls 'questionable research practice' (QRP). This includes practices such as demanding honorary authorship, the failure of an author to report a conflict of interest, non-reporting of contradictory data, republication of previously published results, vilification of whistleblowers, publishing of misleading summaries in abstracts, and improper use of statistics.

While less than 1% of researchers engage in outright research misconduct, it is estimated that between 10% and 50% of researchers are involved in some form of QRP. In the past, research misconduct has attracted the majority of attention on this issue, but Steneck emphasizes that preventing QRP is actually more important. To see why, one need only consider the very high proportion of researchers involved in QRP who go on to commit research misconduct.

The rules that researchers must follow are extremely complex, involving laws set down by governments, and policies established by individual research institutes, not to mention journal policies on authorship and the principles of individual research laboratories. Therefore, the first thing that should be done is to set down the rules for researchers to follow when conducting research, and with these rules offer clear guidance. It is also extremely important that this guidance be easy to find.

Researchers must also be educated to carry out research in a responsible manner. In the US, research institutes that receive government research funds have an obligation to provide education, through training via seminars and the web, on how to conduct research responsibly. The problem, however, is that there is no model curriculum, and thus the quality of teaching varies a great deal, without proper assessment of its effectiveness. Furthermore, mentoring—the guidance of researchers by their supervisor or group leader—receives very little support, despite the fact that it is considered the most essential element in ensuring responsible research.

Steneck points out that there are four issues that should be considered when planning 'responsible conduct of research' programs. The first is the 'what' that needs to be taught. Is the aim to teach rules to be followed in pursuing research, or is it to foster ethical reasoning? The second issue regards the scope of teaching. Ideally one would like to target all employees with any connection to research, but the costs of teaching rise in proportion to the number of people who receive it. Web-based approaches may cut the costs of teaching, but they are also less effective. High-quality teaching costs money. The third issue regards the goal of teaching. Is the priority to create an environment that encourages everyone to carry out their research in an ethical way, or is it to prevent research misconduct? Finally, the last issue to consider is the question of what method to adopt in assessing the effectiveness of training.

Conducting research abroad without fear of misconduct

Steneck notes that many foreign researchers who come to the US to pursue research tend to face a common set of problems, and that these problems can also be expected to be faced by anyone conducting research in another country. The greatest problem is the barrier of language. If a researcher's language skills are lacking, then they are forced to invest time and money in language study. The second problem is a lack of understanding and guidance about foreign research systems. Researchers must receive training about these systems in their own country before they head abroad. The host institution must also make an effort to explain the system clearly. The third problem is the unreasonable expectation placed on the researcher by theit home country. There are cases in which home countries set requirements for young researchers sent to the US from overseas regarding the number of papers they must publish before their return. This can impose an incredible pressure on the researcher, and in some cases has contributed to the decisions leading to misconduct. It is important that expectations are set in a reasonable way, and that they be realistic and feasible.

The role of a good research environment and good mentors

To prevent research misconduct, it is essential that research mentors have appropriate mentoring skills, and that these capabilities be properly evaluated. More attention must also be focused on creating a research environment that fosters responsible research practices. And, as Steneck points out, we must be cognizant of what in fact draws researchers toward misconduct. One reason may be in the regulations that govern research, which are often illogical and overly complicated. Another may be that supervisors are unfair, or that researchers in the lab are treated unfairly. Or it may be that the research system itself is illogical. These excuses are often given as reasons for engaging in research misconduct. If this is indeed the case, then what were the researcher's mentors doing when the research misconduct occurred? According to one study, 75% of the mentors in question were not properly reviewing the source data, and 66% had not explained to the researcher the specific research standards to follow in conducting research. Add to this that half of all researchers who have committed research misconduct indicated that they had experienced stress in their work environment; it is very important for mentors to make a greater effort to reduce the level of stress in the work environment.

One study investigated the degree to which researchers follow rules in pursuing their research. Respondents indicated that they themselves did not always live up to these norms, but further noted that other researchers were more lax and set even lower standards for responsible behavior. Preventing research misconduct in this type of environment would appear to be almost impossible.

Another problem is that it is not easy to be a whistleblower on misconduct. In one study, 10.4% of coordinators in clinical trials responded that they would not do anything if they encountered misconduct, and only 25.7% responded that they would report the incident to the appropriate department. Patients' lives are put at risk by misconduct in clinical trials. The failure to properly report misconduct is a major problem in research today.

Stenecks stresses that measures must be applied to help researchers avoid being drawn down the slippery slope toward research misconduct. Education and guidance by mentors are extremely important to achieving this goal.

Ideas on better laboratory management

Prior to Steneck's lecture, the professor took part in a roundtable discussion with a group of RIKEN research laboratory heads including Soh Osuka, manager of the RIKEN Committee for Research Strategies, and Kohei Tamao, director of the RIKEN Advanced Science Institute (ASI). In Japan, noted Osuka, there is very little education regarding laboratory research management or research misconduct, a deficiency that RIKEN hopes to address. In the laboratory of the Disease Glycomics Team at the ASI, represented at the discussion by Shinobu Kitazume, deputy team leader, when a lab member comes up with data that contradicts a working hypothesis, "we devote more time to it, and consider it together as something that will determine the future direction of the lab," she explained. Toshihiko Hosoya, leader of the Hosoya Research Unit at the RIKEN Brain Science Institute, identified two issues that he focuses on in running his lab. "One is to make sure all conclusions are solid, and the other is to make significant contribution to the understanding of the real brain," he said, pointing out that all data that lab members generate are thoroughly validated. Tamao similarly conveys to all members of his lab at the ASI the idea of 'Scientists in Society'. "As in society, all members of the lab, including the laboratory head, have the right to express their ideas freely, and experience the enjoyment of pursuing research, with an understanding that all members of the lab are equal," he said. "Creating this atmosphere is essential." All three laboratory leaders were adamant that there is no place in the lab for competition over the same research themes. "It is very important to make sure that there is no overlap of the research topics pursued in the same laboratory," Tamao said. "The best way to advance science and nurture researchers is through the sharing of data among researchers, who each pursue their own independent, well-defined topics." Steneck commended the group on taking their laboratories' research work very seriously and doing everything they could to help out other lab members, yet with each retaining their own research style. "It is important that the principal investigator of the research laboratory strives to create a positive research environment," he said.

RIKEN forges ties with Max Planck and Technische Universität München

On 19 January 2010, RIKEN and the Max Planck Society for the Advancement of Science (MPG) signed a memorandum of understanding toward the establishment of a new joint center focused on research in chemical biology. The memorandum was signed by RIKEN President Ryoji Noyori, MPG President Peter Gruss and RIKEN Advanced Science Institute Director Kohei Tamao at the MPG headquarters in Munich, Germany.

On the same day, RIKEN also signed a comprehensive cooperation agreement with the Technische Universität München (TUM). While RIKEN and the TUM have cooperated in the past in the area of accelerator science, the new agreement aims to expand this cooperation to a broad range of fields including chemistry, the life sciences and engineering.

Both partnerships set the seeds for new cross-border research between Japan and Germany and present exciting new possibilities for the future of basic and applied science.

RIKEN and The University of Tokyo release full-length human cDNA clones

RIKEN and The University of Tokyo have partnered to release full-length human cDNA clones compiled under the Genome Network Project (GNP), an initiative of Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT). The set of 80,000 full-length human cDNA, which covers nearly the entire human genome, was made available on 15 March 2010 for general use through the RIKEN BioResource Center (BRC).

Initiated in 2004 on the heels of the first complete sequencing of the human genome, the GNP aims to clarify the structure of gene and protein–protein interactions toward applications in treatment and drug development. By its completion in 2008, the project had succeeded in generating important scientific findings, producing vast amounts of experimental data in the process. To organize this data, the GNP created the Genome Network Platform, a visual interface enabling users to search genes, browse the genome, and analyze expression profiles.

It is through this platform that the 80,000 full-length human cDNA clones are being released. Two types of clones make up this new resource: a collection of 30,000 full-length human cDNA clones compiled by groups at The University of Tokyo and the RIKEN Omics Science Center, and 50,000 Gateway® entry clones reconstructed based on the cDNA clones. The Gateway® entry clones include clones made from cDNA created by the Research Association for Biotechnology under the New Energy and Industrial Technology Development Organization (NEDO) Full-length Human cDNA Sequencing Project. Researchers interested in obtaining the cDNA clones from either of these sets should visit the website of the Genome Network Platform (genomenetwork.nig.ac.jp), operated by the National Institute of Genetics, and place an order through the RIKEN BRC. Details on ordering cDNA clones can be found at the BRC's DNA Bank website (www.brc.riken.jp/lab/dna/en/GNPcloneen.html).

Full-length cDNA clones offer researchers a powerful means to investigate the expression of messenger RNA, study proteins and especially protein function, and isolate specific genes. As one of the most important basic resources for genomic research, the provision of these new clones offers a valuable opportunity for researchers, promising key advances in treatment and drug discovery.

Joint research begins on individual-level mechanisms of gene expression

The RIKEN Omics Science Center (OSC) has partnered with American company Complete Genomics to develop next-generation technology for the rapid analysis of individual-level human gene expression. The partnership combines the OSC's expertise in omics science with technology for the rapid sequencing of complete human genomes developed by Complete Genomics to explore new possibilities in the study and application of cutting-edge gene expression analysis.

Collaborative research in the new project will seek to clarify the underlying mechanisms of complex gene expression in humans. To do so, complete genomes of a number of individuals, sequenced by Complete Genomics, will be analyzed for differences in genetic information and RNA expression using RIKEN's CAGE (cap analysis of gene expression) technology. While technology for the analysis of complete human genomes is today increasingly widespread, the current collaboration takes such sequencing one step further, focusing on RNA levels (transcriptome) representative of detailed differences in gene arrangement, with important applications to genetic profiling.

In the future, through collaborative research using technology developed independently by the two organizations, RIKEN and Complete Genomics envision further cooperation toward the creation of a commissioned genome analysis service. The first stage of the new partnership was launched on 7 January 2010.

Cutting-edge environmental research on display at AAAS annual meeting

A global organization dedicated to advancing science around the world, the American Association for the Advancement of Science (AAAS) attracts thousands of leading scientists, engineers and educators to its annual meeting every year.

In keeping with this year's theme of 'Bridging Science and Society', RIKEN's booth at the 2010 AAAS meeting showcased cutting-edge environmental research by researchers at RIKEN centers and institutes. This research included new bio-based resources that make use of the symbiotic systems of termites, new plants created using radioactive isotope beams, and a novel technology to extract useful compounds from jellyfish, which are currently in overabundance in the seas around Japan. The information and exhibits on display attracted a great deal of attention from students, researchers and journalists, many of whom also inquired about possible research and industry collaborations, as well as career opportunities at RIKEN.

Katsuhiko Mikoshiba
Laboratory of Developmental Neurobiology
RIKEN Brain Science Institute
Wako, Saitama, Japan

Dear Prof. Mikoshiba,

It has been more than five months since I left Japan and I am back to my previous life. Nothing changed here; Paris is a beautiful city as always. I am now working on the next step of my career, but I already find it hard after the threshold set by your laboratory.

Back to September 2008: I had been thinking seriously of going to Japan for scientific purposes for almost three years. I had very few contacts through my university network, but just when I was starting to feel hopeless, I noticed something on the announcement board when attending a lecture at the Pasteur Institute. I saw 'it', the famous name I had heard about: Katsuhiko Mikoshiba. I could not believe it, you were coming to the institute for a lecture. The presentation was really interesting, especially because so many different projects joined together were held in your laboratory. I gathered my energy to initiate a dialogue with you. You sounded so nice and glad that foreign students could also be interested in your laboratory.

A few weeks later, you gave me a positive answer, and, on the 31st of December, I finally arrived on the other side of the globe. I knew no-one in Japan, but the very kind people in your laboratory had already made contact with me. In my first amazing days in Japan, I discovered typical Japanese food and had parties with great people, and I even tasted a homemade end-of-year dinner.

Then my six months project started, and you personally explained to me the details of the IRBIT project and its purpose, and assigned me to the supervisor you thought was the best for me. Soon, I felt independent in my organization and ideas; I could also ask anyone for help or answers if I had questions. I really made a huge step forward in my capacity to be a researcher.

The RIKEN institute is such a great place to work in; it is a reserve of smart and diverse people working on stunning research projects and ready to discuss with you in any corridor corner… my time in your laboratory gave me the opportunity to meet very passionate people, and they made me understand my own passion about science. I felt really motivated to find my own path, started to feel more confident to start my career for real and continue as a PhD student as soon as possible. I am very grateful for the opportunity you gave me, because it changed my life and it was an important contribution to the person I am.

I had a wonderful time in Japan and it was very hard to leave. I discovered Tokyo for real as an 'insider', and also experienced many other incredible places. As a great conclusion to my stay, my friends from the laboratory organized a farewell party for me, and it was so amazing that I will never forget it.

Thank you for everything. I hope you are doing well and that you will continue to instill your passion in young people.

Regards,

Laura Gainche
PhD student
Sleep Laboratory
University of Melbourne
Melbourne, Australia

P.S. After six months in Paris I was fortunate enough to secure a position as a PhD student at the University of Melbourne in Australia. Thanks to your help and inspiration, I am now firmly on my own path and enjoying my prospects for the future.

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RIKEN, Japan's flagship research institute, conducts basic and applied experimental research in a wide range of science and technology fields including physics, chemistry, medical science, biology and engineering. Initially established as a private research foundation in Tokyo in 1917, RIKEN became an independent administrative institution in 2003.

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For further information on the research presented in this publication or to arrange an interview with a researcher, please contact
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