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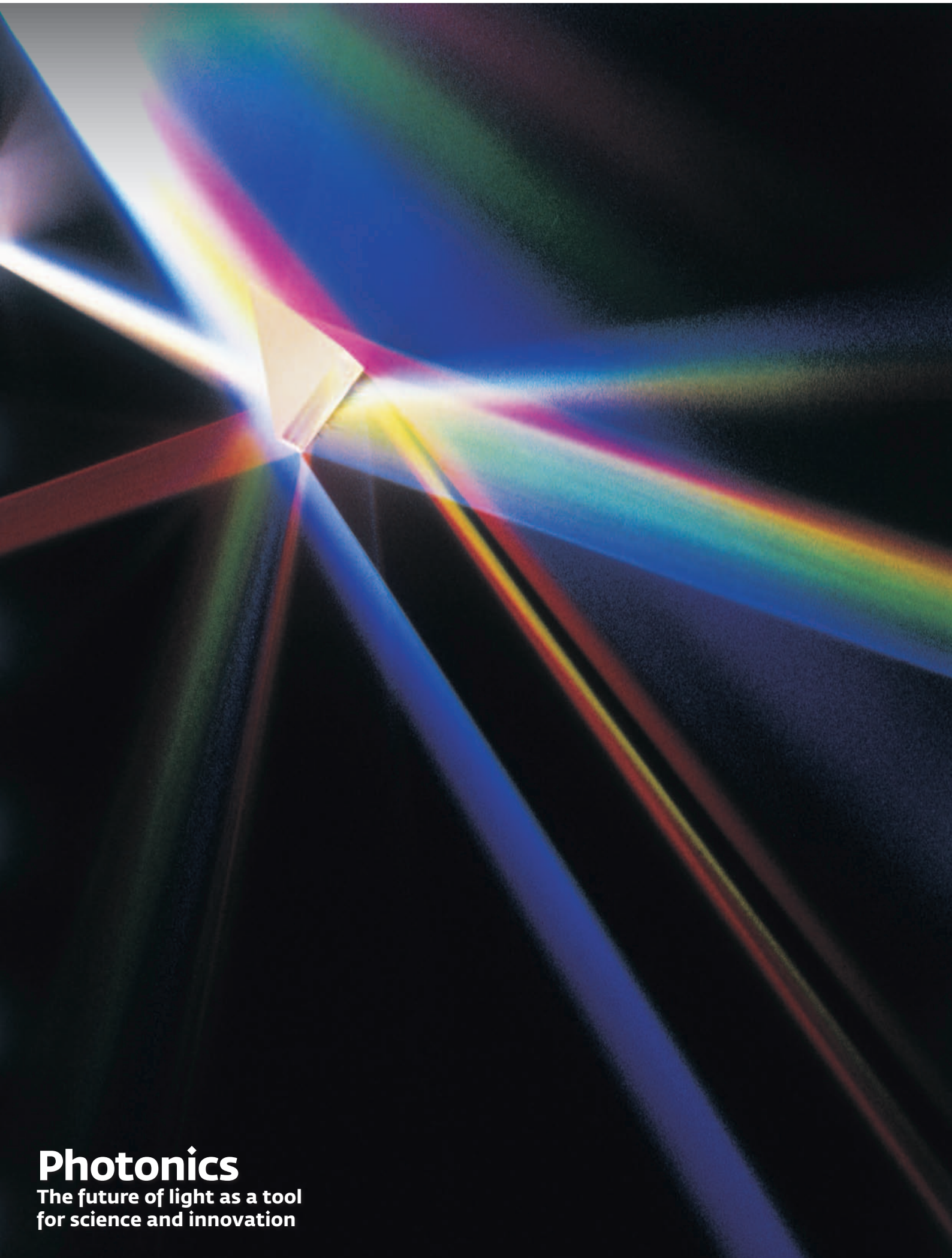

# Photonics

**The future of light as a tool for science and innovation**

Control room at the Radioactive Isotope Beam Factory in Wako

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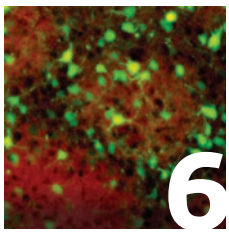


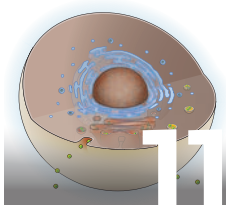


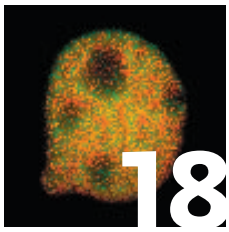




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# RIKEN RESEARCH

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Photonics

# Making the invisible visible

KATSUMI MIDORIKAWA
Director
RIKEN Center for Advanced Photonics

Photonics, the science and technology of light, is not only a fascinating and important topic for basic research but is also indispensable for sustaining modern society. Applications developed in this area are the foundation of many breakthroughs in healthcare, advanced manufacturing, metrology, security and sensing. The importance of such applications is inspiring researchers in the field to further push the possibilities of light.

Light is a packet of photons with increasingly important applications for both the scientific community and general public. For example, photonics researchers are harnessing photons as a means to visualize the motion of electrons in materials, as well as for use as noninvasive biological probes in medical imaging in humans. The use of photons has even revolutionized the way we send and store digital data, cost-effectively manufacture complex devices and generate electricity.

Photonics has also infiltrated our everyday lives. For instance, the circuit board of your cellular phone is likely to have been drilled by a laser and your car was probably laser welded. Using the Internet or sending an e-mail employs photons to carry the information. A hospital visit can involve x-ray cameras and optical endoscopes to look inside your body. If the nineteenth century was the century of steam and the twentieth century was the century of the electron, the twenty-first century will be remembered as the century of light (Fig. 1).

## A century of light takes flight

Just over 50 years ago—in 1960—the first successful operation of a laser took place. Since then, laser research has expanded in a number of different directions: high-energy photonics, quantum metrology, nanophotonics and terahertz photonics.

The earliest lasers emitted photons with the energy of about 1 electronvolt, but today a strongly focused laser beam can generate high-energy radiation and accelerate particles up to gigaelectronvolt energies. Photonics researchers can now create short pulses of light that are just attoseconds ($10^{-18}$ seconds) in duration. These ultrashort pulses are useful as probes for investigating ultrafast dynamics within atoms and molecules.

In the field of metrology, laser light is used to cool atoms and molecules down to extremely low temperatures of just a few nanokelvin. This capability makes it possible to realize ultraprecise optical clocks that are essential for tasks in communication and metrology, or to achieve new states of matter, such as Bose–Einstein condensates, and thus explore new features of quantum physics.

Laser research has also stimulated the development of nanophotonics. A long-held, universal law in optics is that the resolution of an optical image is limited by diffraction; thus, features smaller than half the wavelength of the light used cannot be visualized. This law means that the first lasers, which had a wavelength of just 1 micrometer, were unable to image nanostructures. Now, these tiny structures can be seen using lasers, thanks to the development of near-field optical technology and super-resolution imaging techniques, which can directly resolve subwavelength structures.

Advances in laser research have also provided the ability to generate radiation with a very long wavelength. Such lasers were developed by extending the concept of the maser, an amplifier that operates in the microwave region. Several of the first lasers, including the ruby, HeNe (helium–neon) and Nd:YAG (neodymium-doped yttrium aluminum garnet) lasers, operated only in the visible and near-infrared regimes of the electromagnetic spectrum. Consequently, the region that lies between the infrared and microwave bands—the so-called terahertz region—was left behind.

The terahertz region is very important, however, especially for sensing, imaging and spectroscopic applications. Many important chemicals, such as pharmaceuticals or explosives, have strong absorption signatures in the terahertz band. Terahertz waves are also capable of penetrating various materials, including clothes

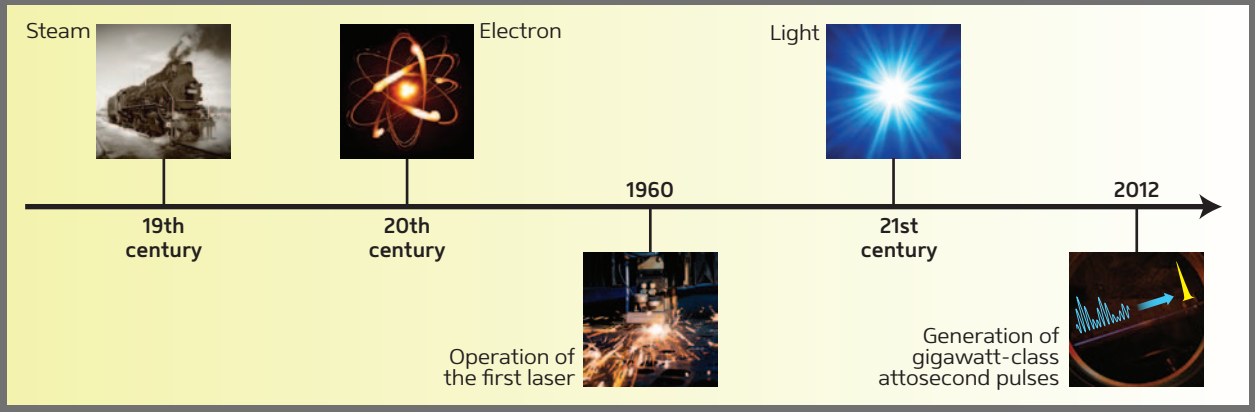


**Figure 1: The century of light**

and paper, making the region important for security screening and structural monitoring. Nowadays, convenient and compact sources of terahertz radiation are available. The semiconductor quantum cascade laser, for example, greatly opens up this region of the electromagnetic spectrum.

## Leading light

Researchers at RIKEN have achieved important results in each of these four research areas—particularly in high-energy and attosecond photonics. Attosecond pulses were first generated in the early 2000s and ever since then scientists have been striving to produce ever shorter pulses. The shortest pulse generated to date is around 70 attoseconds long—10 attoseconds shorter than the previous record set in 2008. Despite the progress in reducing the pulse duration, the energy and intensity of attosecond pulses has not changed accordingly, hampering the application of attosecond pulses.

RIKEN recently developed new methods to generate intense attosecond pulses and now has the capability to produce microjoule- and gigawatt-class isolated pulses. Such pulses can probe the dynamics of electronic processes and 'see' the motion of electrons within a material. The ability to probe and understand the flow and dynamics of electrons greatly assists the development of high-performance catalysts and electronic devices. In the area of nanophotonics, RIKEN has developed super-resolution imaging devices that facilitate imaging at a resolution of just a few nanometers, allowing individual carbon nanotubes to be seen.

In the terahertz area, RIKEN has made two big advances: the development of high-performance quantum cascade lasers operating in the terahertz region, and the generation of intense terahertz radiation. Increasing the operating temperature of quantum cascade lasers toward room temperature—around 300 kelvin—will make them more convenient and practical to use. The latest devices developed at RIKEN have now reached an operating temperature of around 160 kelvin. In the next 5 to 10 years, a temperature of 200 kelvin or more should be achievable. It will then be possible to use solid-state thermoelectric coolers known as Peltier devices instead of cryogenic gases to cool the lasers—an advance that will greatly expand the application of these lasers.

Another important development in the field has been the use of a new materials system for making quantum cascade lasers. In particular, RIKEN has successfully fabricated such lasers from gallium nitride (GaN). The wavelength range of operation of a laser is limited by the materials used. Employing GaN expands the wavelength possibilities into the 5–10 terahertz frequency band, which cannot be reached by traditional quantum cascade lasers based on the aluminum gallium arsenide (AlGaAs) materials system.

In addition, RIKEN has established ways to generate very intense terahertz waves that are useful for driving nonlinear processes and performing spectroscopy. This includes 100-kilowatt terahertz sources based on frequency conversion with a power output similar to that of a free-electron laser, a much larger and far more expensive instrument.

## Bright future ahead

Photonics researchers will continue to find ways to make the invisible visible. If the motion of electrons can be captured using an attosecond pulse and the dynamics of matter can be understood on a new timescale, better and faster electronics devices or chemical catalysts can be designed. The realization of a compact attosecond source, similar to the type of femtosecond laser commonly found in a laboratory today, is another challenge being tackled by scientists at RIKEN.

Many other applications will benefit from advancements in photonics research. For example, a compact and portable optical lattice clock that can work in the field, not just the laboratory, would be very useful. The merging of laser science and particle acceleration technology will also provide exciting opportunities. And laser-based accelerators may hold the answer to reducing the size and cost of traditional high-energy particle accelerators.

The development of more sophisticated, noninvasive imaging techniques for biomedicine is also vital. In many advanced countries, including Japan, the cost of medical care is rapidly increasing. New photonics imaging technology will form a key part of addressing this urgent issue. Ultimately, future photonics research may result in the realization of a noninvasive photonic sensor that provides information about human health simply by touch.

Biology

# Neurons branch out the right way

## An evolutionarily conserved molecular mechanism underlying dendrite orientation plays a key role in fine-tuning critical sensory development

During development, the embryonic brain generates vast numbers of immature neurons, which migrate away from their birthplace, sometimes over long distances. Once they reach their final destination, the neurons sprout fibers that extend toward other cells, forming connections. Developing somewhat haphazardly at first, these connections are soon refined by sensory experiences, which mold them into extremely precise neural circuits.

Each neuron has two types of nerve cell fiber: densely branched dendrites that receive signals and a single axon that conveys signals to other cells. Decades of research have revealed many details about how axons in the developing brain extend and find the correct pathway, but very little is known about the comparable process in dendrites. Tomomi Shimogori and colleagues from the RIKEN Brain Science Institute have now identified a molecule that orients dendrites during connection development[1].

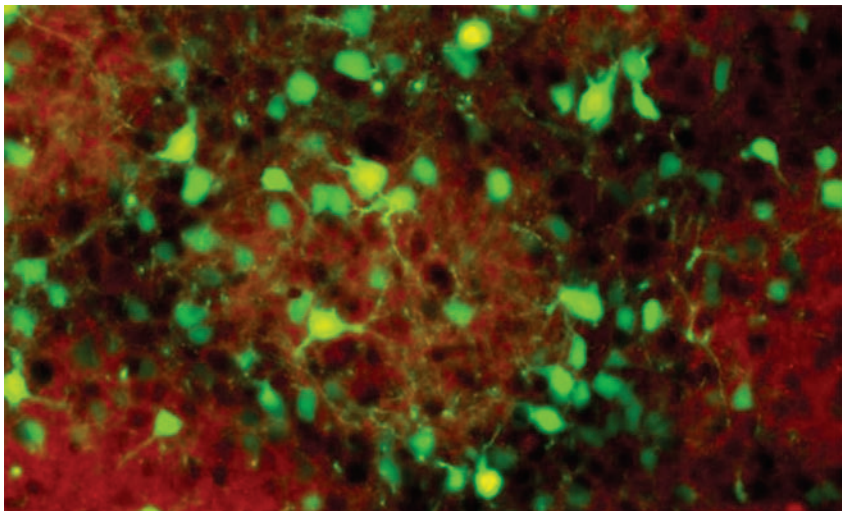

Figure 1: In normal mice, neurons (green) in the barrel cortex orient their dendrites toward the source of incoming sensory information (red).



### Orienting connections from the deep brain

Shimogori and her colleagues focused on a region of the mouse brain called the somatosensory barrel cortex, which receives and processes touch information from the whiskers via a deep brain structure called the thalamus. In the developing barrel cortex, as in other sensory regions of the neonate brain, newly formed connections are refined by incoming sensory information, which causes the dendrites to orientate in the direction of higher sensory inputs (Fig. 1).

The researchers first screened for proteins that are selectively expressed in the barrel cortex during early stages of postnatal development. They found that a molecule called Btbd3 (known as BTBD3 in humans) is expressed from two days after birth, when axons from the thalamus begin to grow into the barrel cortex to form connections.

To determine the role of Btbd3 in neuronal circuit formation, the researchers used a technique called short hairpin RNA inhibition to block synthesis of the protein. Examination of the animals' brains at 6 days of age revealed an increase in the number of dendrites in the barrel cortex and that many of the fibers were incorrectly oriented. The normal fiber structure could be restored with the addition of human BTBD3 protein, suggesting that the altered dendrite structure occurred specifically as a result of Btbd3 inhibition.

### Branching out

Previous work by other researchers has shown that nervous impulses from axons originating in the thalamus are necessary to induce structural changes in the dendrites of cells in the barrel cortex and that this activity is mediated by a receptor called NMDAR1. Shimogori's team therefore examined Btbd3 activity in mutant mice lacking the NMDAR1 receptor in the cortex.

By examining the animals' brains, they showed that the dendrites of cells in the barrel cortex were more extensively branched in NMDAR1-deficient mice than in normal mice and that the branches

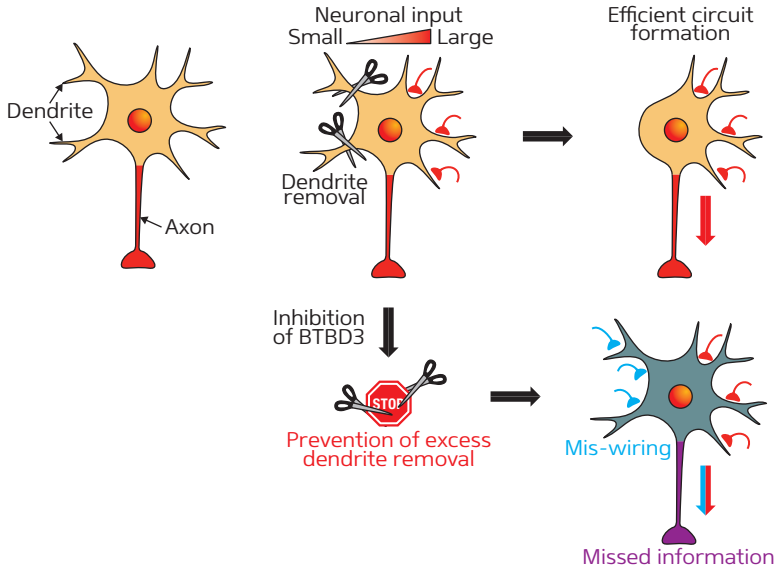


**Figure 2: The BTBD3 protein molds precise neuronal circuits by orienting dendrites toward axons that are firing nervous impulses.**



were also randomly orientated. As this result was similar to that of the Btbd3 inhibition experiment, the researchers tested the expression level of Btbd3 in the mutant barrel cortex. They found that while the cells possessed normal dendrites and the correct level of Btbd3, the protein was distributed differently inside them. In normal animals, the protein was typically localized within the nucleus, but in mice lacking the NMDAR1 receptor in the cortex, the protein was found in the cytoplasm—the fluid-filled area surrounding the nucleus.

These results suggest that nervous impulses travelling from the thalamus to the barrel cortex normally trigger the transfer of BTBD3 from the cytoplasm to the nucleus, where it produces changes in genetic activity that lead to the trimming of excess dendrite branches.

Next, the researchers introduced Btbd3 protein into the primary visual cortex of embryonic mice, which does not normally express Btbd3. This part of the brain processes information from the eyes and contains dendrites that are arranged symmetrically around the immature neurons. The addition of Btbd3 caused all the dendrites to become oriented in the same direction, further suggesting that BTBD3 normally acts to orientate growing dendrites toward active axons.

## Role in sensory function

Investigation of the developing visual cortex in ferrets revealed—in contrast to mice—high levels of BTBD3. Neurons in the ferret visual cortex normally have symmetric dendrites. Following damage to cells that receive inputs from one eye or the other, however, the dendrites become reoriented in a specific direction. This reorientation did not occur when BTBD3 synthesis was blocked before the cells received neuronal input.

Together, the findings support the conclusion that BTBD3 plays a critical role in orienting growing dendrites toward axons that are firing nervous impulses (Fig. 2). The differences between BTBD3 protein expression in mice and ferrets also point to an important evolutionary function: mice are far more dependent on the sense of touch than the sense of vision, whereas the opposite is true of ferrets.

"BTBD3 remodels dendrites toward higher neuronal activity, setting up a sharp functional map in the cortex," says Shimogori. "This provides high-acuity sensory function in cortical areas that have BTBD3 expression."

In mice, precise connections are far more important in the barrel cortex than the visual cortex—and vice versa for ferrets and other species that are more dependent on vision. The findings therefore suggest that the function of BTBD3 is highly conserved, and is expressed only in those areas of the brain where experience-dependent fine-tuning of neuronal circuits is required for the survival of the organism.

"We are now exploring the mechanism by which animals obtain BTBD3 expression in specific cortical areas," says Shimogori. "This will reveal how different animals evolved different functions in different parts of the neocortex."

1. Matsui, A., Tran, M., Yoshida, A. C., Kikuchi, S. S., U, M., Ogawa, M. & Shimogori, T. BTBD3 controls dendrite orientation toward active axons in mammalian neocortex. *Science* **342,** 1114–1118 (2013).

## ABOUT THE RESEARCHER


Tomomi Shimogori was born in Chiba, Japan. She graduated from the Hoshi University School of Pharmacy and Pharmaceutical Sciences in 1993 and obtained her PhD in pharmaceutical science from Chiba University in 1998. After six years of postdoctoral training at the Department of Neurobiology at the University of Chicago, United States, she returned to Japan to join the RIKEN Brain Science Institute (BSI) as a unit leader, exploring the mechanisms of thalamic development and their contribution to cortical evolution. In 2010, she was promoted to team leader of the BSI's Laboratory for Molecular Mechanisms of Thalamus Development. Her research focuses on the patterning mechanisms of the developing mouse thalamus, differences between the developing thalamus of the mouse and chick, and the role of thalamocortical axons in cortical plate development.

# Mapping mechanisms to medications

By charting shifts in gene expression, researchers can gain deeper insights into drug mechanisms and side effects

The timing of gene activity is primarily controlled by regulatory sequences known as promoters, which contain various binding sites for different proteins that help switch genes on or off. The majority of these binding sites are likely to appear in numerous different promoters, such that a single cellular signal can orchestrate the activity of multiple genes in parallel.

Researchers led by Hideya Kawaji from the RIKEN Preventive Medicine and Diagnosis Innovation Program and Harukazu Suzuki from the RIKEN Center for Life Science Technologies have now devised a strategy for quantifying how different drugs and drug candidates modulate activities across the entire genome within cells[1]. In principle, this information could be utilized to predict the efficacy of drug candidates toward a given disease or the risk of unwanted side effects.

Their approach is based on cap analysis of gene expression (CAGE), a method originally developed by RIKEN scientists to measure changes in gene output. By mapping these shifts back to their context in the genome, the researchers were able to identify exactly which promoters were being modulated in response to different compounds. In an initial study, they treated cultured breast cancer cells with wortmannin, gefitinib and U0126—three compounds with known antitumor effects. This CAGE-based approach, in combination with a new generation of DNA sequencing technology, proved both reproducible across multiple experiments and highly sensitive, detecting even the subtle perturbations in promoter function that arose from low doses of a given drug.

Figure 1: By tracking how drugs alter gene activity, scientists can obtain insights into how drugs work, as well as their potential for adverse effects.



For each of these drugs, Kawaji, Suzuki and their colleagues were able to identify specific effects on more than 100 different promoters. The results were consistent with the mode of action and targeted genes previously reported in extensive studies of these compounds. For example, the signaling pathway targeted by gefitinib in turn modulates two separate pathways inhibited by wortmannin and U0126, and modeling based on the CAGE data confirmed that the great majority of promoters affected by these latter two drugs could indeed account for most of the effects observed for gefitinib.

"This is a very important finding," explains Suzuki, "because it means that we can now predict the targets and mechanisms of new drugs using the profiles of known drugs."

The researchers' approach could also provide a deeper understanding of minor or 'off-target' drug effects that are unrelated to the disease being treated but nevertheless exert potentially meaningful physiological effects. "This is very important in many aspects of drug research, such as side effects and synergic effects with other drugs," says Suzuki.

1. Kajiyama, K., Okada-Hatakeyama, M., Hayashizaki, Y., Kawaji, H. & Suzuki, H. Capturing drug responses by quantitative promoter activity profiling. *CPT: Pharmacometrics & Systems Pharmacology* **2,** e77 (2013).

# Harnessing the power of skyrmions

## Magnetic vortices known as skyrmions could form the basis for future high-density, low-power magnetic data storage

With the ever-increasing amounts of digital information being processed, transferred and stored by computers comes a commensurate demand for increased data storage capacity. For magnetic data storage, such as the ubiquitous hard disk drive, this requires not only physically smaller memory elements, or bits, but also reduced switching power to avoid heat issues. Yoshinori Tokura and colleagues from the RIKEN Center for Emergent Matter Science, in collaboration with a research team from the University of Tokyo, have now shown that structural control of small magnetic vortex structures called skyrmions could lead to a compact, low-power alternative to conventional magnetic data storage[1].

Skyrmions are very stable magnetic structures that can form within a chiral crystal lattice. "Each skyrmion can be considered as a single particle and could represent an information bit," says Kiyou Shibata from the University of Tokyo. "The small size of skyrmions is also of great advantage to high-density integration in devices."

Skyrmions occur rarely in certain magnetic compounds. They only began to attract interest for practical applications when the RIKEN researchers, in previous work, were able to demonstrate that skyrmions can exist near room temperature and can be manipulated using very low electrical current densities of about 100,000 times less than those required for controlling conventional ferromagnetic structures.

In another step toward achieving better control over the properties of skyrmions, Tokura, Shibata and their colleagues studied a range of manganese–germanium magnets by preparing magnetic compounds, in which they increasingly replaced manganese with iron. Using a powerful Lorentz microscope capable of visualizing magnetic structures on a nanometer scale, they then studied skyrmions in the various magnets.

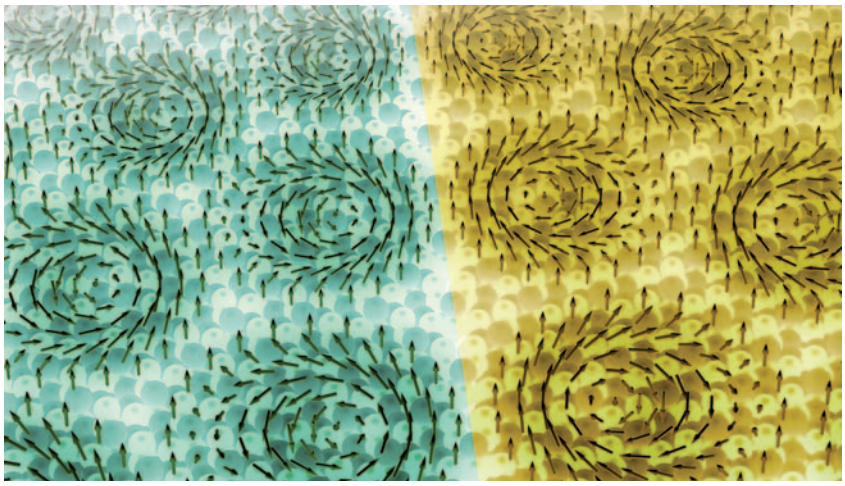

**Figure 1: Skyrmions in a manganese–iron–germanium magnet. Arrows represent the magnetic fields that make up each skyrmion. The size of the skyrmions changes gradually as the composition varies, and the chirality or 'handedness' of the skyrmions changes with the chirality of the crystal lattice.**



The researchers observed that the size of skyrmions changes continuously with composition and that a ratio of about 80% iron and 20% manganese changes their orientation (Fig. 1). This finding is explained by an iron-dependent change in the magnetic coupling between the magnetic properties of the electrons in the magnet and their movements around the atomic core.

It will now be possible to consider practical schemes to design devices based on skyrmions. For example, skyrmions with the desired size and orientation can be created by tuning the composition of the magnet. "The next stage of our research will focus on the manipulation of skyrmions. In particular, the dynamics of isolated skyrmions in confined structures have been predicted theoretically and would be a good subject for experimental research."

1. Shibata, K., Yu, X. Z., Hara, T., Morikawa, D., Kanazawa, N., Kimoto, K., Ishiwata, S., Matsui, Y. & Tokura, Y. Towards control of the size and helicity of skyrmions in helimagnetic alloys by spin–orbit coupling. *Nature Nanotechnology* **8,** 723–728 (2013).

# Data mining for food security

## A new database catalogs thousands of genetic variants in cassava—one of the world's primary food sources

Cassava is a woody shrub that is native to South America and is extensively cultivated in tropical regions worldwide, with an annual crop production of over 200 million tons. A team of researchers led by Tetsuya Sakurai from the RIKEN Center for Sustainable Resource Science has now completed the largest study to date of cassava DNA sequence variations[1].

The cassava plant, *Manihot esculenta*, has a tuberous root that serves as a primary food source for hundreds of millions of people, and the starch extracted from it is widely used in the food, paper and textile industries. Cassava is also highly resistant to drought, and its tubers can remain healthy in dry soil for up to several years. It therefore provides food security as it is a rich source of carbohydrate that can be drawn upon to prevent or relieve famine.

Sakurai and his colleagues retrieved more than 80,000 partial cassava DNA sequences from GenBank, a publicly available genetic sequence database run by the US National Institutes of Health. Using a computational approach to examine the sequences, they identified and characterized 10,546 DNA sequence variations called single nucleotide polymorphisms (SNPs), as well as 647 insertions and deletions.

They found that 62.7% of the SNPs occurred in protein-coding regions of the genome and that genes conferring disease resistance contained a significantly higher ratio of 'non-synonymous' DNA sequence variations, which alter the coding sequence, than 'synonymous' variations, which do not.

Figure 1: Cassava is one of the world's primary food sources.



The researchers organized and integrated all of this information into the Cassava Online Archive, a comprehensive database that is freely available online at cassava.psc.riken.jp. The database can be searched by keyword, specific genetic variation, cassava variety and several other criteria. It also includes information about all the variants identified, such as their exact location on the genome and how they can be isolated.

Variations in protein-coding sequences of the genome probably have subtle influences on protein structure and function that are likely to play an important role in cassava's ability to adapt to environmental changes. The Cassava Online Archive could therefore help researchers to gain a better understanding of how the crop can survive in harsh conditions.

"The Cassava Online Archive is an ongoing project," says Sakurai. "We have plans to append gene expression profile data and aim to develop the database as a one-stop shop for cassava research."

1. Sakurai, T., Mochida, K., Yoshida, T., Akiyama, K., Ishitani, M., Seki, M. & Shinozaki, K. Genome-wide discovery and information resource development of DNA polymorphisms in cassava. *PLoS ONE* **8,** e74056 (2013).

# In search of a sugar's secrets

## The discovery of the origin of enigmatic 'waste' sugar molecules within the cell also hints that they serve some as-yet-undefined function

Within a cellular compartment called the endoplasmic reticulum (ER), certain proteins become decorated with sugar molecules by a process called *N*-glycosylation, before being delivered to the cell surface. This process also gives rise to a population of untethered sugar molecules known as free oligosaccharides (fOSs). Tadashi Suzuki, Yoichiro Harada and colleagues from the RIKEN–Max Planck Joint Research Center for Systems Chemical Biology have now determined how fOSs are produced and at the same time have uncovered some important new questions[1].

The oligosaccharyltransferase (OST) enzyme complex facilitates *N*-glycosylation by transferring preassembled sugar structures from dolichyl pyrophosphoryl-linked oligosaccharide (DLO) molecules onto the target protein. Scientists had long believed that fOSs result from the breakdown of DLOs until Suzuki discovered an enzyme in the cytoplasm called peptide:*N*-glycanase (PNGase) that can generate fOSs by acting on improperly folded glycosylated proteins. While initially skeptical, the scientific community eventually embraced this as the primary mechanism for fOS production. Suzuki, however, suspected that the biological picture was even more complicated, particularly since yeast cells lacking PNGase still produce low levels of fOSs.

To clarify the situation, he and Harada set out in search of this yet-undiscovered source of fOSs. First, they verified that PNGase-deficient yeast cells produce fOSs within the ER, which are subsequently exported to and degraded in the cytosol. Prior studies suggested that OST—the same enzyme that drives *N*-glycosylation—can also break down DLOs to yield fOSs. After obtaining initial data to support this hypothesis from experiments with mutant yeast strains, Harada went through the laborious process of isolating the intact, eight-protein OST complex. Experiments with purified OST showed that the enzyme can indeed convert DLO molecules into fOSs, providing new support for the DLO-centered model of fOS production.

Importantly, the research revealed that a sharp increase in the concentration of available DLOs does not lead to an equivalent bump in fOS production, suggesting that this is not an accidental process. "Our data suggest that OST-mediated fOS release is a highly regulated reaction," says Suzuki. Given that mammalian cells appear to produce the majority of their fOSs through a similar non-PNGase mechanism, Suzuki hypothesizes that this pathway may serve some specific function in higher eukaryotes, which his team hopes to uncover in future studies. "It looks like a waste if fOSs are merely the junk produced by erroneous activity of OST," he says. "We believe there must be a reason for cells to engage in such an apparent waste of energy."

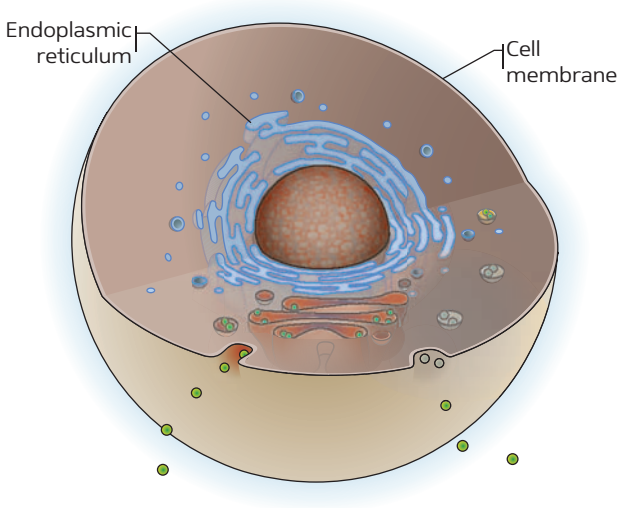


**Figure 1: In the endoplasmic reticulum, *N*-glycosylation of proteins results in the formation of free oligosaccharides.**



1. Harada, Y., Buser, R., Ngwa, E. M., Hirayama, H., Aebi, M. & Suzuki, T. Eukaryotic oligosaccharyltransferase generates free oligosaccharides during *N*-glycosylation. *The Journal of Biological Chemistry* **288,** 32673–32684 (2013).

# The two sides of a lung cancer biomarker

## A genetic variant implicated in lung cancer development is also linked with improved outcomes among affected patients

Lung cancer is the most common cause of cancer death in much of the industrialized world, including Japan. While cigarette smoke is most frequently the source of the disease, genetics can also play a role. Scientists have identified a handful of genetic variants associated with an increased susceptibility to lung cancer among Asian women who do not smoke. A research team led by Toshihisa Ishikawa and colleagues from the RIKEN Center for Life Science Technologies has now identified another gene mutation that puts non-smoking Japanese women at elevated risk of lung cancer, but which is also linked to better prognosis for those who develop the disease[1].

Genetic variants, referred to by geneticists as single nucleotide polymorphisms (SNPs), can be useful as prognostic biomarkers for guiding diagnoses and personalizing therapies. Ishikawa and his colleagues set out to test whether variants in the *NRF2* gene, which encodes a transcription factor involved in cellular antioxidant defense mechanisms, could predict the likelihood of someone developing lung cancer. The researchers analyzed DNA samples from a total of 387 Japanese lung cancer patients —43% women and 40% non-smokers— using a rapid isothermal genetic test developed specifically for this study. The team's rapid genotyping does not require DNA to be isolated or polymerase chain reaction (PCR) amplification and takes only 30–45 minutes to complete.

Looking at position −617 in the *NRF2* gene, the researchers identified 24 patients who had two copies of the adenine nucleotide; the rest had at least one cytosine at this location. Of these 24 individuals, 16 were female non-smokers, and all had a type of lung cancer known as adenocarcinoma.

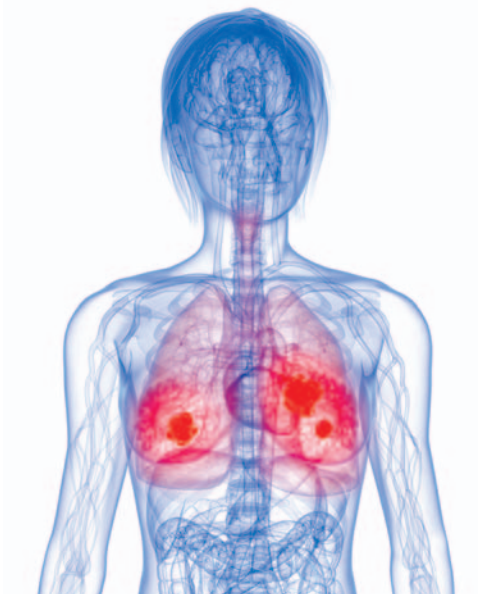

**Figure 1: A mutation linked to lung cancer in Japanese women who do not smoke is also associated with better clinical outcomes.**



Notably, patients of either gender who carried two copies of adenine at position −617 of the *NRF2* gene also had significantly better survival outcomes than other patients. These same individuals were more likely to carry the most common, or 'wild type', version of *MDM2*, a gene regulated by NRF2 that encodes a negative regulator of p53, an important tumor suppressor protein. Both the −617A variant in the *NRF2* gene and the wild-type version of *MDM2* are more often found in east Asian populations.

"Genetic testing of these SNPs should be performed to predict the prognosis of lung cancer patients in east Asian countries, such as Japan, South Korea and China," suggests Ishikawa. "It would be desirable to include such genetic information in each patient's record as guidance for medical doctors to provide individualized treatment."

1. Okano, Y., Nezu, U., Enokida, Y., Lee, M. T. M., Kinoshita, H., Lezhava, A., Hayashizaki, Y., Morita, S., Taguri, M., Ichikawa, Y. *et al*. SNP (−617C>A) in ARE-like loci of the *NRF2* gene: A new biomarker for prognosis of lung adenocarcinoma in Japanese non-smoking women. *PLoS One* **8,** e73794 (2013).

# Natural deep-sea batteries

A system for generating electricity from deep-sea hydrothermal vents could revolutionize ocean exploration and improve our understanding of extreme ecosystems

Exploring the deep oceans presents huge technical challenges, many of which could be overcome if there were some cheap and efficient way to deliver power to machines while at depth. To tackle this problem, a collaborative research team including Ryuhei Nakamura from the RIKEN Center for Sustainable Resource Science has now demonstrated a remarkable system that uses natural hydrothermal vents on the sea floor to generate electricity[1].

Nakamura and colleagues at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) and the University of Tokyo developed a robust robotic system that essentially works like a household battery. Hydrothermal fluid from deep-sea vents is enriched with reduced or electron-rich ions, while seawater contains oxidized or electron-depleted ions. By placing one electrode in the hydrothermal fluid and another in the seawater nearby, the system creates a chemical gradient that produces an electric current (Fig. 1).

"Our biggest challenge was to construct remotely operated electrochemical systems for fuel cell operation on the deep sea floor," says Nakamura. "We are grateful for the help of Masahiro Yamamoto's group at JAMSTEC, who are world experts in deep-sea exploration."

The researchers tested their system at a natural hydrothermal vent and at an artificial vent drilled during the Integrated Ocean Drilling Program—an international effort to study the world's seabeds. At both sites, the system generated sufficient power to illuminate three light-emitting diode (LED) lamps.

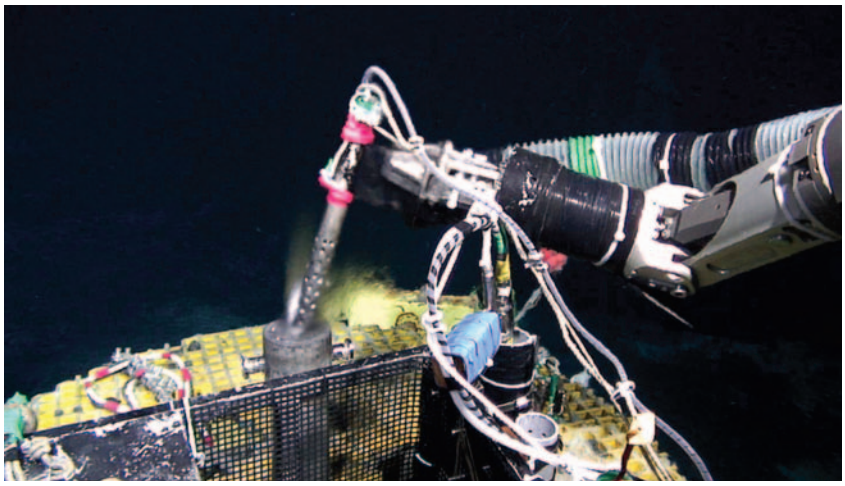

**Figure 1: A robotic system inserts an electrode into hydrothermal fluid released from deep-sea vents in order to generate electricity.**



"This is the first demonstration of an electrochemical fuel cell at a hydrothermal vent," says Nakamura. "Previous attempts have been made based on the difference in temperature between the hydrothermal fluid and seawater, by thermoelectric conversion. Our method is more efficient thanks to special electrodes we made from an iridium-coated titanium mesh that resists corrosion."

Nakamura is hopeful that the new technology will, in addition to benefiting deep-ocean science on a practical level, improve our understanding of how biological ecosystems exploit energy sources in such extreme environments.

"I am very curious about carbon fixation in environments that are isolated from solar radiation, such as the deep ocean," he says. "Because there is no input energy from solar radiation, the reductive energy discharged from the Earth's interior sustains all the biological activity there."

Nakamura and his colleagues have speculated that bacteria, microorganisms and even animals utilize electricity not only as an energy source but also as a signal for communication. "It would be fantastic if we could prove that nature was exploiting electrical energy millions of years before humans," he says.

1. Yamamoto, M., Nakamura, R., Oguri, K., Kawagucci, S., Suzuki, K., Hashimoto, K. & Takai, K. Generation of electricity and illumination by an environmental fuel cell in deep-sea hydrothermal vents. *Angewandte Chemie International Edition* **52,** 10758–10761 (2013).

# Nanotechnology gives a boost to next-generation batteries

## Ruthenium oxide nanoparticles that prevent rigid crystal formation make lithium–oxygen batteries more efficient

Non-aqueous lithium–oxygen ($Li–O_2$) batteries could store energy at densities rivaling gasoline. Commercializing this emerging technology, however, will require breakthroughs that will allow the batteries to be recharged efficiently. Hye Ryung Byon and Eda Yilmaz at the RIKEN Byon Initiative Research Unit have taken a major stride toward this goal by significantly enhancing the recharge efficiency of $Li–O_2$ batteries through judicious application of catalytic ruthenium oxide ($RuO_2$) nanoparticles[1].

$Li–O_2$ batteries eliminate the heavy metal oxide cathodes used in conventional lithium-ion batteries to let lithium react directly with atmospheric oxygen on cathodes made from light, porous materials such as carbon nanotubes. When the battery discharges, lithium ions and oxygen gas react to form lithium peroxide ($Li_2O_2$) crystals on the cathode. To recharge the battery, the insulating $Li_2O_2$ crystals must be decomposed—a reaction that requires significant recharge potentials, which can shorten battery life.

Byon and Yilmaz tried to improve the battery recharge efficiency by adding $RuO_2$ nanoparticles to the carbon nanotube cathodes. "$RuO_2$ has an optimal surface energy for oxygen adsorption and is a good catalyst for oxidation reactions," explains Yilmaz. However, because most ruthenium-based catalyses are performed in aqueous solutions, the team had to tread carefully to understand what would happen when $RuO_2$ was surrounded by solid $Li_2O_2$.

Experiments revealed that the new $RuO_2$/carbon nanotube composite considerably lowered the battery recharge potential compared to cathodes made from nanotubes alone. To understand why, the researchers collaborated with the Synchrotron Radiation Center at Ritsumeikan University in Kyoto to characterize the discharge products using a number of techniques, including x-ray absorption spectroscopy and electron microscopy. These tests revealed that the $Li_2O_2$ deposits on the $RuO_2$-loaded nanotubes had an amorphous morphology quite unlike that seen in any other $Li–O_2$ battery system.

The electron microscopy images showed that $Li_2O_2$ particles that formed on the bare nanotube cathodes had large, halo-shaped crystals. On the $RuO_2$/carbon nanotube cathodes, however, a formless layer of $Li_2O_2$ coated the entire nanotube (Fig. 1). The team notes that this $Li_2O_2$ layer has a large contact area with the conducting carbon nanotube cathode. Consequently, $Li_2O_2$ decomposition can be achieved with less energy, resulting in improved battery efficiency.

"This is one of the first studies showing how catalysts affect non-aqueous $Li–O_2$ batteries; until now there has been little focus on the impact of $Li_2O_2$ structure on battery performance," says Byon. "This research might act as a guideline for future alternative approaches."

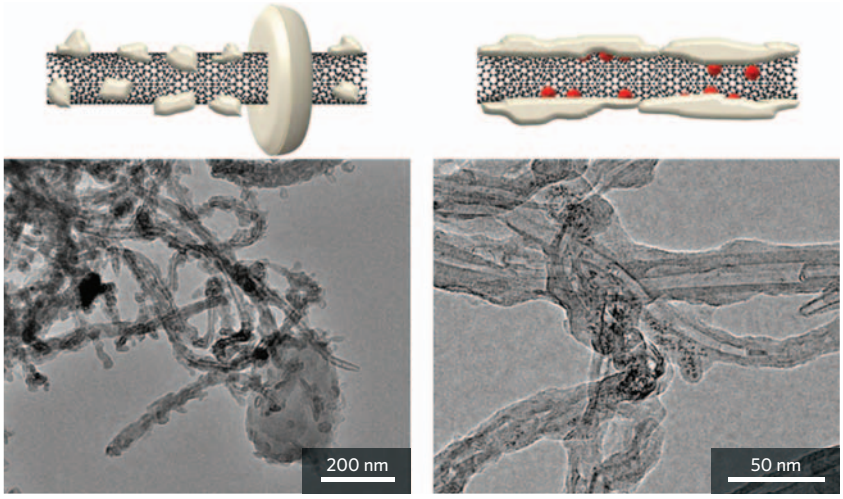


**Figure 1: Schematic views (top) and transmission electron microscopy images (bottom) showing rigid crystals that form on bare carbon nanotubes (left) and amorphous deposits on carbon nanotube cathodes with ruthenium oxide ($RuO_2$) nanoparticles (right) after discharge of lithium–oxygen ($Li–O_2$) batteries.**



1. Yilmaz, E., Yogi, C., Yamanaka, K., Ohta, T. & Byon, H. R. Promoting formation of noncrystalline $Li_2O_2$ in the $Li–O_2$ battery with $RuO_2$ nanoparticles. *Nano Letters* **13,** 4679–4684 (2013).

# Mapping the promoterome

## A comprehensive new map details the dynamics of gene activity during embryonic development

Embryonic development involves the tightly coordinated activity of thousands of genes, each switched on at a specific time and place in the growing organism under the control of regulatory DNA sequences called promoters. An international team of researchers including Piero Carninci from the RIKEN Center for Life Science Technologies has now produced the first vertebrate 'promoterome', or genome-wide map of how promoter usage changes during development[1].

Carninci and his colleagues obtained RNA samples from zebrafish at 12 key stages of development and used a technique called cap analysis of gene expression (CAGE) to determine promoter usage in each sample. The samples contain messenger RNA transcripts—temporary copies of DNA sequences that carry the information for protein synthesis—and other long non-coding RNAs. Transcripts can be identified and isolated by virtue of a common motif called the 5' cap and then sequenced to establish which promoter was used to synthesize them.

Analysis of the data revealed a number of hitherto unknown features of gene regulation. After the very early stages of development, RNAs inherited from the mother are destroyed and substituted by RNAs produced by the embryo. These RNAs are produced by a different set of promoters than those involved in the production of maternal transcripts. This is the first time that such a switch in promoter usage has been mapped at a broad scale and with such high accuracy.

The researchers compared the data to equivalent datasets collected previously for fruit flies, pufferfish and humans. The comparison suggests that all vertebrates have remarkably similar gene regulation mechanisms, which are fundamentally different from those of invertebrates.

They also identified a previously unknown regulatory sequence within vertebrate promoters. This sequence, which is rich in the DNA bases adenine and guanine, is likely to be a common regulatory element in all vertebrate species.

The zebrafish promoterome is the first large-scale map of any fish genome, and the results suggest that there are important features that are conserved in the basic structure of the promoters. "We identified short stretches of DNA that are used in the same way in distant genomes," says Carninci. "In particular, the elements that cause the gene to initiate RNA transcription."

Carninci believes that the application of CAGE will be highly beneficial for the identification of regulatory DNA sequences in many organisms. "We are now expanding the utilization of CAGE to several other organisms," he notes.

**Figure 1: Comparison of the promoterome of zebrafish (top left) and pufferfish (right) with those of humans reveals that all vertebrates have remarkably similar gene regulation mechanisms.**



1. Nepal, C., Hadzhiev, Y., Previti, C., Haberle, V., Li, N., Takahashi, H., Suzuki, A. M. M., Sheng, Y., Abdelhamid, R. F., Anand, S. *et al.* Dynamic regulation of the transcription initiation landscape at single nucleotide resolution during vertebrate embryogenesis. *Genome Research* **23,** 1938–1950 (2013).

# Clearing up the clouds

## Simulations of deep moist convection need to be run at grid resolutions finer than 2 kilometers to accurately predict the effects of climate change

Deep moist atmospheric convection controls the development of major weather systems like hurricanes, drives the global transport of energy within the climate system and strongly influences the uncertainty of projected climate change. As computational power advances, the direct simulation of cloud processes within climate change models is on the horizon. Yoshiaki Miyamoto, Hirofumi Tomita and their colleagues from the RIKEN Advanced Institute for Computational Science reveal that in order to realistically simulate the critical features of cloud convection, models will ultimately need to be run at a grid resolution no coarser than 2 kilometers[1].

Unless cloud simulations improve, uncertainties will remain unacceptably high for many pressing topics, such as the response of regional precipitation and global mean temperature to increases in greenhouse gas concentrations. Yet despite decades of research, the role of clouds in a changing climate remains unclear, largely reflecting a disconnect between cloud processes operating at the scale of individual water droplets and the 50- to 100-kilometer resolution used in many simulations of future climate.

Miyamoto and his colleagues showed that an intermediate resolution can be used to drive deep moist convection, thus providing a clear target for future climate model development. The team simulated 12 hours of global cloud processes with the Nonhydrostatic ICosahedral Atmospheric Model (NICAM) at a range of grid spacings of 0.87 to 14 kilometers. They found that simulations at 0.87 kilometers resolved deep moist convective processes ranging from individual subkilometer convective cells to the detail of tropical cyclones spanning hundreds of kilometers (Fig. 1).

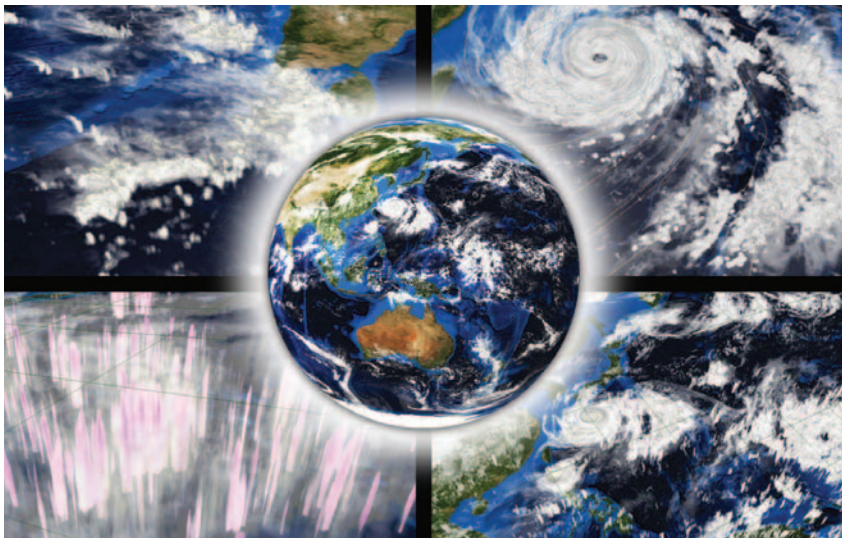

**Figure 1: The global Nonhydrostatic ICosahedral Atmospheric Model, when run with a 0.87-kilometer grid size, simulates realistic features of major weather structures.**



At resolutions coarser than 2 kilometers, crucial cloud features were lost. "In the real world, convection is intensely variable and clouds have wide gradients in water vapor density and vertical velocity," says Miyamoto. "Coarser grids create unrealistically sharp transitions in cloud properties, with negative consequences for the realistic representation of related processes like condensation and precipitation."

New theoretical insights could also arise from the team's work. "Our finding that convective features change drastically at resolutions of 2 kilometers or more opens up new avenues for research into the interactions between convection and global atmospheric circulation that would have been invisible at coarser resolutions."

Hitting the 2-kilometer target will be a challenge. Even with ever-expanding computational power, 2-kilometer simulations of climate for a period of a few years, let alone the next century, are probably a decade or more away. Another option, according to Miyamoto, would be to simulate a limited area at a 2-kilometer spacing within a coarser global grid.

1. Miyamoto, Y., Kajikawa, Y., Yoshida, R., Yamaura, T., Yashiro, H. & Tomita, H. Deep moist atmospheric convection in a subkilometer global simulation. *Geophysical Research Letters* **40,** 4922–4926 (2013).

# Modifying one cell factor alters many others

## Genetically engineering cells for specific functions can have unexpected side effects

Using a widely studied species of cyanobacterium, researchers from the RIKEN Center for Sustainable Resource Sciences have shown how difficult it is to alter the metabolism of a unicellular organism with the aim of producing a particular product without affecting other aspects of its functioning[1].

Takashi Osanai and his team genetically engineered a strain of *Synechocystis* cyanobacterium to stimulate the breakdown of sugar and the production of biopolymers. Although the modification enhanced biopolymer production as intended, they found that the change also affected the size and shape of the cell, as well as photosynthesis, respiration and other aspects of its metabolism.

The genetic modification Osanai and his team engineered in *Synechocystis* involved overexpression of the *sigE* gene, which encodes a protein that regulates RNA synthesis known as a sigma factor. Under transmission electron and scanning probe microscopy, they found that the cell size of the engineered strain was larger than normal (Fig. 1) and there was evidence that the cell division process had been affected by the modification. Photosynthetic activity also tended to be higher when nitrogen was depleted but lower when nitrogen levels were normal and under high light conditions. Furthermore, hydrogen production increased at low oxygen levels. Several regulatory proteins were also seen to change with the elevated levels of the sigma factor protein.

According to the researchers, the results suggest a close relationship between metabolism, photosynthesis, cell form and hydrogen production.

**Figure 1: Scanning electron microscopy images showing the difference in size between normal (left) and *sigE*-overexpressing *Synechocystis* cells (right).**



Despite all these differences, however, the modified strain remained just as viable as the normal strain over a range of nitrogen levels.

Apart from demonstrating the challenge in isolating cell factor-related alterations, the work also highlights the potential of cyanobacteria for the production of hydrogen for plastics and renewable energy applications. "We succeeded in increasing the production of hydrogen using the modified cyanobacteria," Osanai says. "The hydrogen was produced using light energy and the cells were cultured with carbon dioxide as a carbon source. Thus, we could possibly use our cyanobacterial strain to produce renewable energy that could replace fossil fuels and even nuclear power."

At present, the amount of hydrogen produced by this modified cyanobacterium is quite low, but Osanai is focused on exploiting the opportunity. "We will now try to increase hydrogen productivity by additional genetic engineering," he says. "We will also try to increase the synthesis of bioplastics. A deeper understanding of the mechanism of the relationship between all the factors we have uncovered will be important."

1. Osanai, T., Kuwahara, A., Iijima, H., Toyooka, K., Sato, M., Tanaka, K., Ikeuchi, M., Saito, K. & Hirai, M. Y. Pleiotropic effect of *sigE* over-expression on cell morphology, photosynthesis and hydrogen production in *Synechocystis* sp. PCC 6803. *The Plant Journal* **76,** 456–465 (2013).

# Tuning epigenetics to treat disease

MINORU YOSHIDA

Group Director
Chemical Genomics Research Group
RIKEN Center for Sustainable Resource Science



**Many diseases occur due to abnormalities in patterns of gene expression caused by epigenetic changes as a result of aging, stress or other factors. To address these changes and advance the field of medicine, Minoru Yoshida is leading research into the control of epigenetics using chemical compounds. Together with colleagues at the Chemical Genetics Laboratory, he is also investigating the activation of 'dormant genes' in plants and microorganisms to produce useful substances and to enable the cultivation of crops on arid and high-salinity soils.**

## The compounds that control cancer

Every cell in the body houses DNA, but the expression of this genetic information varies from cell to cell. In liver cells, for example, those genes that are essential to the liver are expressed. This inherited 'memory' of gene expression patterns is known as epigenetics.

Research into cancer cell differentiation initially emerged from the discovery in the 1980s of a phenomenon whereby cancer cells cultured in the presence of a certain compound transformed into normal cells. The finding catalyzed a worldwide search for compounds that 'normalize' cancer cells, and eventually propelled research into epigenetics.

Among those investigating the newly uncovered phenomenon was Teruhiko Beppu, the head of a laboratory at the University of Tokyo. A direct successor of the late Kin-ichiro Sakaguchi, a world authority on applied microbiology who also worked at RIKEN, Beppu led an experimental study to culture cancer cells in the presence of extracts from various microorganisms.

The team eventually succeeded in normalizing the leukemia cells with an extract derived from a microorganism, but was yet to identify which specific compound in the extract had been responsible for the normalization. "At the time, I was about to undertake a master's program at Beppu's laboratory and asked him to allow me to take over the study, but he refused," recalls Yoshida. "It was only after I proceeded to embark on my doctoral degree that he gave me permission to conduct the study."

In 1987, Yoshida demonstrated that trichostatin A, a microbe-derived compound, had caused the leukemia cells to normalize. However, since trichostatin A was already a known compound, Yoshida asked laboratory head Beppu to let him continue to investigate the mechanism by which the compound normalizes cancer cells. "In natural product chemistry, an emphasis is placed on discovering new compounds. It would therefore have come as no surprise to me if he had rejected my request. Fortunately, he encouraged me to continue my research."

## Breaking through into epigenetics

In 1990, Yoshida identified the mechanism by which trichostatin A normalizes cancer cells. This discovery spurred epigenetics research into becoming the active global field that it is today.

"All DNA in a wide variety of cells that were differentiated from a single fertilized egg conveys the same information. Despite this, liver cells and nerve cells possess totally different natures. This difference arises as a result of epigenetics," explains Yoshida.

A portion of the genes in DNA carries information on protein biosynthesis. In liver cells, only those genes for proteins essential to themselves are expressed; the genes essential only to nerve cells are never suddenly expressed. "Liver cells memorize the expression pattern of the genes essential to themselves without writing it in their DNA sequence. Even after cell division, this memory is retained," Yoshida elaborates further.

But the location of this memory was not known until recently. "DNA is made compact by its winding around proteins called histones. A wide variety of chemical substances, including acetyl groups and methyl groups, attach to the histone–DNA complex. These chemical modifications were considered to be important to epigenetics but the details of their role were not known," says Yoshida.

In his investigation of trichostatin A, Yoshida found that the compound inhibits histone deacetylase (HDAC) from fulfilling its function of eliminating the acetyl groups bound to a histone. Further study of the trichostatin A compound helped to elucidate the relationship between histone chemical modifications and gene expression. "The acetyl groups bound to a histone serve as flags to indicate the command: 'Read this gene.' Accordingly, molecules that read the flag come together, and the gene is expressed. Conversely, there are flags that indicate the command: 'Do not read this gene.' The flagging pattern differs depending on the type of cell," notes Yoshida.

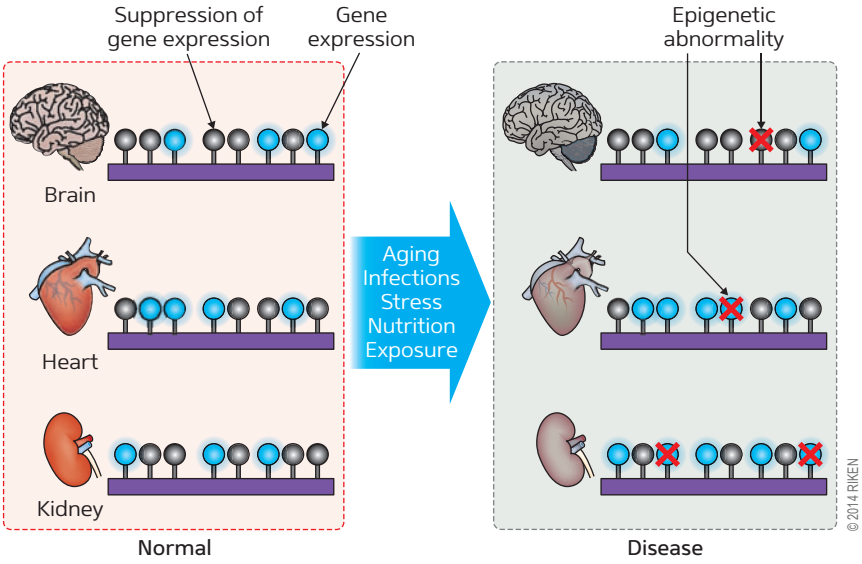


**Figure 1: Epigenetics and disease**

Many diseases are believed to occur as a result of abnormalities in gene expression patterns caused by epigenetic changes due to aging, infections, stress and other factors.

## Flagging cancer

The flagging system also offered an explanation for how trichostatin A normalizes cancerous cells. "Cells transform into cancer cells when their DNA is injured by exposure to ultraviolet rays, carcinogens or other factors," notes Yoshida. In normal cells, however, 'cancer-suppressor genes' work to suppress this transformation.

To express the cancer-suppressor gene, the histone around which the DNA carrying the cancer-suppressor gene is wound is acetylated. "In many cases, cancer transformation is induced when the expression of cancer-suppressor genes no longer functions. Many cancer cells seem to eliminate the acetyl groups using HDAC to prevent cancer-suppressor genes from being expressed. Because trichostatin A inhibits HDAC, the acetyl groups are not eliminated and cancer-suppressor genes are allowed to be expressed. This intervention normalizes the cancer cells," asserts Yoshida.

Epigenetic alterations have also been observed in gastric mucosal cells of the stomach that have been infected with *Helicobacter pylori*. "These modifications can contribute to increased susceptibility to gastric cancer. In addition, in many cancer cells, genes not normally read in that specific cell are expressed. In this situation, for example, genes typically expressed in the testis can be expressed in cells outside of the testis," notes Yoshida.

Yoshida suggests that all diseases, not just cancer, are influenced by epigenetic changes. "Many diseases do not manifest themselves by nature. Diseases occur as a result of abnormalities in gene expression patterns caused by epigenetic changes due to aging, infections, nutritional conditions, stress and other factors," (Fig. 1).

## Treating intractable diseases with 'chemical epigenetics'

In light of this observation, Yoshida and his colleagues began to apply 'chemical epigenetics'—the control of epigenetics using chemical compounds—with the aim of contributing to the treatment of diseases that are intractable using conventional therapies. The compounds would target epigenetic-related enzymes and molecules, such as HDAC (Fig. 2).

Yoshida's research team demonstrated that, in addition to trichostatin A, two compounds of microbial origin—trapoxin and FK228—also inhibit HDAC and clarified the mechanism of their action. Unfortunately, neither trichostatin A nor

trapoxin evolved into cancer therapeutics. However, FK228 and suberoylanilide hydroxamic acid (SAHA), a compound similar to trichostatin A, are already in clinical use for cancer therapeutics.

As modifiers of epigenetic processes, FK228 and SAHA could potentially have therapeutic effects not only against cancers but also against various other diseases. "These compounds have been reported to be effective against neurodegenerative diseases, such as Alzheimer's and Huntington's disease," adds Yoshida. However, FK228 and SAHA still pose the problem of also acting on HDAC. Thus, to increase the therapeutic effect of these compounds with minimal adverse reactions, the specific epigenetic abnormality that is the cause of each disease must be controlled with pinpoint accuracy—a goal yet to be achieved, points out Yoshida, since the epigenetic changes that cause individual diseases still remain unclear.

Elucidating epigenetic changes in the cell differentiation process would also aid drug discovery and regenerative medicinal research. In fertilized eggs and cells in the early stages of development, the epigenetic state is 'reprogrammed' to allow all genes to be expressed. The genes undergo various chemical modifications, and the cells differentiate into a wide variety of types while changing their gene expression pattern.

Induced pluripotent stem (iPS) cells, with their potential for differentiating into all types of cells, are also considered to represent a state of reprogrammed epigenetics. Epigenetics could be used to differentiate iPS cells into any selected type of cell and subsequently utilized to treat diseases. However, Yoshida notes that "the epigenetic changes in the differentiation process are still unclear."

In 2009, Yoshida and his colleagues developed Histac, a fluorescent probe that visualizes histone acetylation in living cells (Fig. 3). "We are also developing a fluorescent probe for histone methylation. These techniques for visualizing chemical modifications are powerful tools for elucidating epigenetics," says Yoshida.

## Chemical genomics project

In addition to chemical epigenetics, the Chemical Genetics Laboratory at RIKEN is also engaged in chemical genetics. The discipline explores the mechanisms of various biological phenomena by treating cells with compounds produced by organisms, like trichostatin A, and determining which target proteins they act on, and what changes result in their phenotypes.

Genes and proteins exhibit their functions not by working alone but by interacting with other biomolecules to form a network. Since identifying the targets of compounds is not an easy task, Yoshida and his colleagues chose yeast for their experimental work. Yeast contains around 5,000 distinct, active genes and their functional network has been almost completely clarified. In addition, yeast permits easy target-identification experiments because of the availability of knockout yeasts, each lacking one of the 5,000 genes.

"A compound's action differs if a gene for a target or a protein that interacts with the target is lacking. In some cases, an organism can survive without gene A but dies if gene B is also lacking," explains Yoshida. "Researchers at the University of Toronto in Canada created double-knockout yeasts that concurrently lack two genes for most of the 5,000 by 5,000 gene combinations, and determined their phenotypes, which they published in a database," he continues. "Hence, we developed an experimental system for concurrently examining the phenotypic changes caused by treating 5,000 knockout yeasts with a certain compound. By checking our results against the phenotypic information of the double-knockout yeast available in the University of Toronto database, we can estimate the location of the target in the functional network on which the compound has acted."

Yoshida's team at the Chemical Genetics Laboratory is also using the experimental system they developed in another initiative. In collaboration with the Antibiotics Laboratory at RIKEN, the group started a chemical genomics project to estimate the biological targets of 40,000 natural compounds—mostly of microbial derivation—maintained in a library by RIKEN. The effort will create a powerful tool for elucidating not only chemical epigenetics but also all biological phenomena. "We expect this project to discover compounds that act on unknown targets and candidate compounds that selectively act on particular portions of the network, which will serve as drugs with fewer adverse reactions," explains Yoshida, whose team has tested 10,000 compounds so far.

Furthermore, Yoshida is planning to conduct the same experiments with human cells. "It is necessary to use human cells to search for drug target networks not found in yeast, such as the cerebral nervous system, or to identify

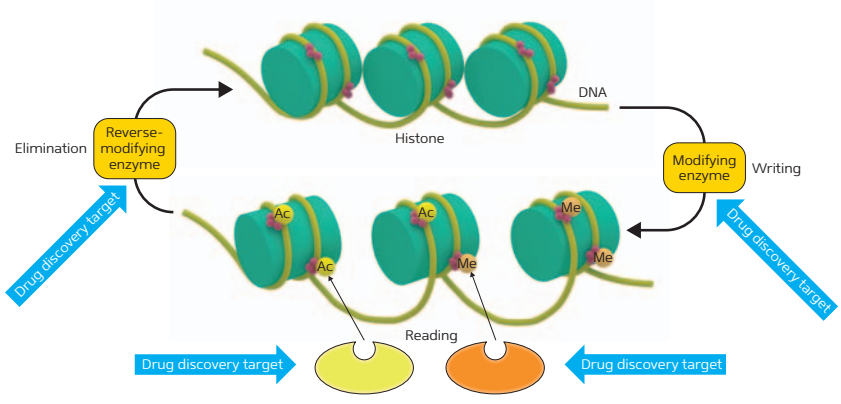




**Figure 2: Epigenetic-related enzymes and molecules that are potential targets for drug discovery**
Researchers can control diseases using epigenetics by targeting the modifying enzymes that add or 'write' acetyl (Ac) or methyl groups (Me) onto histones, and the molecules that read or the enzymes that reverse these chemical modifications.

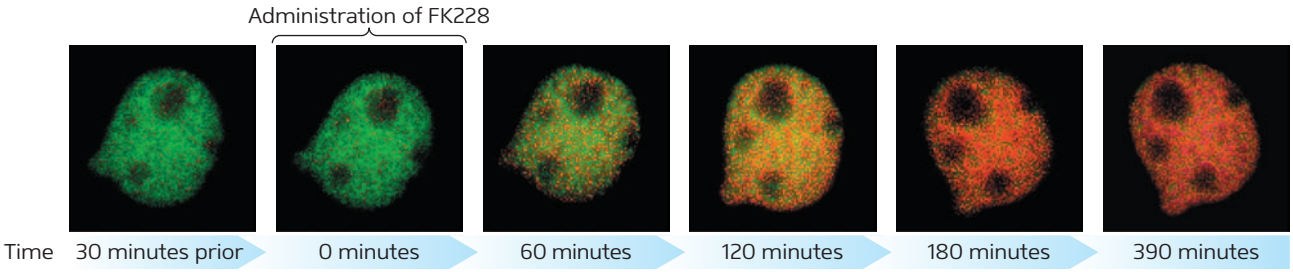


**Figure 3: Visualization of histone acetylation using Histac**

Living cells containing Histac, a fluorescent probe that visualizes histone acetylation, were treated with the histone deacetylase inhibitor FK228 and examined for changes in histone acetylation. The initial state with a low level of acetyl groups attached (green) turns red as acetylation progresses due to the action of FK228.

the utility of compounds for drug discovery. We have already begun an experimental study based on our idea."

## Activating 'dormant genes'

In April 2013, RIKEN founded the Center for Sustainable Resource Science (CSRS), where Yoshida heads the Chemical Genomics Research Group. "At the CSRS, researchers are working to develop a method for activating 'dormant' genes by controlling plant epigenetics to grow crops in arid lands and on soils of high salinity."

Yoshida believes that, unlike gene recombination, which integrates a gene or other genetic material from one organism with another, the approach of activating a gene that an organism essentially already possesses by controlling its epigenetics should be more widely accepted in society. "Already, seedless grapes are grown by administering a plant hormone. Likewise, a crop can be made more resistant to the stresses of drought and salinity by culturing it with the addition of a small amount of a certain compound," maintains Yoshida. "We also aim to express dormant microbial genes to produce candidate compounds for new drugs."

In the past, searching for natural compounds of microbial origin, such as penicillin, was the main method of drug discovery. Now, however, there is considerable research into artificially synthesized small compounds that act on disease-causing target proteins due to the decrease in discovery of new natural compounds. "However, it is not easy to synthesize a small molecule that selectively acts on a particular target. Therefore, creating new drugs is challenging. Against this background, we are working to create new compounds that act on target proteins by controlling epigenetics to activate dormant microbial genes. As a result, natural compounds, including those of microbial origin, will again play the leading role in drug discovery," suggests Yoshida.

## Revolutionizing the life sciences

In 2011, the Laboratory of Molecular Genetics at RIKEN's Tsukuba campus discovered a phenomenon whereby a stress-altered gene expression pattern is inherited by the offspring of *Drosophila* via epigenetics. "It was a major discovery that overturned the conventional theory that the characteristics acquired by a parent through its experience are not inherited by its offspring," reveals Yoshida.

Increasing evidence has shown that epigenetics plays a role in more than just the determination of gene expression patterns. Genetic information written in DNA is transcribed into RNA. Unwanted portions, known as introns, are then cleaved off, and only the necessary portions, or exons, are joined together. Proteins are synthesized in accordance with the information present in the exons. "This process is called splicing. By altering the mode of splicing, different proteins can be produced from the same gene. This explains the fact that more than 100,000 different proteins are produced from approximately 22,000 human genes. It has also become evident that splicing patterns are written on histones," says Yoshida.

Yoshida and his colleagues are currently conducting an experimental study, which uses compounds to inhibit molecules that act in splicing, and have found a fascinating relationship between splicing and epigenetics. These forthcoming findings, and the pioneering discoveries by Yoshida and his colleagues over the past decades, induce much optimism. "We can look forward to a future when chemical epigenetics and chemical genomics will revolutionize medicine, the life sciences and our approaches to environmental problems," Yoshida proclaims.

### ABOUT THE RESEARCHER

**Minoru Yoshida was born in Tokyo, Japan, in 1957. He received his PhD in 1986 from the University of Tokyo's Graduate School of Agricultural and Life Sciences under the guidance of Teruhiko Beppu. In 1995, Yoshida was promoted to associate professor in the University of Tokyo's Department of Biotechnology, and in 2002, he moved to RIKEN as chief scientist of the Chemical Genetics Laboratory at the former RIKEN Advanced Science Institute. In 2008, he was also appointed as group director of the Chemical Genomics Research Group, now part of the RIKEN Center for Sustainable Resource Science. Yoshida's current research focuses on a wide range of areas in chemical biology, including epigenetics, metabolism and post-translational modifications in proteins.**


# Leading the life sciences at RIKEN

MIKAKO SHIROUZU

Director
Division of Structural and Synthetic Biology
RIKEN Center for Life Science Technologies



**What made you decide to become a scientist?**

In high school, we were required to join a school club. I was unable to join my first choice so instead became a member of the biology club. We worked in pairs to conduct experiments but the friend I was partnering with left to study abroad, meaning that I had to do everything on my own.

Performing experiments alone was challenging but I was really happy when they were successful. I realized how much fun performing experiments could be and thought that it would make a great career.

**What is your role at RIKEN?**

I currently serve as director of the Division of Structural and Synthetic Biology in the RIKEN Center for Life Science Technologies (CLST) and am also a deputy director of the center. I am involved in decision-making and the coordination of operations for the CLST and various research groups. I also chair meetings to promote research initiatives.

An enjoyable aspect of my work is helping our principal investigators and researchers to resolve the issues that they face. I hope that my current efforts are building the foundation of a continuing collaboration with many other scientists at RIKEN.

**How did you join RIKEN?**

In 1993, when I was still a PhD student, my supervisor—Professor Shigeyuki Yokoyama—established the Cellular Signaling Laboratory at the RIKEN Wako campus. I came to RIKEN that fall as a member of the lab and in 1995 was hired as a researcher. In 2000, I was transferred to the Yokohama campus as part of a research group within the Genomic Sciences Center. I worked on the RIKEN Structural Genomics/Proteomics Initiative, which was running as a major part of the National Project on Protein Structural and Functional Analyses (NPPSFA), also known as the 'Protein 3000' project. The equipment we used was incredible, and it was a very rewarding experience for me.

**How did you become interested in your current field of research?**

As a university student, I was interested in the mechanisms behind various biological phenomena, such as physiological processes involved in the onset of disease. I wanted to understand these phenomena on a molecular level, which is why I chose my initial research lab. The lab focused on analyzing molecular structures, so I became interested in working to elucidate a structural basis for the mechanisms of cellular signal transduction.

I found it especially fulfilling to be able to determine many protein structures for the Protein 3000 project even though my contributions to the project were not vast. It was interesting when the activity of a protein matched our predictions but equally intriguing when structures turned out to be something we never expected.

**What is the best thing about working at RIKEN?**

RIKEN is very flexible and supportive of researchers who wish to continue their research while on maternity leave. Even when I was away from the campus, I could conveniently access what I needed and communicate instructions for the lab and discussions over the phone and by e-mail.

**CONTACT INFORMATION**

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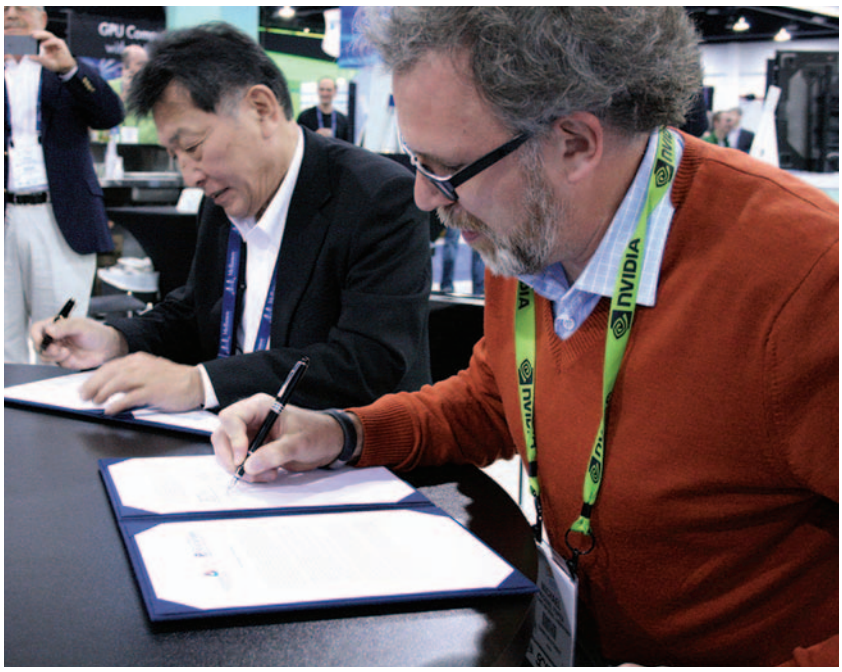
# RIKEN and Argonne formalize alliance in petascale computing

The RIKEN Advanced Institute for Computational Science (AICS) and the Argonne Leadership Computing Facility (ALCF) in the United States signed a Memorandum of Understanding (MoU) to support collaborative projects aimed at expanding the use of petascale computing in the scientific and engineering communities. Petascale computers are capable of processing data at a rate of at least one quadrillion floating point operations per second (petaflops).

Kimihiko Hirao, director of the AICS, and Michael Papka, director of the ALCF, signed the MoU at the supercomputing conference SC13, held in the United States in November 2013. The MoU identifies areas of mutual interest in petascale computing for the two leading institutes and is expected to bring several new research capabilities to the AICS.

Among the distinguished guests who attended the signing ceremony were AICS Deputy Director Akinori Yonezawa, AICS Policy Planning Division Head Hiroshi Kataoka, Argonne Associate Laboratory Director Rick Stevens, ALCF Deputy Director Susan Coghlan and ALCF Director of Science Paul Messina.



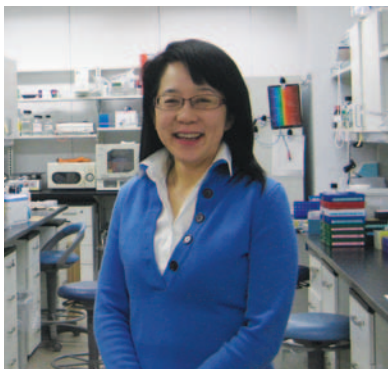



Kimihiko Hirao (left) and Michael Papka (right), directors of the RIKEN Advanced Institute for Computational Science and the Argonne Leadership Computing Facility, respectively, sign a Memorandum of Understanding to advance cooperation in petascale computing.

## RIKEN on *Nature*'s radar for 2014

The interdisciplinary research journal *Nature* has identified Masayo Takahashi from the Laboratory for Retinal Regeneration at the RIKEN Center for Developmental Biology as one of five people to watch in 2014, for her leadership of the first clinical study to use induced pluripotent stem (iPS) cells.

Takahashi, in collaboration with the Institute of Biomedical Research and Innovation (IBRI) and supported by the Kobe City Medical Center General Hospital, plans to test the feasibility and safety of using iPS cells to treat age-related macular degeneration (AMD), a common cause of blindness among the elderly. Launched in 2013, the study will create iPS cells derived from patients afflicted with AMD and differentiate them into one-cell thick sheets of retinal epithelium to transplant into the affected area of the eye.



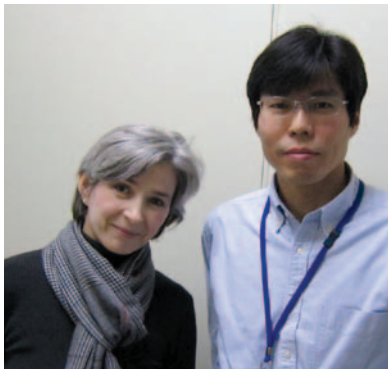


Masayo Takahashi, one of *Nature*'s five people to watch in 2014.

Also receiving a special mention in *Nature*'s 2013 Review of the Year was a captivating image of the Detector Array for Low Intensity Radiation 2 (DALI2) at the Radioactive Isotope Beam Factory, jointly operated by RIKEN and the University of Tokyo. The image was selected by *Nature*'s art and design team for highlighting one of last year's triumphs in science—the identification of 34 as a new 'magic number' of nuclear stability in an exotic calcium isotope, to which DALI2 was critical.

## RIKEN scientists among 2013 NISTEP Researcher Award winners

Japan's National Institute of Science and Technology Policy (NISTEP) under the Ministry of Education, Culture, Sports, Science and Technology (MEXT) awarded the 2013 NISTEP Researcher Award to two scientists at the RIKEN Center for Integrative Medical Sciences. Kenya Honda, head of the Laboratory for Gut Homeostasis, and Sidonia Fagarasan, head of the Laboratory for Mucosal Immunity, received the award for developing a technique to elucidate the role of microorganisms that inhabit the human digestive track in the immune system. The method combines metagenomics—analysis of genes contained in the body and other organisms it hosts—with metabolomics—the study of the metabolic products of cellular processes—using next-generation gene sequencing technologies.





Sidonia Fagarasan (left) and Kenya Honda (right) from the RIKEN Center for Integrative Medical Sciences received the 2013 NISTEP Researcher Award.

The integrated approach enabled the pair to gain an understanding of the complex molecular and cellular mechanisms that regulate the environment in the digestive system, creating avenues for the treatment of a variety of illnesses, such as allergic and inflammatory bowel diseases.

The NISTEP Researcher Awards are presented at the end of every year to recognize advances in science and technology in Japan. Honda and Fagarasan were among nine teams to receive the award in 2013.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