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Unraveling splicing in plant development

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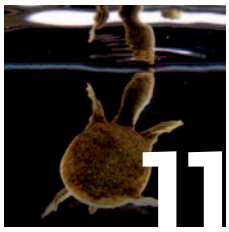

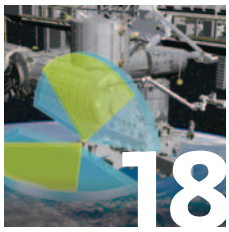

RIKEN RESEARCH

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Biology

Multifunctional molecules aid mutation analysis

Specialized molecular probes that light up on binding to their target allow scientists to quantify DNA samples and detect specific mutations in a single test

Over the past decade, DNA sequencing technology has become faster and more powerful while also growing more affordable and accessible. As a result, researchers have been able to chart a multitude of genomic variations that could potentially contribute to the development of various cancers as well as a host of hereditary diseases.

Identifying a candidate mutation is only the beginning, however, as independent assays are required to confirm the link between gene and disease. Matthias Harbers and Kengo Usui from the Division of Genomic Technologies at the RIKEN Center for Life Science Technologies have now developed a special class of probes that could accelerate the development of such assays by allowing scientists to simultaneously quantify target DNA sequences while also detecting the presence of mutations of interest[1].

Scientists have used variations of a technique called real-time polymerase chain reaction (PCR) to amplify target DNA while monitoring the progress of the amplification. These data are used to determine the original amount of genetic starting material, which can be valuable information in a diagnostic context. Real-time PCR is performed using short stretches of DNA called 'primers', which bracket the target sequence of interest and initiate amplification. Harbers and Usui previously worked with a special class of fluorescently labeled molecules called 'exciton-controlled hybridization-sensitive fluorescent oligonucleotides' (ECHOs), which feature a pair of dye molecules that quench each other's fluorescence when in close proximity. When the ECHO binds DNA, the dye molecules become separated as they insert themselves into the double-helix structure, generating a signal that is proportional to the target DNA's amplification. To efficiently couple this quantitation with the ability to directly detect the presence or absence of mutations, the researchers set out to devise a modified ECHO variant that combines both functions into a single assay.

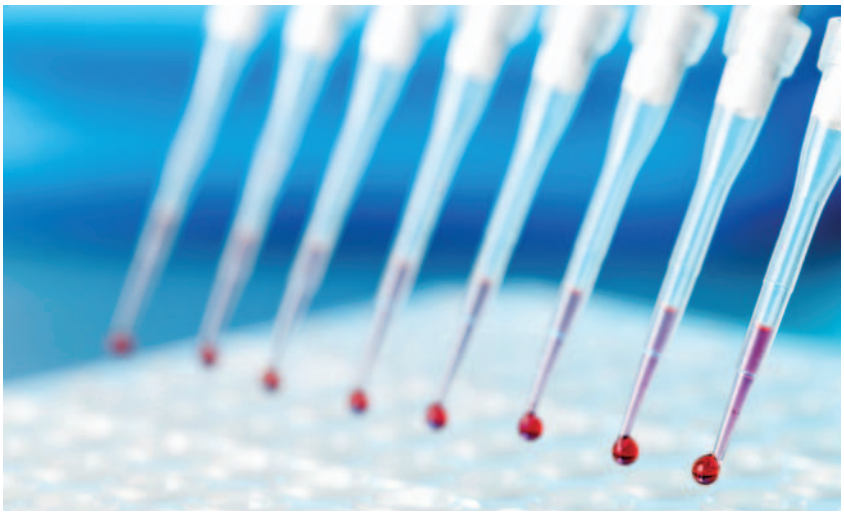


Figure 1: Specialized fluorescent probes allow scientists to quantify and detect mutations in target DNA with a single PCR-based test.

Dual-purpose probes

DNA strands that are directly complementary to each other bind more tightly than those that contain mismatches. Scientists can use targeted probes to detect the presence or absence of mutations based on this principle through a technique called melting curve analysis. In contrast, PCR primers generally need to be a perfect match for their target sequence to work reliably. Harbers, Usui and their colleagues therefore opted to modify ECHOs so that they serve as detection probes that act in parallel with a separate set of real-time PCR primers.

The ECHO-based 'Eprobes' developed by the research team are designed to bind to potential mutation-containing sites within the region being amplified, allowing amplification detection in real time (Fig. 2). However, Eprobes also contain a chemical group that prevents them from being incorporated into the amplified DNA and instead causes them to dissociate from the target as the sample is heated during the PCR process. Once

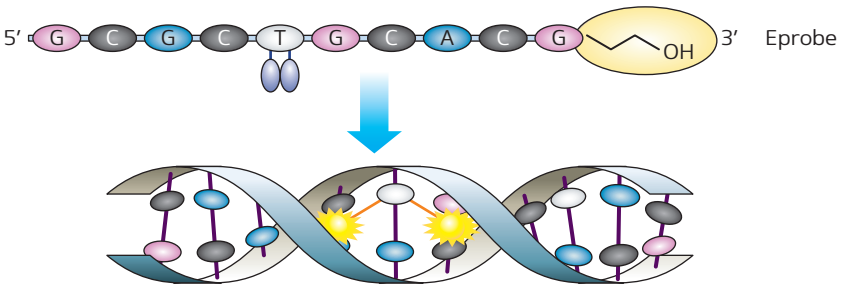


Figure 2: Eprobes contain a pair of dye molecules that interfere with each other's fluorescence (top) until they bind to a complementary DNA target sequence (bottom)—a process that separates the two dyes and generates a signal. A chemical blocking group (yellow oval) prevents the Eprobes from being incorporated in the PCR, so that they can be used for mutational analysis after the real-time PCR reaction is complete.

the PCR has concluded, the Eprobes go back to detecting mutations. "Since their fluorescence activity is still maintained after PCR, we can carry out melting curve analysis as a post-PCR assay in the same tube without additional modification of the reaction mix," says Usui.

As an initial demonstration, the researchers designed a series of Eprobes to recognize a segment of the gene encoding the epidermal growth factor receptor (EGFR) protein, targeting a mutation associated with cancer risk. The Eprobes performed well in initial experiments, achieving sensitive detection of amplification over the course of the real-time PCR process. Importantly, these Eprobes also proved to be capable of distinguishing between samples that contain only mutant target DNA from those that contain both normal and mutated sequences. Such discrimination is important for many diseases, where a patient's prognosis can differ depending on whether one or both copies of a chromosome carry a particular mutation. Furthermore, unlike other fluorescent detection tools, Eprobes are completely dependent on DNA binding to generate a signal and are therefore far less likely to produce false positive results that could confound diagnosis.

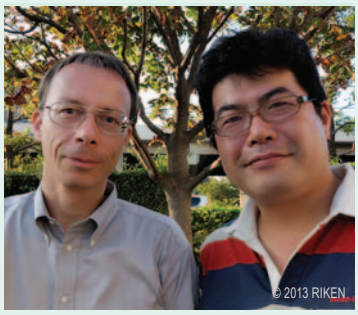
Monitoring multiple mutations

By combining Eprobes labeled with different fluorescent dyes that exhibit different melting behaviors, scientists could conduct 'multiplexed' assays to look at several different mutations or genomic regions simultaneously. To demonstrate this concept, the researchers combined a pair of Eprobes and primer sets to simultaneously investigate both *EGFR* and *KRAS*, another gene prone to oncogenic mutations. "At this point, we have two dyes that work well and have also determined that it may be possible to work with Eprobes of three different melting temperatures in one assay," says Harbers. "This would in theory enable 'six-plexing' and could be extended once we have more dyes." Harbers further notes that the expansion of these multiplexing capabilities will be a key objective in enabling Eprobes to achieve their potential as diagnostic tools.

The research team is currently working with clinicians at Gunma University Hospital to test the sensitivity of these reagents for detecting relatively scarce mutations within the highly heterogeneous context of a tumor. "The frequency of mutation in samples from cancer patients is usually quite low—under 5 per cent—and the detection of such rare mutations is our next challenge," says Usui. This technology is also undergoing commercial development through K.K.DNAFORM, a RIKEN venture company, and scientists may soon be able to obtain customized Eprobes for a variety of imaging applications. For example, sequence-specific Eprobes could be designed for mapping chromosomal regions of interest, allowing scientists to directly identify clinically relevant chromosomal abnormalities using a microscope. "Any disease for which the outcome or treatment course can be determined by mutation detection could potentially be targeted by our technology," notes Usui.

1. Hanami, T., Delobel, D., Kanamori, H., Tanaka, Y., Kimura, Y., Nakasone, A., Soma, T., Hayashizaki, Y., Usui, K. & Harbers, M. Eprobe mediated real-time PCR monitoring and melting curve analysis. *PLoS ONE* **8,** e70942 (2013).

ABOUT THE RESEARCHERS



Matthias Harbers was born in Göttingen, Germany, in 1960. He received his PhD from the University of Hamburg in 1989. Harbers worked in Sweden and France before joining RIKEN as a visiting scientist at the Omics Science Center, which was later reorganized into the RIKEN Center for Life Science Technologies. As a member of the FANTOM Project, Harbers has contributed to the development of full-length cDNA cloning and analysis methods (Cap Analysis of Gene Expression (CAGE) technology). His current work focuses on advancing molecular diagnostic methods.

Kengo Usui was born in Kasugai in 1974. After obtaining his PhD from Gifu University in 2003, Usui worked as a researcher for the Japan Science and Technology Agency's CREST program, where he developed self-assembling proteins known as 'Nanolego'. In 2008, he joined the RIKEN Omics Science Center and has since been conducting research into the development of a rapid genetic diagnostic system. Usui became unit leader of the Genetic Diagnosis Technology Unit at the RIKEN Center for Life Science Technologies in 2013.

Rapid recall to fight familiar foes

By tinkering with immune cell development, researchers learn how the body mounts an accelerated response to recurring threats

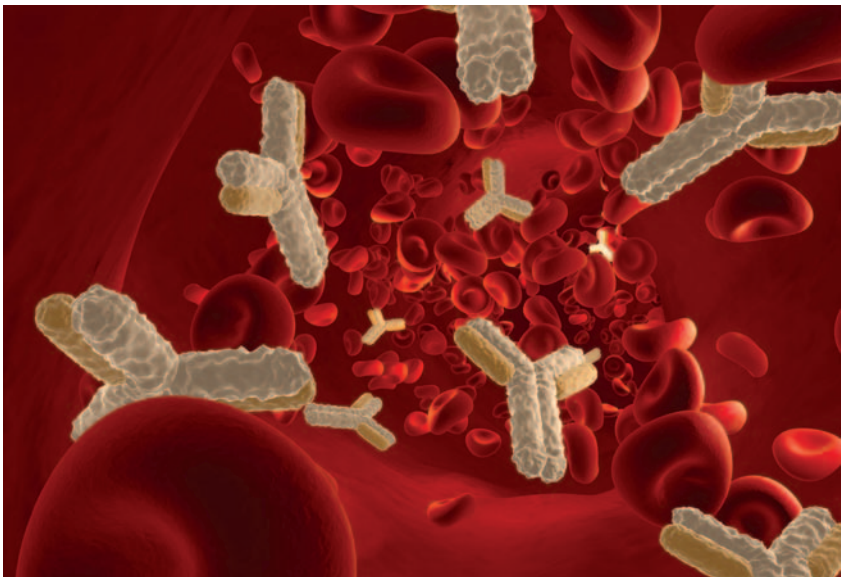


Figure 1: Memory B cells quickly recognize threats previously encountered by the immune system to mount a rapid and robust antibody response.

The immune system's first encounter with a potential threat is a valuable learning experience. Through a process of genetic recombination, our immune B cells can potentially produce a wide array of B-cell receptor (BCR) molecules, each recognizing a distinct molecular target. When a naive B cell bumps into its specific target, it initiates an immune response that yields antibodies with the same specificity as its BCR, but also gives rise to 'memory cells' that can quickly recognize the target if it appears in the future. Working with genetically modified mice, Tomohiro Kurosaki's team at the RIKEN Center for Integrative Medical Sciences in Yokohama has now gained insight into how memory cells mobilize[1].

"Memory B cells achieve rapid and robust antibody production during a secondary immune response, but its molecular mechanism was unknown," explains Kohei Kometani, a researcher in Kurosaki's lab and lead author of the study.

Naive B cells typically produce BCRs and antibodies that belong to the immunoglobulin M (IgM) class of proteins. In contrast, memory B cells produce an alternative immunoglobulin G (IgG) subtype of BCRs and antibodies through an additional gene recombination process called 'class switching'. Some researchers believe that the IgG form of BCR contains structural elements that stimulate the memory B-cell response, while others have favored cellular mechanisms besides IgG-induced signaling.

In initial experiments, the researchers worked with IgM naive and IgG memory cells that specifically recognize a known antigen, nitrophenol. As expected, the former cells proliferated in response to nitrophenol, while the latter promptly developed into antibody-secreting plasma cells, as occurs in a typical secondary immune response. To determine whether IgG is specifically responsible, Kurosaki's group generated cloned mice derived from an IgG memory cell that recognizes nitrophenol.

The resulting animals produced nitrophenol-specific IgG naive B cells, which do not normally occur in nature. Remarkably, these cells responded to nitrophenol by proliferating in essentially the same fashion as IgM naive cells, suggesting that IgG alone does not drive the memory response. A comparative analysis of gene expression revealed an alternative mechanism, controlled by a protein called Bach2. "We found that Bach2 is reduced in memory B cells, and is important for their enhanced antibody production," says Kometani.

By revealing this IgG-independent mechanism, these results should help resolve the long-standing debate over memory cell function. However, this finding is just a starting point, and Kurosaki's group is now engaged in exploring the upstream factors that switch off Bach2 production in memory cells.

1. Kometani, K., Nakagawa, R., Shinnakasu, R., Kaji, T., Rybouchkin, A., Moriyama, S., Furukawa, K., Koseki, H., Takemori, T. & Kurosaki, T. Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. *Immunity* **39,** 136–147 (2013).

Improvements in efficiency stack up

An unexpected but welcome change in polymer structure opens a new avenue in the search for improved organic solar cell efficiency

Solar cells based on organic polymers are of great interest because the materials are both cheaper to make and easier to process than those used in traditional inorganic solar cells. To date, however, the very best power conversion efficiencies for polymer solar cells remain below the threshold for practical application. Itaru Osaka from the Emergent Molecular Function Research Group at the RIKEN Center for Emergent Matter Science and co-workers have now serendipitously discovered that changing the polymer's structure results in a significant enhancement of power conversion efficiency[1].

When light energy is absorbed by the polymer in a polymer solar cell, electrons are excited to higher energy levels to produce a high-energy electron and a corresponding electron 'hole'. To convert the light energy into electrical current, these electrons and holes have to move through the polymer to the electrodes before they recombine and the energy is lost. Much research has been devoted to understanding how to improve this conversion process.

Osaka and his colleagues had been working with a particular type of copolymer containing a repeating naphthodithiophene–naphthobisthiadiazole structure called PNNT-DT. "PNNT-DT has very low solubility," explains Osaka, "so we were interested in attaching additional alkyl side chains to the polymer to improve its processability." As expected, this modification significantly improved the solubility of the polymer, but also significantly and unexpectedly improved the power conversion efficiencies of solar cells made with the polymer.

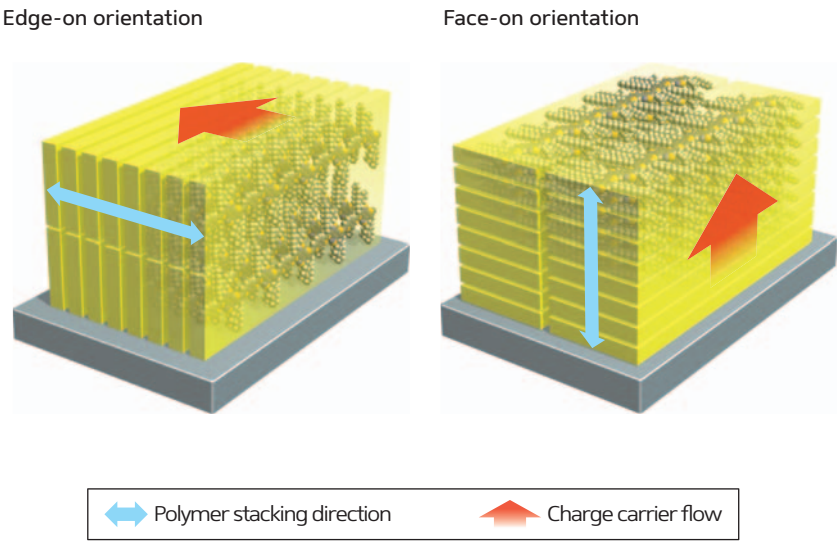


Figure 1: A small change in polymer structure affects how the polymer chains stack together in a thin film, resulting in a dramatic improvement in solar cell efficiency.

In the solar cells, the polymer is deposited as a thin film, and analysis revealed that these new 'alkylated' polymers were arranged so that the polymer chains lay flat in stacks on the surface rather than aligned perpendicular to it. This causes the charge carriers—electrons and holes—to move perpendicular to the surface rather than parallel, improving the power conversion efficiency (Fig. 1). "This unexpected change in orientation produced solar cells with an efficiency of up to 8.2% compared with just 5.5% for the unalkylated material," says Osaka.

Ultimately, Osaka and his co-workers hope to exploit this dramatic efficiency enhancement in other polymers in order to move closer to producing polymer solar cells that can truly compete with the 15% or greater efficiency of inorganic solar cells. "We need a greater understanding of why this switch in polymer orientation occurs, and then we need to apply it to other polymers that can absorb a wider range of visible light wavelengths," he says.

1. Osaka, I., Kakara, T., Takemura, N., Koganezawa, T. & Takiyama, K. Naphthodithiophene–naphthobisthiadiazole copolymers for solar cells: alkylation drives the polymer backbone flat and promotes efficiency. *Journal of the American Chemical Society* **135,** 8834–8837 (2013).

A new twist in plant development

An enzyme that unravels RNA molecules has a crucial role in plant development

Before messenger RNA molecules can be translated into amino acids and eventually functional proteins, the primary gene transcripts must be spliced to remove non-coding sequences. Misato Ohtani from the RIKEN Center for Sustainable Resource Science and colleagues have now identified a key protein involved in splicing and shown that it is essential for several aspects of plant development[1].

Splicing is carried out by the spliceosome, which detects the non-coding sequences—called introns—and splices the primary RNA transcripts by a precisely regulated step-by-step process. The spliceosome itself consists of five different RNA–protein complexes, called snRNPs. These complexes have over 50 other associated proteins, all of which are vital for proper spliceosome assembly. However, the roles of only a few of these proteins have so far been characterized.

Ohtani and her colleagues investigated one of these co-factors of spliceosome assembly by performing genetic analyses on mutated *Arabidopsis thaliana* plants displaying temperature-sensitive developmental defects (Fig. 1). Their target was the mutated gene associated with these defects, called *ROOT INITIATION DEFECTIVE1* or *RID1*. They found that the associated DNA sequence corresponds to a protein of the DEAH-box RNA helicase family of enzymes, which catalyze the twisting and unraveling of the two strands of messenger RNA molecules to allow splicing.

To confirm the RID1 enzyme's role in splicing, Ohtani and her colleagues used genetic engineering to introduce non-coding sequences into the gene encoding yellow fluorescent protein (YFP), and then transiently introduced the construct into normal and mutated plant cells. In wild-type plants incubated at 22 or 28 °C, almost all of the YFP messenger RNA was spliced correctly. In the mutants, however, splicing efficiency was markedly reduced at 28 °C.

The researchers then examined the distribution of the enzyme by fusing part of its gene to the *YFP* gene, or the gene encoding an enzyme called β-glucuronidase. In seedlings, RID1 was found in the shoot, roots and tissues that eventually form the leaves, and during reproductive stages in the flower buds and parts of the female reproductive system.

Figure 1: Wild-type (top row) and *rid1* mutants (bottom row) grown at 22 °C for 2 weeks and then at 22 °C (left) or 28 °C (right) for an additional 2 weeks. The *rid1* mutants stopped growing and died after exposure to higher temperature.

Finally, the researchers investigated genetically engineered plants lacking the *RID1* gene. Those with one copy of the gene developed normally, but those missing both had abnormally shaped female gametophytes, which usually form the egg cells.

"We subsequently performed a genome-wide transcriptome analysis to find out which genes were affected in our mutants," says Ohtani. "We have already identified several candidates, and are currently testing their functions."

1. Ohtani, M., Demura, T. & Sugiyama, M. *Arabidopsis* ROOT INITIATION DEFECTIVE1, a DEAH-Box RNA helicase involved in pre-mRNA splicing, is essential for plant development. *The Plant Cell* **25,** 2056-2069 (2013).

Seeing the brain at greater depth

An agent that enhances light transmission through brain tissue enables microscopy of the mouse brain from top to bottom

Brain tissue is opaque, so classical microscopy methods require slicing the brain into ultrathin slivers to allow light to shine through. Techniques have been developed to enhance brain tissue transparency, but the chemicals used have a range of limitations. Takeshi Imai and colleagues from the Laboratory for Sensory Circuit Formation at the RIKEN Center for Developmental Biology have now developed a 'clearing' agent that resolves many of these limitations[1].

Existing brain clearing agents have a range of undesirable side-effects, such as causing the brain tissue to swell or shrink, quenching the signal of fluorescent probes used to label individual neurons, rendering the brain tissue very fragile and difficult to work with, or requiring very long incubation times to clear the brain tissue. Some of the existing methods also require special preparation methods that are prohibitive to most researchers.

Imai and his colleagues searched for a new clearing agent by testing various combinations of chemicals. The combination they found to be most effective consisted of the sugar fructose mixed with a thiol compound to prevent the tissue from turning brown or from fluorescing on its own. The researchers showed that this solution, called SeeDB (short for 'see deep brain'), could enhance the transparency of mouse embryos and young mouse brains. Moreover, despite adult brain tissue having fiber tracts that cannot be cleared by other methods, SeeDB was able to render adult mouse brains completely transparent. Another advantage of the new clearing agent is its ease of preparation, which allows researchers to produce and use it in their lab.

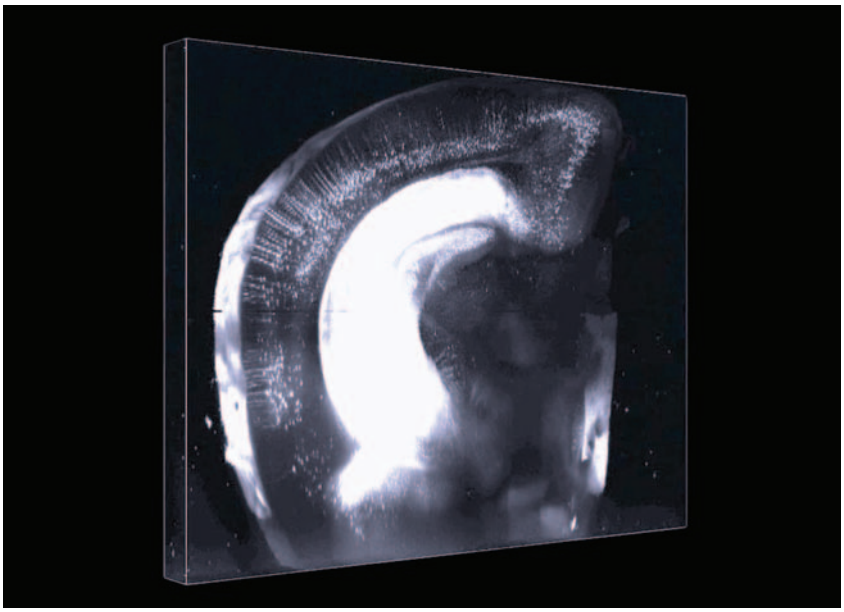


Figure 1: A fluorescent cross-section of a mouse brain after treatment using the SeeDB clearing agent.

The researchers then used SeeDB to clear brain tissue from an adult mouse containing fluorescently marked neurons. Using a standard microscope lens and brain tissue incubated in SeeDB at room temperature, they were able to view 4 millimeters into the brain. Using a custom-made microscope lens and brain tissue incubated in SeeDB at body temperature, they were able to obtain images from the top to the bottom of the adult mouse brain—about 6 millimeters in depth (Fig. 1).

SeeDB will allow researchers to obtain clearer pictures of neuronal circuitry during development and disease in many species. The next challenge, notes Imai, will be to accelerate the imaging process. "With existing fluorescence microscopes, it takes a very long time—10 to 20 hours—to get images of just a part of the mouse brain," he explains. "The development of high-speed microscopes and computers for data analysis will be essential for large-scale imaging in the future."

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Drug treatment improves survival of insulin-producing cells

Pretreatment with a calcium-blocking drug improves the effectiveness of islet transplantation for diabetes in mice

The transplantation of insulin-producing islet cells from a donor pancreas could help diabetes sufferers avoid the need for daily insulin injections. However, the use of this experimental procedure is hampered by an immune response in the recipient that often rejects the transplanted islet cells. Masaru Taniguchi from the Laboratory for Immune Regulation at the RIKEN Center for Integrative Medical Sciences and Yohichi Yasunami from Fukuoka University have now led research that has improved the efficiency of the procedure by pretreating the islet cells with a drug that blocks the sodium–calcium exchanger (NCX) protein[1].

Islet transplantation often involves the use of islet cells from two or three different donors in order to achieve sufficient cell engraftment. "This low efficiency has been a major obstacle facing clinical islet transplantation," says Taniguchi.

Taniguchi, Yasunami and their respective lab groups set out to improve the islet transplantation procedure by pretreating islet cells prior to transplantation with a drug that blocks the NCX protein. This pretreatment protected the cells from innate immune responses in the liver, the site of islet transplantation, which led to longer-term survival of the cells in mouse models of diabetes.

"Pretreatment of donor islets with an NCX inhibitor prior to transplantation prevents early loss of transplanted islets and affords a new strategy to improve the efficiency of islet transplantation," says Taniguchi. Notably, the method allows for improved engraftment efficiencies without requiring transplant recipients to take any additional anti-rejection drugs. "Our new strategy to target donor islets does not add further risks to recipients," notes Taniguchi.

The drug pretreatment is thought to work by stopping NCX from boosting the intracellular levels of calcium ions. Normally, this calcium influx leads to low oxygen conditions, which trigger the release of a protein called high-mobility group box 1 (HMGB1) from islets soon after their transplantation. HMGB1 in turn activates immune cells, causing the early loss of transplanted islets. The NCX-blocking drug, called SEA0400, can prevent this cascade of events.

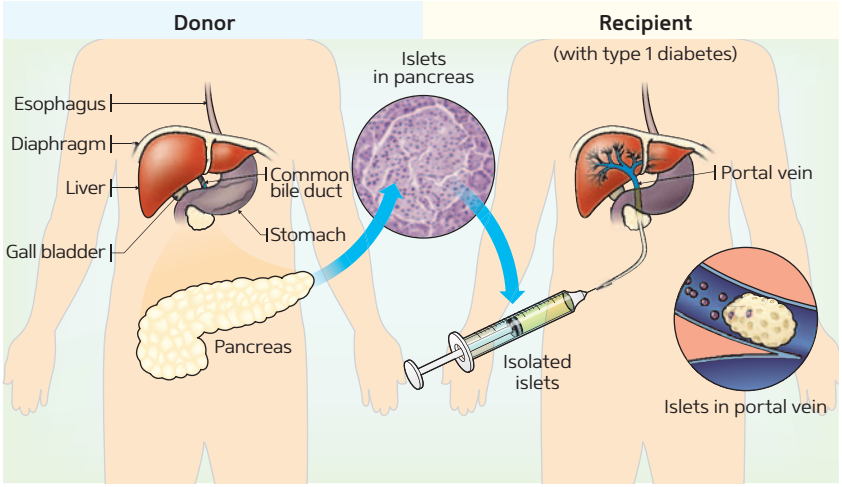

Figure 1: The process of clinical islet transplantation.

After transplanting pretreated and untreated islets into diabetic mice, the research team found that mice receiving SEA0400-treated islets displayed normal blood sugar control. In contrast, the control animals, which received the same number of untreated islets, experienced elevated blood sugar levels due to a lack of functional insulin-producing cells, which presumably had been attacked by the immune system. This same effect was seen whether the transplanted islets were human or murine in origin.

1. Mera, T., Itoh, T., Kita, S., Kodama, S., Kojima, D., Nishinakamura, H., Okamoto, K., Ohkura, M., Nakai, J., Iyoda, T. *et al.* Pretreatment of donor islets with the Na^+/Ca^{2+} exchanger inhibitor improves the efficiency of islet transplantation. *American Journal of Transplantation* **13,** 2154–2160 (2013).

Unraveling the secrets of the turtle shell

A long-lived controversy over the origins of the turtle shell has now been resolved

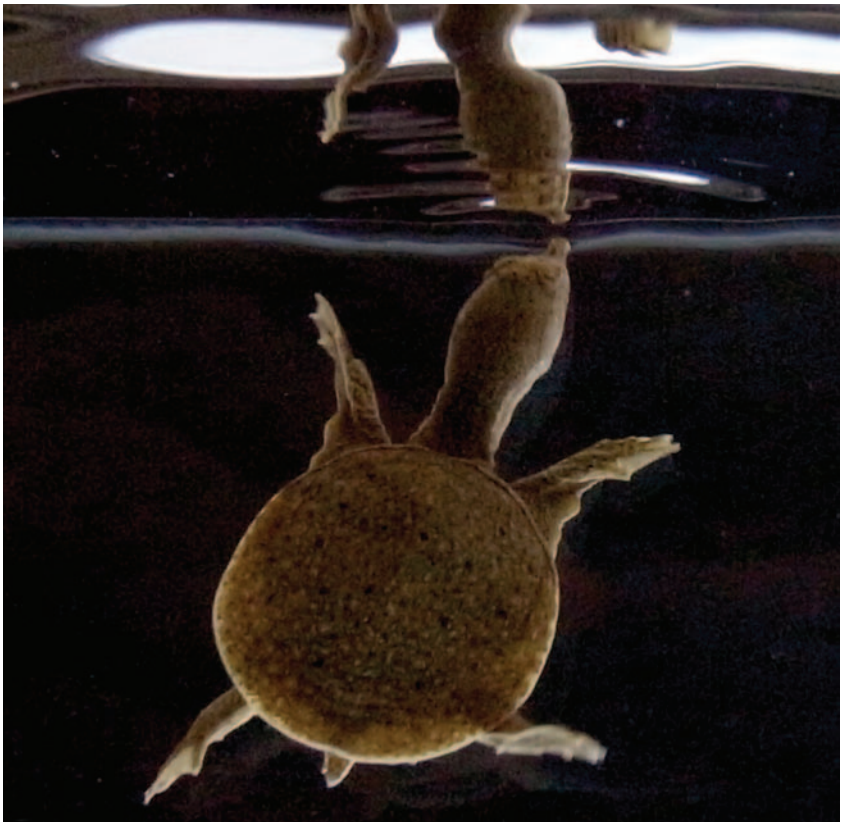

Figure 1: The Chinese soft-shelled turtle *Pelodiscus sinensis*.

For over 200 years, scientists have been split over the developmental origins of the turtle shell, unsure if it is an elaboration of the internal skeleton or an exoskeletal structure like that in crocodiles and alligators. New research by Tatsuya Hirasawa of the Laboratory for Evolutionary Morphology at the RIKEN Center for Developmental Biology and colleagues has now settled this controversy by revealing that the shell develops in the embryo as a modification of the rib cage and vertebrae[1].

Through detailed observations of the embryological development of the Chinese soft-shelled turtle *Pelodiscus sinensis* (Fig. 1), the researchers found that the ribs initially develop just as in other vertebrates, attached to the backbone with intercostal muscles in between. However, they observed that the intercostal muscle tissue degenerates during further development and is invaded by bony projections called trabeculae, which gradually fuse to form a bony plate. The trabeculae are produced in an area surrounded by bone membrane outside the skin tissue, or dermis. In contrast, the exoskeletal components in the alligator, called osteoderms, develop in the dermis layer of the skin. The observations confirm the evolutionary continuity between the axial endoskeleton and the turtle carapace, bridging the gap in body plan between the turtle and other tetrapods.

Having determined that the carapace develops embryologically as a modification of the internal vertebrate skeleton, the researchers began investigating the evolution of the turtle carapace by studying fossil turtles and earlier marine reptiles. They found that the form seen in the earliest known turtle species, *Odontochelys*, in which the carapace does not form a closed shell, is best explained by similar embryological development to the modern turtle. Earlier marine reptiles already contained examples of the large plate-like ribs that eventually became incorporated into the carapace. Recent genomic analyses support the possibility that the turtles and these marine reptiles are likely to be relatively closely related in evolutionary terms.

The researchers are now planning to expand their work. "We will further investigate the evolution and development of the ribs and the body wall of the turtle," says Hirasawa. "In the embryonic development of the turtle, the rib does not extend ventrally, which is very strange. We call this turtle-specific phenomenon the 'axial arrest' of the rib. The signs of axial arrest are perhaps recognizable in some fossil marine reptiles. We want to unravel the evolution of this axial arrest, by a combination of developmental biology and paleontology."

1. Hirasawa, T., Nagashima, H. & Kuratani, S. The endoskeletal origin of the turtle carapace. *Nature Communications* **4,** 2107 (2013).

A better trigger for targeted drug delivery

Protein-based drug carriers activated by the body's biochemical energy carrier open up new possibilities for targeted drug delivery

Biomolecular 'nanocarriers' formed by the careful assembly of protein subunits are common in nature and perform a range of essential roles in biological processes, powered by the biological energy carrier adenosine-5'-triphosphate (ATP). Takuzo Aida from the RIKEN Center for Emergent Matter Science and colleague Shuvendu Biswas from the University of Tokyo have now led the development of a prototype artificial nanocarrier that uses ATP to fuel the delivery of a therapeutic payload[1].

Targeted drug delivery using artificial nanocarriers is a burgeoning area of clinical research, particularly for the treatment of cancer. Most nanocarriers designed to date to specifically target tumors generally rely on subtle changes in pH to sense cancerous environments. However, the pH difference around cancers is typically not large enough to selectively activate drug delivery by the nanocarriers.

Local ATP levels, on the other hand, vary significantly throughout the body. ATP concentrations are over 200 times higher within cells compared to the extracellular environment and inflammation around diseased tissue triggers an immune response that increases local ATP production.

"We envisioned that if our nanocarrier sensed high-concentration ATP and broke up into short-chain fragments because of motions generated by its constituents, we could develop a conceptually new drug delivery system for tumor tissues and cells," says Aida.

The researchers assembled their nanocarriers using barrel-shaped chaperonin proteins linked together by magnesium ions to form tubular structures. In nature, explains Aida, chaperonins trap denatured proteins inside their cavity through hydrophobic interactions and assist refolding. Once the trapped proteins are refolded, the chaperonins bind to ATP and undergo a machine-like opening motion that expels the guest proteins out of the cavity. The goal of the researchers was to exploit this ATP-enabled motion in their artificial nanocarrier to control payload transport and release.

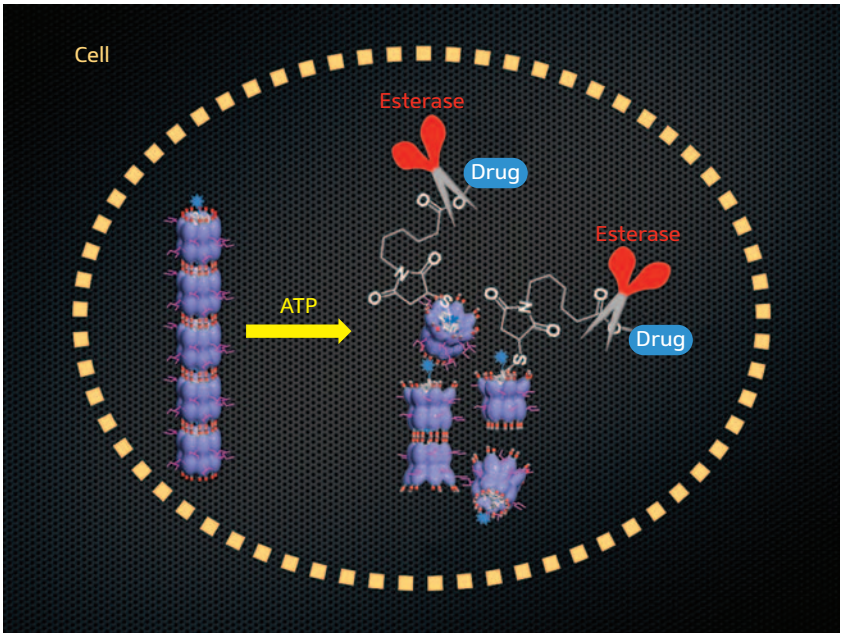

Figure 1: A chaperonin-based drug delivery system. A drug compound is attached to an irreversibly denatured protein via an ester linker. The protein sits inside the chaperonin cavity, which opens under the action of ATP in the intracellular environment. An esterase enzyme then cleaves the ester linker, freeing the drug.

Modified from Ref. 1 © 2013 S. Biswas *et al.*

In their proof-of-concept study, the researchers showed that a denatured fluorescent protein loaded into the nanocarrier recovered its folding and luminescent properties through the action of ATP, demonstrating that the chaperonins retained their biological function. They also showed that fluorescent dye administered to tumor-carrying mice using the nanocarriers selectively accumulated in cancer tissues and also the liver. "The liver absorbs most of the nanocarriers to excrete them from the body, so that only a small number of carriers reaches tumor tissues and cells," says Aida.

The team is currently undertaking further development of their system for drug delivery. "Research for delivering real drugs is now underway," says Aida.

1. Biswas, S., Kinbara, K., Niwa, T., Taguchi, H., Ishii, N., Watanabe, S., Miyata, K., Kataoka, K. & Aida, T. Biomolecular robotics for chemomechanically driven guest delivery fuelled by intracellular ATP. *Nature Chemistry* **5,** 613–620 (2013).

An energy-efficiency lead for nitrogen fertilizer production

The production of useful nitrogen compounds from atmospheric nitrogen could become considerably less energy-intensive thanks to a novel polyhydride complex

Figure 1: Worldwide production of nitrogen fertilizer for agriculture exceeds 100 million tons a year.

Nitrogen and phosphorus fertilizers are essential in modern agriculture and crucial to meeting the ever-growing global food demand. Nitrogen fertilizer, in the form of ammonia, is produced now in the same way that it has been for close to a century—by the energy-intensive Haber-Bosch process, which uses high temperatures and pressures to split nitrogen gas molecules. Takanori Shima, Zhaomin Hou and colleagues from the RIKEN Center for Sustainable Resource Science have now made a discovery that could allow ammonia and other nitrogen-bearing compounds to be produced energy-efficiently at room temperature[1].

Nitrogen gas (N_2), comprised of a pair of nitrogen atoms, is abundant in the atmosphere, but converting it to a useful solid form by breaking the triple bond between the nitrogen atoms consumes considerable energy. Chemists have had difficulty in finding ways to break the triple bond at mild temperatures without resorting to special electron- and proton-donating reagents, which are generally nonrecyclable and expensive.

Hou and his colleagues instead considered multinuclear transition metal hydrides—cage-like compounds in which several metals are linked together by multiply bonded hydrogen atoms. These hydrides can generate sufficient electrons to break the triple bond and also act as a hydrogen source for the fixation of nitrogen as ammonia. The team also suspected that the metal centers could enhance N_2 activation through cooperative effects seen in biological nitrogen fixation and the Haber-Bosch process.

Drawing on their expertise in rare-earth hydrides, the researchers produced a novel complex based on titanium—a transition metal that readily forms nitrogen bonds. Their experiments involved mixing the titanium precursor with hydrogen gas and N_2 in a pressure reactor at room temperature. Rather than the pure metal hydride they expected, the reaction produced a strained cubic structure containing four bridged titanium hydrides and, intriguingly, a pair of nitrogen–proton (N–H) units.

To understand how this complex formed, the team went back and monitored each step of the reaction using x-ray crystallography and isotope-labeled spectroscopy. They determined that N_2 was activated and cleaved by binding simultaneously to three titanium atoms and that the hydrides migrated from titanium to the nitrogen portion of the complex. "This is unprecedented—to observe N_2 activation, bond cleavage and N–H bond formation steps in one reaction," says Shima.

Although the researchers are still investigating why the trinuclear titanium complex has such particular affinity for N_2 compounds, they are certain that these materials will provide a unique opportunity to develop innovative nitrogen fixation strategies.

1. Shima, T., Hu, S., Luo, G., Kang, X., Luo, Y. & Hou, Z. Dinitrogen cleavage and hydrogenation by a trinuclear titanium polyhydride complex. *Science* **340,** 1549–1552 (2013).

From a drop of blood, a clone

A process for generating clones from blood may make it easier for scientists to preserve valuable lines of research animals

Over the last few decades, scientists have drawn upon a powerful arsenal of biotechnology techniques to establish a wide variety of genetically engineered mouse strains. These animals represent invaluable resources for studying mammalian development and disease, but many of these mouse lines are infertile or challenging to breed by conventional means. Atsuo Ogura and colleagues at the RIKEN BioResource Center have now developed a simplified cloning strategy that should make it easier for scientists to protect their painstakingly developed mouse lines[1].

"We have been undertaking somatic cell nuclear transfer experiments for the preservation of valuable mouse genetic resources," explains Ogura. Somatic cell nuclear transfer (SCNT) is the standard technique for mammalian cloning, in which a nucleus from a donor cell of interest is transferred into an unfertilized egg from which the nucleus has been removed. The resulting cell acts like a fertilized egg and gives rise to a clone of the donor animal.

Ogura was interested in the possibility of using blood to obtain nuclei for SCNT as a more straightforward and less invasive donor cell harvesting procedure. As an initial test, the researchers collected drops of blood from the tails of donor animals and attempted to derive clones from various subtypes of blood cells. For comparison, they performed parallel SCNT experiments using cumulus cells, which normally act as support cells for the oocyte and are commonly used as donors for cloning.

Figure 1: A female mouse cloned from a single peripheral blood cell.

Although the success rate was generally higher with cumulus cells, Ogura was pleased to find that his team could successfully generate clones (Fig. 1) from two different blood cell types—granulocytes and lymphocytes. "Peripheral blood cells, especially granulocytes, are terminally differentiated cells and have a short life span," he explains. "It was surprising that they can give rise to a new life by nuclear transfer." Ogura's team successfully used granulocyte-based SCNT to clone four different genetically modified mouse lines. These initial results indicate that the technique should be broadly applicable, and Ogura proposes that it could even help preserve endangered species in the wild.

The use of peripheral blood offers notable advantages for cloning compared with the collection of other source tissues, which can sometimes require euthanization of the donor animal. Although other, non-SCNT, techniques are available for rescuing 'endangered' mouse lines, such methods require viable male germ cells and are therefore not universally applicable. Ogura's priority now is to boost the efficiency of peripheral blood SCNT so that the technique's reliability is on par with its simplicity.

1. Kamimura, S., Inoue, K., Ogonuki, N., Hirose, M., Oikawa, M., Yo, M., Ohara, O., Miyoshi, H. & Ogura, A. Mouse cloning using a drop of peripheral blood. *Biology of Reproduction* **89,** 24 (2013).

Visualizing how infection exacerbates lung disease

A new mouse model combined with advanced three-dimensional imaging allows researchers to investigate how infection worsens emphysema

Chronic obstructive pulmonary disease (COPD) is a leading cause of death worldwide. The disease, characterized by constricted airways due to bronchitis and emphysema, is commonly seen in smokers but can also be brought on by environmental pollutants. The symptoms of the disease are known to be worsened by bacterial and viral infections of the airways, but exactly why remains largely unclear. A research team led by Naoyuki Taniguchi from the RIKEN Global Research Cluster has now developed a mouse model that allows COPD exacerbation to be studied in living animals[1].

Acute exacerbation of COPD as a result of infection of the airway is known to be a major cause of death in COPD patients. It is also known to accelerate the progression of the disease, but despite the disease's prevalence, surprisingly little is known about the mechanism of exacerbation or how to treat it. "Steroid therapy is common for COPD exacerbation, but its effectiveness is not clear," says Taniguchi. "It is reported to be effective in only 20% of patients, and sometimes causes serious side effects."

The development of a suitable animal model to specifically study COPD exacerbation is therefore of great interest to researchers looking for clinical treatments for these acute episodes.

Satoshi Kobayashi and Reiko Fujinawa from Taniguchi's research group combined a simple mouse model of COPD exacerbation with an imaging technique called micro-computed x-ray tomography (micro-CT), which allowed them to monitor pathological changes by visualizing three-dimensional variations in tissue density. They produced their mouse model by exposing healthy mice to the chemical elastase, which results in the development of emphysema-like lung disease. Exacerbation of the disease was then triggered by exposing the mice to a single dose of lipopolysaccharide (LPS), a toxic component of bacterial cell walls that is known to cause inflammation.

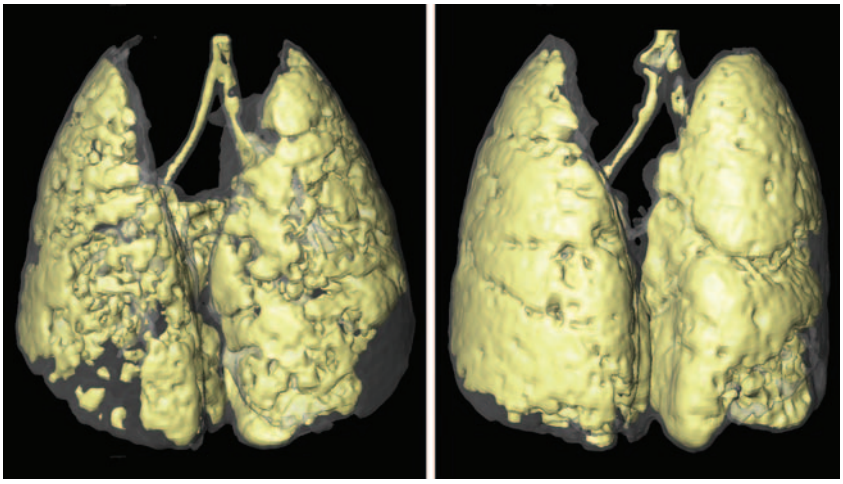

Figure 1: Three-dimensional visualizations of emphysema (yellow) in mice treated with elastase, showing accelerated progression of disease after 12 weeks in mice exposed to LPS (right) compared with mice treated only with saline solution (left).

The experiments showed that lung inflammation was exacerbated in mice exposed to LPS, with an associated increase in two different types of inflammatory cells and an increase in proinflammatory proteins and signalling molecules. Micro-CT imaging at 3 and 12 weeks following LPS exposure confirmed the acceleration of emphysema-like symptoms (Fig. 1).

The observed pathological changes mimic those found in human COPD exacerbation, meaning that this model could be used to help researchers gain a better understanding of the underlying mechanisms of COPD and perhaps to develop new treatments for it. "Using this model mouse," says Taniguchi, "we are looking for biomarkers of the exacerbation process, and our final goal is to develop new therapeutics."

1. Kobayashi, S., Fujinawa, R., Ota, F., Kobayashi, S., Angata, T., Ueno, M., Maeno, T., Kitazume, S., Yoshida, K., Ishii, T., *et al.* A single dose of LPS into mice with emphysema mimics human COPD exacerbation as assessed by micro-CT. *American Journal of Respiratory Cell and Molecular Biology* advance online publication, 3 July 2013 (doi:10.1165/rcmb.2013-0074OC).

Honing in on developmental epigenetics

A technique for investigating epigenetic modifications in small numbers of cells reveals unusual similarities in gene expression between male germ cells and certain cancer cells

Germ cells have unique molecular features that enable them to perform the important task of transmitting genetic information to the next generation. During development from their embryonic primordial state, germ cells are thought to be reprogrammed by epigenetic modification of their DNA. However, due to the significant technical challenges associated with epigenetic analysis of rare cells such as primordial germ cells, the exact nature and effect of such epigenetic changes remains poorly understood. Kuniya Abe, Rieko Ikeda and colleagues from the RIKEN BioResource Center have now developed a method that enables as few as 100 cells to be analyzed for epigenetic changes, providing new insight into these processes[1].

Epigenetic changes regulate gene expression without altering the underlying DNA sequence. They involve highly specific chemical modifications of DNA and DNA-bound proteins that activate or deactivate individual or related sets of genes. DNA methylation is one such epigenetic mechanism, and the technique developed by Abe's team is an adaptation of an existing microarray-based assay method for detecting DNA methylation. "Our modification of the state-of-the-art approach might be useful for basic research as well as diagnostic applications when less than a nanogram of genomic DNA is available," says Abe.

Using their technique on embryonic mouse cells, the researchers compared the extent to which the genomic DNA of somatic cells, stem cells and both male and female germ cells at different stages of development is tagged with methyl groups. Looking specifically at germ cells (Fig. 1), they found that large stretches of DNA on the X chromosome of male germ cells were depleted of methyl groups. These large 'hypomethylated' domains frequently included genes with germ cell- or testis-specific expression, including cancer testis antigen (CTA) genes, which are expressed in male germ cells and cancer cells but not in normal somatic cells. Genes in these regions were also expressed despite the presence of a histone modification that normally suppresses gene expression.

"The mechanistic basis for the relationship between hypomethylation and the expression of CTA genes remains to be established," says Abe. "We suspect that a change in nuclear architecture that controls the accessibility of the genes to the transcriptional apparatus might be involved."

Abe also notes that if CTA gene expression does indeed contribute to oncogenesis, then understanding the epigenetic regulation of these genes in germ cells could provide insight into the molecular events that cause certain types of cancer.

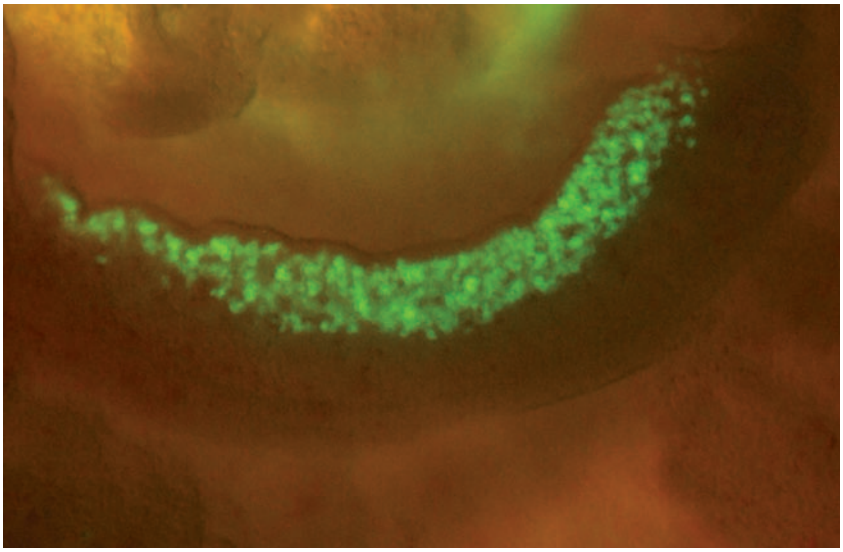

Figure 1: Fluorescent mouse primordial germ cells approximately 12 days after fertilization.

1. Ikeda, R., Shiura, H., Numata, K., Sugimoto, M., Kondo, M., Mise, N., Suzuki, M., Greally, J. M. & Abe, K. Large, male germ cell-specific hypomethylated DNA domains with unique genomic and epigenomic features on the mouse X chromosome. *DNA Research* advance online publication, 15 July 2013 (doi:10.1093/dnares/dst030).

RIKEN Center for Developmental Biology

Realizing the potential of regenerative biology

At the heart of biomedical research and innovation in Kobe, the Center for Developmental Biology is in an excellent position to apply its pioneering research to regenerative medicine

The Center for Developmental Biology (CDB) is dedicated to uncovering the fundamental processes that underlie animal development as well as the complex mechanisms involved in organ formation and stem cell and regenerative biology, with the ultimate goal of applying the latest research to regenerative medicine. A core institute for basic research and the inaugural center of RIKEN's Kobe campus, the CDB was launched in 2000.

Eying stem cell research

Researchers at the CDB have access to supporting laboratories that specialize in mutant mouse production, genetic sequencing and microbial analysis, which helps them to achieve their research aims. Consequently, several CDB projects are at the forefront of stem cell research. For example, building on their work with mouse embryonic stem cells, researchers at the CDB's Laboratory for Organogenesis and Neurogenesis have induced human embryonic stem cells to self-organize into retinal tissue.

In 2013, the CDB launched a joint clinical study with the Institute of Biomedical Research and Innovation to investigate whether differentiated cell sheets derived from induced pluripotent stem (iPS) cells can be safely transplanted into elderly patients suffering from a degenerative eye disease. Led by the CDB's Laboratory for Retinal Regeneration, the study takes advantage of the center's close proximity to hospitals and research institutions that specialize in clinical trials. The CDB's strategic location at the core of Kobe's biomedical cluster on Kobe Port Island—home to over 200 biomedical companies—offers many opportunities for collaboration with industry and places the center in an ideal position to translate its research into clinical therapies.

Embracing young talent

With an annual budget of over 3 billion yen, the CDB supports 30 active research laboratories and employs 435 staff members, including 213 women and 43 foreign researchers. The center also hosts postgraduate students and even offers young scientists the opportunity to become principal investigators. The annual CDB Symposium draws an international audience, and seminars throughout the year create regular opportunities for knowledge sharing.

Over the next five years, the CDB will continue to advance research in its core areas of developmental and stem cell biology. Exciting projects are underway in the emerging field of self-organizing embryonic stem cells, and the center is enhancing its use of mathematical methods to better understand the principles that determine an organism's size and shape.

Additionally, the CDB plans to strengthen and expand mutual partnerships at home and abroad, such as the cooperative research agreements it signed with three Spanish institutes in 2012. Through its summer schools for high school students and Joint International Graduate School Program, the center continues to welcome the next generation of Japanese and foreign researchers. By encouraging a constant influx of new talent and ideas, the CDB will ensure its long-term sustainability and, with it, enduring innovation in the field of developmental biology.

CONTACT INFORMATION

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Monitoring the rapidly changing Universe

KAZUO MAKISHIMA

Team Leader
MAXI Team
RIKEN Global Research Cluster

To the naked eye, stars appear unchanging for many decades or even hundreds of years. Yet in the x-ray spectrum, celestial bodies emerge suddenly and change brightness and shape on the order of days or even seconds. RIKEN developed the Monitor of All-sky X-ray Image (MAXI) experiment to observe these unpredictable x-ray objects. Using MAXI, a team led by Kazuo Makishima has observed many different phenomena, including stars spiraling into a black hole, and discovered traces of hypernova explosions.

MAXI monitors the whole sky

The origin of MAXI dates back to the 1980s. In 1985, Japan joined the International Space Station (ISS) project and the National Space Development Agency of Japan (NASDA, currently the Japan Aerospace Exploration Agency (JAXA)) began to develop the Japanese Experiment Module (JEM), also known as the Kibo laboratory. Kibo, which continues as an active part of the ISS, includes two experimental areas: an inboard pressurized cabin and an outboard experimental platform exposed to space (the Exposed Facility). In 1996, NASDA solicited ideas from the public as to the equipment that should be mounted in the Exposed Facility, and Masaru Matsuoka, chief scientist of the Cosmic Radiation Laboratory at RIKEN at that time, proposed equipment for observing the whole sky in x-rays—an idea that would eventually become MAXI.

Makishima explains: "The ISS orbits the Earth about once every 92 minutes, with its bottom side facing the Earth. As a result, to observe a single celestial body, a telescope in the ISS must be moved in the opposite direction so as to cancel out the movement of the ISS. Thus, many astronomers thought that the ISS was unsuitable for astronomical observation. Dr. Matsuoka, however, thought the opposite and conceived of mounting the observation equipment on the side opposite to the Earth so that the whole sky could be observed in a single orbit around the Earth. We cannot predict when and where an x-ray object will emerge and how it will change, so all-sky observation is an effective tool for detecting x-ray objects."

In 1997, it was officially decided that MAXI would be mounted on the Exposed Facility of Kibo. Matsuoka moved to NASDA in 1999, and in 2001 Makishima was appointed as chief scientist of the Cosmic Radiation Laboratory, taking over the development of the x-ray detector at the heart of the MAXI system. In June 2009, MAXI was launched by a US space shuttle and began operation in August 2009.

At present, JAXA is in charge of MAXI's operation and the RIKEN MAXI team is providing support as well as managing, releasing and analyzing observed data in collaboration with JAXA, Osaka

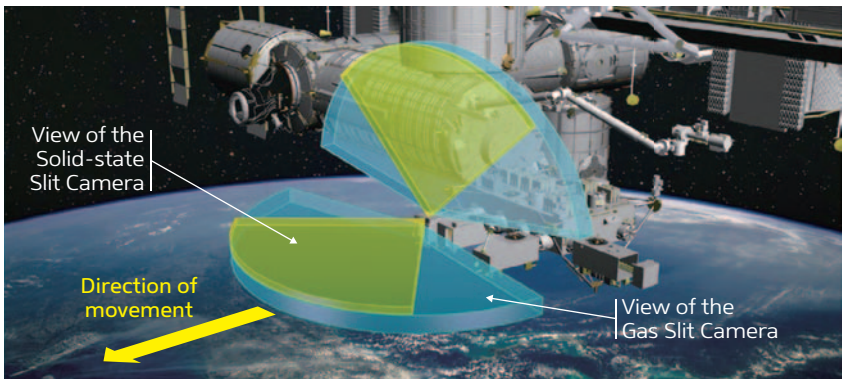

Figure 1: The Monitor of All-sky X-ray Image (MAXI) experiment
The monitor is mounted on the extravehicular experimental platform (Exposed Facility) of Japan's Kibo laboratory, part of the International Space Station (ISS). MAXI observes the whole sky as the ISS orbits the Earth.

University, Tokyo Institute of Technology, Aoyama Gakuin University, Nihon University, Kyoto University, the University of Miyazaki and Chuo University. For each orbit of the ISS around the Earth, MAXI collects a single complete set of observation data for the whole sky (Fig. 1). The new dataset is then compared with previous datasets. Whenever any celestial body is observed to be different in brightness or emerges suddenly, MAXI's findings are sent out as alerts and followed up by detailed observations using ground-based telescopes and artificial satellites.

Stars spiraling into a massive black hole

"RIKEN is strongly associated with the K computer and the life sciences, so many people are surprised to hear that RIKEN is also studying the Universe," says Makishima. "Actually, RIKEN has a long history in space research. RIKEN's Yoshio Nishina (1890–1951) pioneered cosmic-ray research in Japan, and I heard that it was Minoru Oda (1923–2001), director of RIKEN from 1988 to 1993, who advised creating the Exposed Facility on the Kibo laboratory. I would like more people to know that RIKEN performs space research." MAXI is indeed helping to raise the profile of RIKEN's space research and has contributed to many discoveries since it commenced operation. "MAXI's most dramatic achievement," says Makishima, "was the observation of an x-ray object in 2011."

At 21:57 JST on 28 March 2011, the US gamma-ray-burst observation satellite Swift detected x-rays in the direction of the constellation Draco. After receiving a report of the detection, the MAXI team reviewed their observation data and found an increase in x-ray intensity several hours before the discovery was made by the Swift satellite (Fig. 2). "The celestial body was named Swift J1644+57 and found to be at a distance of 3.9 billion light years from Earth. This discovery is considered to be a star spiraling into a massive black hole in the center of a galaxy because intense x-ray radiation has been repeatedly observed since then," notes Makishima (Fig. 3).

Discovering the traces of a hypernova explosion

MAXI was also key to the discovery in March 2013 of a celestial structure that appears to be the traces of a hypernova explosion in the constellation Cygnus. The discovery was achieved through the use of one of the two types of x-ray detector mounted on MAXI: the Solid-state Slit Camera (SSC) that has lower sensitivity but higher energy resolution than the other detector, the Gas Slit Camera (GSC), which is used to watch the varying x-ray sky. By analyzing data from the SSC, researchers can perform detailed diagnostics of x-ray-emitting astrophysical plasmas because the SSC is able to detect atomic emission lines. The energies of these emission lines can reveal which elements are involved and their respective temperatures.

A MAXI all-sky image created from observations made over the course of 30 months showed a superbubble—a massive structure that emits low-energy x-rays—in the direction of the constellation Cygnus (Fig. 4). The MAXI team first confirmed that the superbubble was not a supernova explosion, which occurs when a star with a mass exceeding eight times that of the Sun collapses at the end of its life. According to Makishima: "The superbubble in the constellation Cygnus has a linear dimension 40 times larger than our Moon, which is too large for a remnant of a single supernova explosion."

The group further determined that the superbubble was not the result of a series of supernova explosions. "If that was the

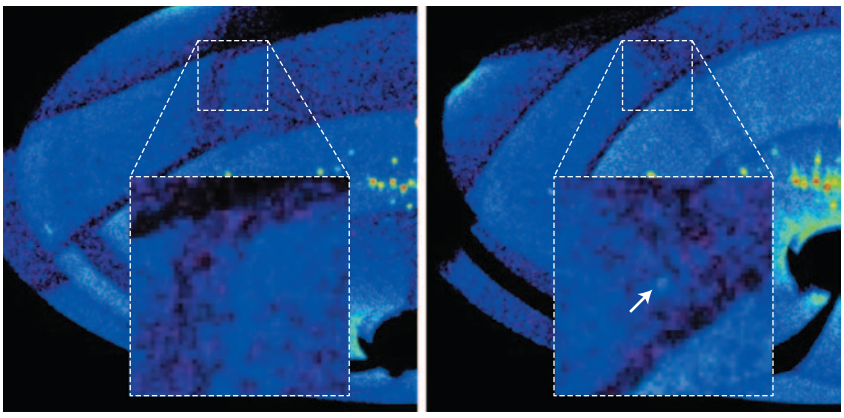

Figure 2: Part of an all-sky image captured by MAXI before and after the appearance of Swift J1644+57
The supermassive black hole Swift J1644+57 was discovered by the Swift satellite on 28 March 2011. The left image shows part of the all-sky image captured by MAXI and an enlarged view around Swift J1644+57 on 21 March 2011—a week before the discovery; no x-ray sources are observed. The right image shows the same enlarged view on 29 March 2011; Swift J1644+57 can be seen in the middle of the enlarged view (indicated by an arrow).

Figure 3: Illustration of stars spiraling into a massive black hole

case, elemental abundance and temperature should vary with location," continues Makishima. "Our investigation, however, revealed that they are almost the same throughout the bubble. We think that the superbubble represents a remnant of a massive supernova explosion attributable to a single, very heavy star with a mass of as much as dozens of times the mass of the Sun." Such a massive supernova explosion is known as a 'hypernova explosion'. Although several hypernova explosions have been discovered, the MAXI team's finding seems to be the first discovery of the traces of a hypernova explosion within our Galaxy.

The magnetic nature of a neutron star

MAXI has also made possible the successful measurement of the magnetic field of a neutron star, a research theme that Makishima has been working on for a long time together with Takehiro Mihara, a senior research scientist at RIKEN. A neutron star is a superdense stellar remnant of the supernova explosion of a heavy star and is strongly magnetic. When charged particles such as electrons and protons gyrate in a magnetic field, they emit or absorb electromagnetic waves of a specific wavelength. In a very strong magnetic field like the one created by a neutron star, this resonant electromagnetic frequency falls within the x-ray range, which can be used to calculate the strength of the magnetic field that produced them.

Makishima used MAXI data to study the magnetic field of many different neutron stars and also to search for undiscovered neutron stars. "In September 2009, MAXI captured an unfamiliar x-ray object. On examination, the object was found to be GX 304-1, which had been observed for the first time in 28 years. I knew the name because GX 304-1 was in an x-ray source catalog that I used when I was younger. It felt like meeting an old friend again."

GX 304-1 is a binary system in which a neutron star orbits a particular type of star (Fig. 5, left) that spins rapidly, forming a gaseous disc. When the neutron star passes through the gaseous disc, the gas flows onto the neutron star, emitting x-rays. However, during the past 28 years, there could not have been any gas flow fueling GX 304-1.

The MAXI data showed that GX 304-1 emits x-rays at an interval of about 132 days (Fig. 5, right). Makishima's team predicted when strong x-rays would be emitted from GX 304-1 and observed the x-ray emission using Suzaku, a Japanese x-ray astronomy satellite. Makishima emphasizes that Japan's MAXI and Suzaku projects complement each other—MAXI excels in all-sky observation while Suzaku can observe a single celestial body in detail. After analyzing the Suzaku data for GX 304-1, the group determined that the magnetic field of the neutron star is ten trillion times stronger than the magnetic field of the Earth.

Makishima is also using his observations of the magnetic fields of many different neutron stars to clarify whether a neutron star can be regarded as an electromagnet or a permanent magnet. The most widely held theory is that a neutron star is an electric magnet and that a flowing electric current generates its magnetic field. If this is true, the intensity of the magnetic field of neutron stars must be distributed continuously from high to low.

"However, past measurements of neutron stars reveal that the magnetic field intensity is clustered over a narrow range," says Makishima. "Thus, we think that a neutron star could be better explained as a permanent magnet. Such a condition, or 'ferromagnetic state', could be realized if the huge number of neutrons that constitute a neutron star were aligned in the same direction. We are investigating how this could happen." So far, Makishima has measured the magnetic field of about 20 neutron stars. By increasing the sample number, he hopes to be able to resolve the origin of magnetic fields in neutron stars, while attracting the interest of nuclear scientists.

Extending the MAXI project

MAXI was initially planned to be in service for only two years. However, thanks to a succession of exceptional results, MAXI's operation is set to be

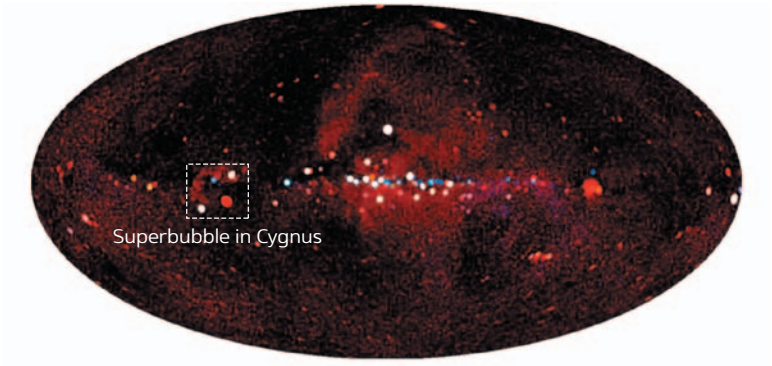

Figure 4: Superbubble in the constellation Cygnus that is considered to be the traces of a hypernova explosion

An all-sky image obtained from 30 months of observations by the Solid-state Slit Camera (SSC) of MAXI. Red indicates that low-energy x-rays are emitted from a region, whereas blue shows that high-energy x-rays are being emitted. Researchers believe that the superbubble in the constellation Cygnus was created when a star with a mass of dozens of times that of the Sun underwent a hypernova explosion 2 to 3 million years ago.

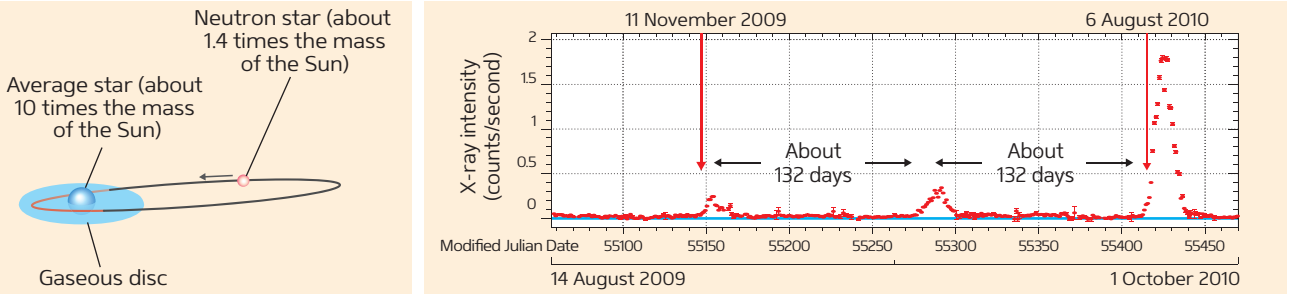

Figure 5: X-ray object GX 304-1 and variations in x-ray intensity observed by MAXI

The x-ray object GX 304-1 is a binary system consisting of an ordinary star and a neutron star. The star rotates at a high speed, producing a gaseous disc around it. When the neutron star passes through the gaseous disc (red line in the left image), gas flows onto the neutron star, causing it to emit intense x-rays. Observations by MAXI clarified that the x-ray intensity from GX 304-1 varies with a period of about 132 days (right).

extended to March 2015. Makishima has big plans for the extra time. "I want to take advantage of the synergy between MAXI and Suzaku to do three things," he says. "The first is to measure the magnetic field of more neutron stars to settle the debate about the origin of their magnetism. The second is to find evidence of black hole mergers."

It is known that there is a massive black hole at the center of many galaxies, but the origin of these black holes is still unknown. One idea postulates that many intermediate black holes with masses of between 100 to 1,000 times the mass of the Sun merged into a single massive black hole. "We think that the very bright x-ray sources observed in the arm regions of nearby spiral galaxies are strong candidates for intermediate black holes," says Makishima. "Some massive black holes are now speculated to be in the final phase of completing their mergers. Their x-ray intensity is expected to show a periodic change as the black holes circle each other. I want to capture those changes with MAXI."

Makishima's third aim relates to heat sources in galaxy clusters. "A galaxy cluster, which is a collection of galaxies, is filled with high-temperature gases that emit strong x-rays. Since x-rays are emitted, the gases should gradually cool down with time. However, they show no signs of cooling—something we discovered with the Advanced Satellite for Cosmology and Astronomy (ASCA or ASTRO-D), the predecessor of Suzaku. We therefore wish to clarify the heat sources in galaxy clusters."

Makishima makes the following prediction: galaxies moving through the x-ray emitting gas experience friction, which may produce the heat necessary to prevent the gas from cooling. If Makishima's prediction is correct, galaxies would slow down and gradually fall to the center of their galaxy cluster. To verify this novel view, Makishima is examining in closer detail whether the hot gas is actually dragged by moving galaxies. Although Suzaku is not sensitive enough to detect this, Makishima has high expectations for ASTRO-H, Japan's next-generation x-ray astronomy satellite, which is scheduled to be launched in 2015.

Future directions

Makishima is participating in the development of ASTRO-H as a professor at the University of Tokyo's School of Science, and the High Energy Astrophysics Laboratory led by Toru Tamagawa at the RIKEN Nishina Center for Accelerator-Based Science is also involved in the project. "I would like to extend the operation of MAXI and use it in collaboration with ASTRO-H," Makishima says. "That way, x-ray astronomy will be able to make further progress."

In addition, a replacement for MAXI, the Wide-Field MAXI, is under discussion and is also likely to be mounted on the Kibo laboratory's Exposed Facility. Some x-ray objects become brighter only for several seconds and unfortunately MAXI only has a narrow viewing field. Therefore, the area that can be instantaneously captured by MAXI accounts for less than 2% of the whole sky, which makes it difficult to successfully observe such short-lived celestial phenomena. With a much larger field of view, the new Wide-Field MAXI will make important contributions to phenomena such as gamma-ray bursts.

Makishima's overall space research goals are wide in scope. "To be honest, in addition to my main research focus on x-ray astronomy, I am interested in astrobiology," he says. "I believe that there is life somewhere else in the Universe besides Earth. Many scientists from many different fields of science are working at RIKEN. Wouldn't it be interesting for RIKEN to draw on this expertise to explore the potential for extraterrestrial life?"

ABOUT THE RESEARCHER

Kazuo Makishima was born in 1949 in Tokyo, Japan. In 1974, he obtained his undergraduate degree from the University of Tokyo, followed by a master's degree in 1978 from the Graduate School of Science at the same university. After working as a research assistant at the Institute of Space and Astronautical Science in Tokyo, he returned to the University of Tokyo's Graduate School of Science in 1986 as an associate professor and completed his PhD. He was promoted to full professor in 1995. From 2001 to 2009, he jointly served as a chief scientist at the RIKEN Cosmic Radiation Laboratory. Since 2010, he has been team leader of the MAXI team and group director of the Coordinated Space Observation and Experiment Research Group at RIKEN.

Promoting sound management at RIKEN

KENJI ITO

Auditor
RIKEN Board of Executive Directors

What is your role at RIKEN?

As an auditor, I examine RIKEN's financial dealings and oversee its activities as a whole. In cooperation with other departments, such as the Auditing and Compliance Office, I audit the appropriateness of RIKEN's governance and management.

How did you join RIKEN, and what kind of support did RIKEN provide?

I have always worked in finance, and in a previous position at The Industrial Bank of Japan, I was in charge of financing the Riken Electric Wire Co., Ltd, a RIKEN business concern. During this period, I learned a lot about RIKEN and discovered the critical contribution it has made to Japan's rapid economic growth.

Though my knowledge is limited when it comes to the technical aspects of what RIKEN does, I thought that my management experience would be beneficial for the organization. Good management is a common challenge in any industry, and it is important and useful to review an organization's situation from an unconventional point of view.

Regarding the actual execution of audits, I receive the full support of the Auditing and Compliance Office. When I arrived at RIKEN, the staff in each office offered detailed explanations of their roles, helping me to gain the information and knowledge necessary for an effective audit.

What have you learned about RIKEN during your time here so far?

Due to the ongoing adverse financial situation in Japan, RIKEN has faced a number of difficulties. Although I have only been working here for four months, I have learned that RIKEN has approached these issues with a sincere attitude. Hence, I would like to express my respect for President Noyori's leadership and the support offered by all the managerial staff.

What is the best thing about working at RIKEN?

I was very impressed by RIKEN's outstanding research, including the discovery of element 113 and the studies of induced pluripotent stem (iPS) cells and plant breeding with heavy-ion beams. Researchers at RIKEN are often behind notable Japanese research findings. I am particularly excited about these achievements when the results are important and I know the researchers involved.

What is your impression of RIKEN's staff?

Researchers at RIKEN feel that they are able to make significant contributions to society and are extremely proud of what they do. RIKEN develops technologies for the production of important products used around the world—something that must make our administrative staff proud of working at Japan's best research institute. Administrative staff also take pride in their own work, especially since RIKEN's projects cannot be carried out without their support.

Prior to joining RIKEN, you worked in a credit rating company. How would you 'rate' RIKEN?

As more than 90 per cent of RIKEN's budget comes from government grants and subsidies, and RIKEN gains additional income from renting out large facilities, it is in fact unnecessary to rate RIKEN. But if RIKEN did need to temporarily borrow a large amount of money from a bank, its rating would be excellent.

CONTACT INFORMATION

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RIKEN forges new ties in India

In September 2013, Ryoji Noyori, president of RIKEN, embarked on a whirlwind tour of India to sign a series of agreements intended to strengthen RIKEN's ties with scientific organizations in the country. Through these collaborations, RIKEN hopes to advance science and technology in Japan and India over the long term.

President Noyori first visited the Indian government's Department of Science & Technology and Department of Biotechnology—both part of the Ministry of Science & Technology—to enter into a Memorandum of Understanding. At the signing ceremony, Noyori delivered a lecture entitled "Science Shapes Our Future," where he proposed a number of themes of importance to humanity as potential areas of cooperation between the two countries—namely, energy, food, health and the environment.

Noyori then traveled to Bangalore in southern India to sign a comprehensive research agreement with three organizations: the National Centre for Biological Sciences (NCBS), the Institute for Stem Cell Biology and Regenerative Medicine (inStem) and the Centre for Cellular and Molecular Platforms (C-CAMP). The agreement included plans to establish a RIKEN–NCBS joint research center, which will initially focus on cell, developmental and regenerative biology and may later expand into other fields, such as brain science.

President Noyori (center) with professors M. R. S. Rao (left) and C. N. R. Rao (right) of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, during a visit designed to strengthen scientific ties between RIKEN and India.

While in Bangalore, President Noyori also signed a supplementary agreement with the Jawaharlal Nehru Centre for Advanced Scientific Research and the Indian Institute of Science—two of India's most prestigious scientific institutions—to launch a joint research center between the three organizations that will specialize in research into materials science and the life sciences. ■

RIKEN–Shanghai Jiao Tong University joint workshop

A joint workshop organized by RIKEN and Shanghai Jiao Tong University (SJTU) took place on 11 September 2013 in Shanghai. Aimed at students and researchers with interests in computer simulation, laser science and neutron science, the workshop offered an opportunity for attendees to meet, exchange views and sow the seeds of future research collaborations.

During an open discussion at the workshop, Jie Zhang, president of SJTU, agreed to establish a joint research center and a collaboration framework, which will include support for young researchers. To close the day, Shoji Nagamiya, science advisor to RIKEN, delivered a talk on accelerator science in Japan to an audience that included students with a keen interest in this area of research.

RIKEN and SJTU have been collaborating since 2008, when the two institutions first entered into a general agreement for cooperation. The partnership has now been extended with another workshop—expected to be held in October and focusing on energy and the environment. ■

SJTU President Jie Zhang (center) and RIKEN Science Advisor Shoji Nagamiya (right of President Zhang) at the joint RIKEN–SJTU workshop in Shanghai in September.

RIKEN signs Memorandum of Understanding with Shanghai Institute of Optics and Fine Mechanics

On 10 September 2013, RIKEN and the Shanghai Institute of Optics and Fine Mechanics (SIOM) signed a Memorandum of Understanding (MoU) and launched the RIKEN–SIOM Joint Laboratory. Established in 1964 as part of the Chinese Academy of Sciences (CAS), the SIOM is China's first and largest institute specializing in laser research. Attendees at the signing ceremony—including representatives from the CAS and China's Ministry of Science and Technology—listened to congratulatory speeches and toured the new facilities.

RIKEN's Katsumi Midorikawa (front left) and Ruxin Li (front right), director of the SIOM, at the MoU signing ceremony.

The joint laboratory will be RIKEN's first with the CAS since the two institutions signed a research cooperation agreement in 1982. Building on several years of combined study into optics and fine mechanics by Katsumi Midorikawa, chief scientist at the RIKEN Laser Technology Laboratory, and Ruxin Li, director of the SIOM, the laboratory aims to accelerate research into laser science and develop related technologies. ■

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