WINTER 2015

RIKEN RESEARCH

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Complex brain activity in mice determines fatal choice

IN CLEAR VIEW
Making brain tissue transparent

MAGIC NUMBER 32
Spelling isotope stability

HORNET POWER
Boosting endurance and fat metabolism with hornet juice

LEGO model of nuclear stability
Researchers at the RIKEN Nishina Center for Accelerator-Based Science have built a LEGO model showing the nuclear stability and atomic mass of isotopes.

RIKEN, Japan's flagship research institute, conducts basic and applied experimental research in a wide range of science and technology fields including physics, chemistry, medical science, biology and engineering.

Initially established as a private research foundation in Tokyo in 1917, RIKEN became a national research and development institute in 2015.

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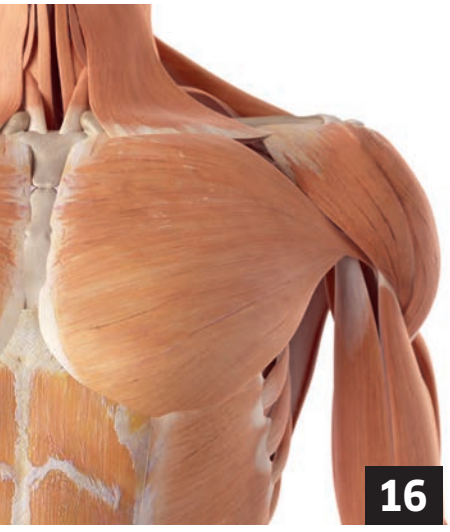

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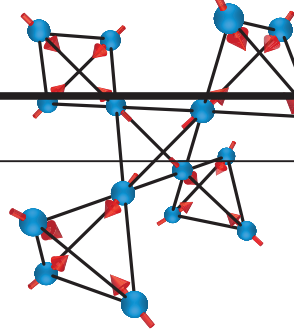

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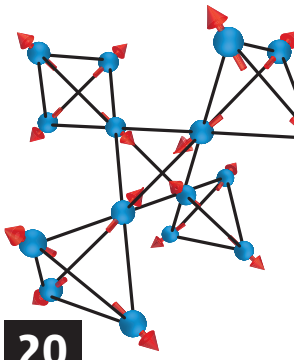

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Cover story: Researchers at RIKEN have developed a sports drink from hornet saliva. **Page 34**

By the time the Winter 2015 issue of *RIKEN RESEARCH* reaches you, orchestras across Japan will be performing Beethoven's ninth symphony, accompanied by professional and amateur choirs singing the fourth movement (*Ode to Joy*). In the spirit of this song, we hope this year has been a joyous and peaceful one for everyone.

This issue sees the start of an exciting new series of articles called "Impact" that looks at the social and economic benefits of RIKEN's research. The first story in this series features the sports drink VAAM, which contains a unique combination of 17 amino acids naturally produced by hornet larvae. VAAM was developed by former RIKEN researcher Takashi Abe in collaboration with Meiji, a Japanese manufacturer of drinks, dairy products and confectionery. The sports drink helped power long-distance runner Naoko Takahashi when she won the women's marathon at the 2000 Sydney Olympic Games.

The "Research highlights" feature recent research at RIKEN that has attracted significant global attention, including an organic molecule whose fluorescence wavelength varies widely when it is subjected to mechanical forces. The "Feature highlight" describes the development of a hydrogel that functions like an artificial muscle, produced by a scientist from the RIKEN Center for Emergent Matter Science. The hydrogel rapidly stretches and contracts in response to temperature changes.

In "Perspectives", Tetsuo Hatsuda, who heads the RIKEN Interdisciplinary Theoretical Science Research Group, describes how collaboration among theorists in the areas of physics, materials science and biological science is helping to answer the most fundamental questions regarding the origin of the Universe and life on Earth. We hope you will enjoy this symphony of our latest achievements.

Scrutinizing biological nanomachines

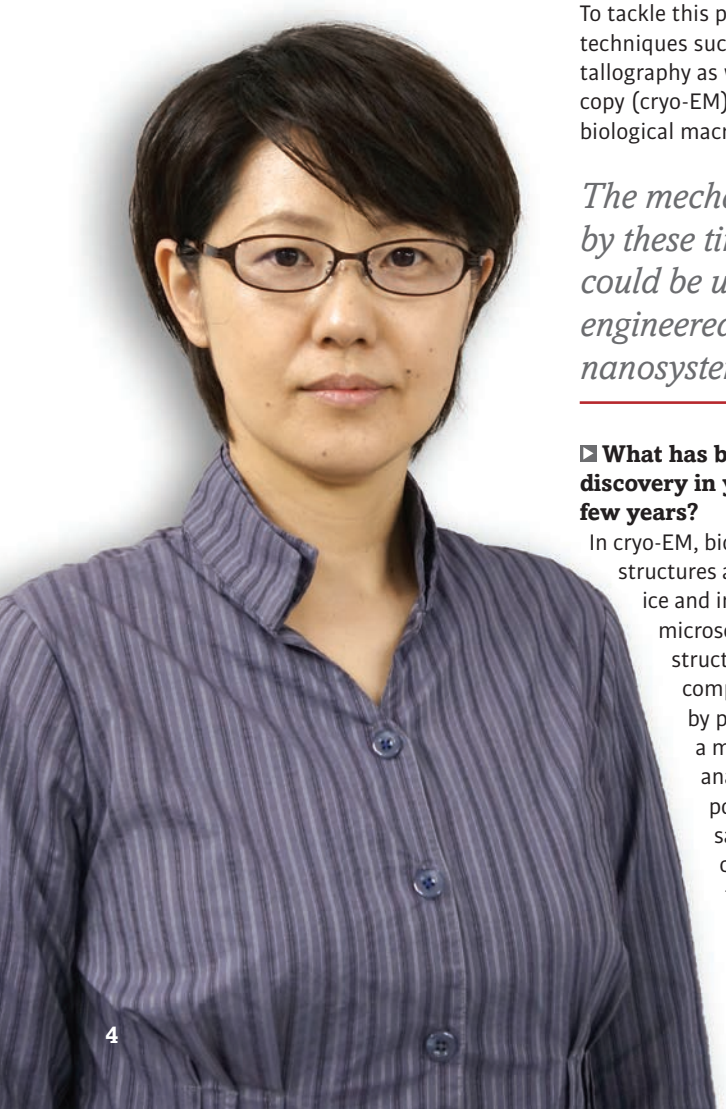
Saori Maki-Yonekura

Research Scientist

Bio-specimen Platform Group, RIKEN SPring-8 Center

Please briefly describe your current research.

I study biological macromolecular structures, which include membrane proteins and macromolecular complexes. In bacteria, membrane protein complexes are involved in the active transport of essential nutrients, such as iron and vitamins, across the outer membrane. The mechanisms employed by these tiny nanomachines could be used to develop engineered nanosystems, but they are difficult to analyze. To tackle this problem, I combine various techniques such as x-ray and electron crystallography as well as cryo-electron microscopy (cryo-EM) to study the structures of biological macromolecules.



The mechanisms employed by these tiny nanomachines could be used to develop engineered nanosystems.

What has been the most interesting discovery in your field in the last few years?

In cryo-EM, biological macromolecular structures are frozen in a thin layer of ice and imaged using an electron microscope. The three-dimensional structure of the macromolecular complex is then reconstructed by processing the images using a method called single-particle analysis. Cryo-EM is a very powerful technique because samples do not need to be crystallized, but its resolution has been limited until recently. By introducing high-resolution and fast-read-out electron detectors, which can correct for the movement of molecules in ice during data collection, researchers have been able to achieve resolutions comparable to, or even surpassing, those of x-ray crystallography.

Electron crystallography of thin three-dimensional (3D) crystals also provides a powerful means for determining the structures of crystals that are too small to observe using x-ray crystallography. This is because protein atoms diffract electrons four to five orders of magnitude more strongly than x-rays. The technique can also be used to visualize the charged states of amino acid residues and metals—information that cannot be obtained by x-ray crystallography.

These two new technologies—single-particle cryo-EM and electron 3D crystallography—could help to reveal the working mechanism of biological macromolecules in more detail. I am applying both methods to study the structures of membrane proteins under different physiological conditions.

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

After completing my master's degree, I worked at a research institute that had the latest electron microscope. I dreamt of using this microscope to determine high-resolution structures of biological macromolecular complexes. Over the course of my research career, I have realized that the combined approach of x-ray crystallography and cryo-EM is very powerful for studying the structures of difficult biological targets.

More recently, I have started using the facilities at the RIKEN SPring-8 Center to combine x-rays and electron beams for structural biology. These include the high-brilliance x-ray sources produced by beamlines BL32XU and BL41XU at the SPring-8 synchrotron radiation facility, the SPring-8 Angstrom Compact free electron LAser (SACLA), and the automated web-based crystal screening system and data collection for x-ray diffraction data, called D-Cha.

How has being at RIKEN helped your research?

RIKEN offers a suitable environment for researchers to concentrate on their studies. Despite belonging to a small laboratory, I can learn about the latest techniques from the many experts in x-ray crystallography at the RIKEN SPring-8 Center. ■

Microbeams enter the life sciences

Réka Judit Bereczky

Foreign Postdoctoral Researcher

Ion Beam Breeding Team, RIKEN Nishina Center for Accelerator-Based Science

Please briefly describe your current research.
The goal of my research as a foreign postdoctoral researcher at RIKEN is to develop narrow beams of charged particles known as microbeams that can be used to expose cells to precise and targeted irradiation.

Obtaining a microbeam with well-defined characteristics is a complex process. My more recent work involves studying the profile and the spectra of the helium-ion microbeam produced by the RIKEN pelletron accelerator in combination with tapered glass capillaries. I also collaborate with other laboratories. For example, together with the National Institute of Radiological Sciences based in Chiba prefecture, I apply proton microbeam irradiation techniques to explore the signaling communication within targeted cells and between targeted and non-targeted cells. This study will bring new insights into radiation-induced cellular responses and will advance our understanding of the health risks associated with radiation exposure.

> *Maybe one day the output of my research will be used to cure diseases.*

How did you become interested in this field?
During my PhD in physics, I did extensive research in the field of charged particle beam transport using glass capillaries. I was really interested in the possible applications of this process. In particular, the application to biological samples appeared as a very appealing field, since I also have a master's degree in biology.

What made you decide to become a scientist?
I was a very curious child and always wanted to understand how things work. I am grateful to my parents who encouraged me to develop this inquisitive nature into a passion for science. When I grew up I understood that science can be useful—even my own work, where I apply physics to biology, can produce helpful results for society. Maybe one day the output of my research will be used to cure diseases.

How and when did you join RIKEN?
I am from Hungary, and I joined RIKEN through the Foreign Postdoctoral Researcher program in September 2014. I first met my colleagues from the Ion Beam Breeding Team at international conferences. We engaged in several discussions and agreed that my experience in both physics and biology would make a valuable contribution to the research activity of the team.

What has been your most memorable experience at RIKEN?
My first tour of the laboratories at RIKEN was very impressive. Discovering all the available facilities, as well as the number and caliber of researchers, really gave me an idea of the outstanding research that is carried out here. A special memory, in between my professional and personal life, is the warm welcome I received from my closest colleagues. Their support helped me to overcome the difficulties of working so far from home and to become operational quickly.

Please tell us about your professional and personal goals.
I would like to establish my own research group in the future that can be a part of an international collaboration network. I am certain that the experiences I gain at RIKEN and the many scientists from different fields and countries whom I meet here will help me to achieve this goal. As personal goal I would like to bring happiness to myself, my family and the people around me, including through my work. ■

Careers at RIKEN

For further information, visit our Careers page:
Website: www.riken.jp/en/careers
E-mail: pr@riken.jp

Fluorescent slime and broccoli DNA at Yokohama Open Day 2015

More than 3,000 visitors attended the RIKEN Yokohama Campus annual Open Day on 29 August, despite the cool weather, overcast skies and light rain. Families with young children arrived in large numbers to enjoy their last weekend before the start of the Fall school term. Kids enjoyed making fluorescent slime, extracting broccoli DNA and learning about molecular structures using beads. Meanwhile, the adults heard lectures and seminars on immunity and gut microbiota, skin allergies, and why identical twins have different personalities. Together, they visited plant science and genome analysis laboratories and toured one of the world's largest nuclear magnetic resonance facilities.

Over 51 hands-on events, facility tours, lectures and seminars were organized for the day, including 36 poster presentations. Students from the nearby Yokohama Science Frontier High School also presented their own research results and helped out as volunteers at various Open Day events.

Yokohama Open Day is a joint effort between RIKEN Yokohama Campus and neighboring Yokohama City University's Tsurumi Campus, which is the main facility for the university's Graduate School of Medical Life Science.

www.yokohama.riken.jp/openday2015/en/

Japan and Singapore celebrate a decade of collaboration

RIKEN Executive Director Yoichiro Matsumoto (right) and A*STAR Chairman Lim Chuan Poh (second from left) at a materials science symposium organized to commemorate 10 years of Japan–Singapore collaboration.

On 3 August, RIKEN and Singapore's Agency for Science, Technology and Research (A*STAR) marked the 10-year milestone of their research partnership by holding a joint symposium in Singapore, focusing mainly on materials science.

RIKEN and A*STAR signed their first Memorandum of Understanding (MoU) in September 2005 to encourage scientific exchange between Singapore and Japan. In 2006, RIKEN opened its first overseas international liaison office in Singapore and has since renewed the MoU three times. The long-term partnership has spawned joint projects in fields ranging from the biomedical sciences to the physical sciences and engineering. It has also offered opportunities for scientific exchange through RIKEN's summer programs in brain science and immunology.

The most recent renewal of the MoU extends the existing partnership to mutual areas of interest in materials science.

"I am pleased that A*STAR's and RIKEN's long-standing partnership has catalyzed many opportunities for collaboration in R&D between Singapore and Japan," says A*STAR Chairman Lim Chuan Poh.

"A major mission of science today is to ensure the continued survival of humanity, and this mission cannot be accomplished without international cooperation," says RIKEN President Hiroshi Matsumoto. "I strongly hope that our partnership will continue to develop, leading to important research breakthroughs that will benefit all of humanity."

www.riken.jp/en/pr/topics/2015/20150807_1/

RIKEN Summer School

The seventh annual RIKEN Summer School was held on 4–5 September at Shinrin-Koen in Saitama Prefecture just north of Tokyo. The two-day event was organized by the RIKEN Global Relations and Research Coordination Office to provide an opportunity for young researchers from different research fields and countries to interact. This year, just over 100 students and researchers attended the event, including International Program Associates (IPAs), Junior Research Associates (JRAs). Foreign Postdoctoral Researchers and Special Postdoctoral Researchers also volunteered to support the event.

Participants gained valuable perspectives from talks by RIKEN President Hiroshi Matsumoto and prominent researchers, including RIKEN Honorary Scientist Takehiko Shibata. They also introduced their research and made new friends at a poster session where prizes were awarded.The best-poster prize went to Toshinobu Shida, a JRA at the RIKEN Brain Science Institute, who studies small, misfolded infectious proteins called prions that cause many neurodegenerative diseases. The field prizes went to IPA Wen Dee Ong (biology), JRA Masaru Tanioka (chemistry), IPA Krishnachary Salikolimi (engineering), JRA Masahito Yoshihara (medical science) and IPA Phong Vi (physics).

www.riken.jp/summerschool/

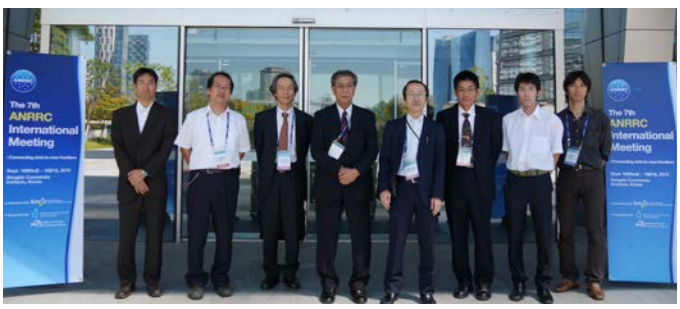
Resource center network stresses quality control

On 16–18 September, close to 200 representatives of biological resource centers in Asia convened at the 7th Asian Network of Research Resource Centers (ANRRC) International Meeting in South Korea, where speakers stressed the importance of establishing quality control standards. The annual meeting was organized by the Korea National Research Resource Center (KNRRC) under the theme of "Connecting dots to new frontiers".

The ANRRC was established in 2009 by a tri-institutional collaborative effort of the RIKEN BioResource Center (BRC), the KNRRC and the Institute of Microbiology, Chinese Academy of Sciences (IMCAS). It facilitates cooperation and networking among repositories of human, animal, plant, microorganism and non-biological materials in the most-species-diverse region in the world. The ANRRC includes 97 institutional members from 14 countries.

At the plenary session in South Korea, Yuichi Obata, director of the BRC, raised the importance of maintaining quality control and transparency in resource centers to ensure that experiments are reproducible. Yeonhee Lee, director-general of the KNRRC, presented models and standards for quality management and discussed the benefits of building networks that support them. The scientific sessions included 44 talks, 61 poster presentations and exhibitions on a wide range of subjects, including biodiversity, standardization, biobanking and information.

www.anrrc.org



Asparagus lowers blood pressure

Asparagus spears contain a sulfur-containing compound that suppresses an enzyme associated with elevated blood pressure, according to a study published in the *Journal of Natural Products* in April 2015.

The benefits of asparagus have a long history. Going back to antiquity, Egyptian Pharaoh Akhenaten and his queen, Nefertiti, are claimed to have called asparagus a "food of the gods." The new compound, which researchers at the RIKEN Center for Sustainable Resource Science (CSRS) and Chiba University have named asparaptine, works by inhibiting angiotensin-converting enzyme (ACE), an enzyme known to contribute to hypertension.

"Sulfur-containing compounds have been reported to have a range of benefits, including anti-inflammatory, anti-oxidative and anti-cancer effects," says Ryo Nakabayashi at the CSRS and the study's lead author. Nakabayashi and his colleagues pioneered an approach called targeted metabolomics, in which they used mass spectrometry to scan for sulfur-containing molecules in *Asparagus officinalis*. They identified several molecules and screened them to test their effectiveness in inhibiting ACE. Asparaptine proved very effective.

"We hope to use this efficient method to find other new substances and to help gain a better understanding of the positive effect of plant compounds on human health," says Nakabayashi.

www.riken.jp/en/pr/topics/2015/20150817_1/

Masayo Takahashi awarded first Ogawa–Yamanaka Stem Cell Prize

Masayo Takahashi, a researcher at the RIKEN Center for Developmental Biology (CDB), has been awarded the inaugural Ogawa–Yamanaka Stem Cell Prize for her "trailblazing work leading the first clinical trial to use induced pluripotent stem (iPS) cells in humans." The prize was established by the Gladstone Institutes in the United States to recognize individuals "whose original translational research has advanced cellular reprogramming technology for regenerative medicine." Takahashi was formally presented with the award at a ceremony on 16 September 2015.

Takahashi, who leads the Laboratory for Retinal Regeneration at the CDB, launched the first-ever clinical study using iPS cells in 2013. The study seeks to test the safety of reprogrammed cells as a treatment for age-related macular degeneration, a major cause of vision loss among senior citizens.

"As both a physician and a scientist, Dr. Takahashi embodies the ideal recipient because her work brings cellular reprogramming to patients," says Gladstone President R. Sanders Williams in a press release from the Gladstone Institutes.

www.cdb.riken.jp/en/news/2015/topics/0911_7778.html

Tracing the spread of vitamin B_1

A technique for tracking vitamin B_1 as it moves through the body has been developed by researchers at the RIKEN Center for Life Science Technologies (CLST). The technique involves 'tagging' the vitamin and its prodrug fursultiamine with a short-lived radioisotope of carbon known as carbon-11, which can be detected using positron emission tomography (PET) imaging.

"The process of synthesizing carbon-11-labeled compounds was very difficult and we look forward to putting it to use," state Hisashi Doi and Yasuyoshi Watanabe at the CLST, who led the research in collaboration with the global pharmaceutical company Takeda. "We have successfully carried out experiments in rats, and now plan to conduct studies in humans to learn more about how vitamin B_1 spreads through the body." Vitamin B_1 is known to be important in preventing fatigue, but the precise mechanism by which it works is not well understood.

Since its earliest days, RIKEN has been a pioneer in vitamin research. In 1910, one of the institute's founders, Umetaro Suzuki, discovered the first vitamin—vitamin B_1—in rice bran, continuing his research in the area at RIKEN. The most recent findings by Doi and Watanabe were published in *The Journal of Organic Chemistry* in June 2015.

www.riken.jp/en/pr/topics/2015/20150806_1/

RIKEN tops supercomputer rankings

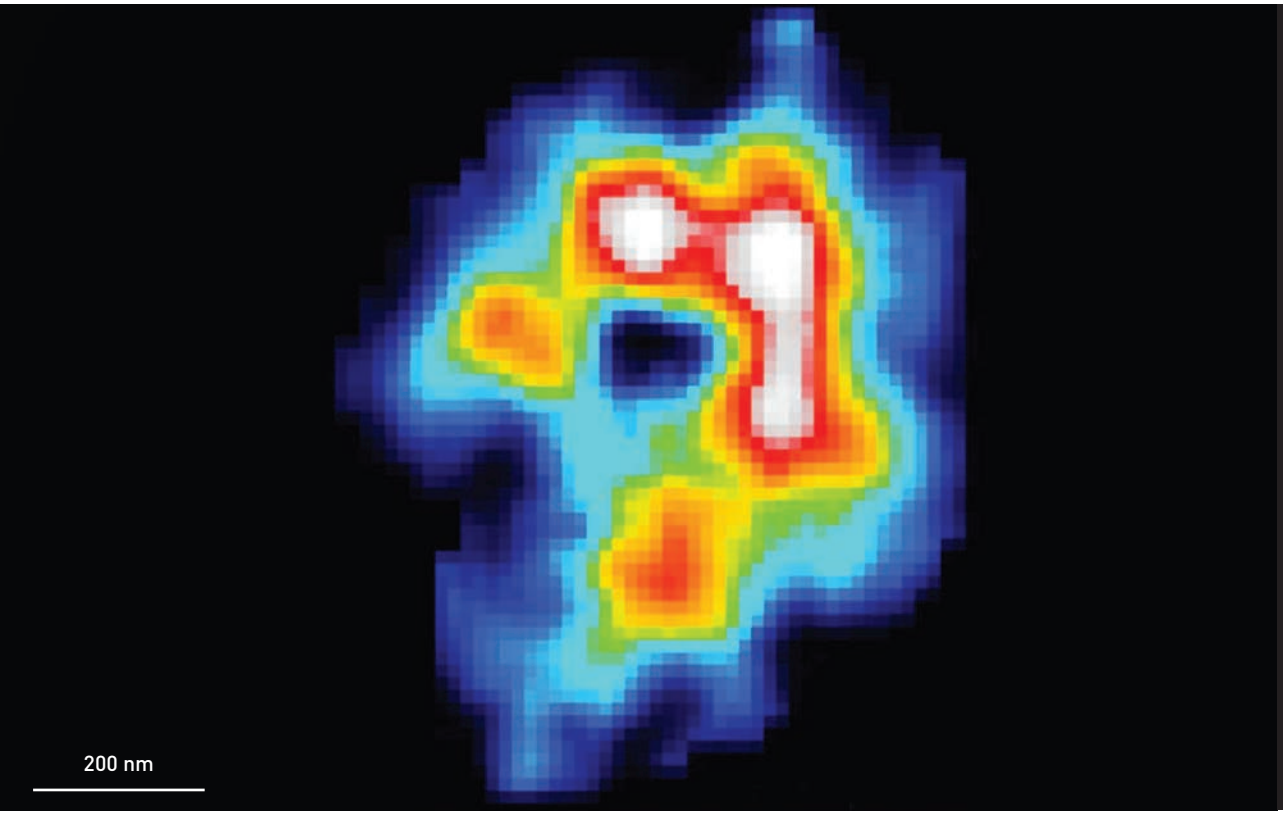
RIKEN's new Shoubu supercomputer claimed the title of most energy-efficient (or greenest) supercomputer in the world in the June 2015 and November 2015 Green 500 supercomputer ranking. Shoubu is named after a wetland plant known in English as sweet flag (*Acorus calamus*) because of the supercomputer's ingenious cooling system that completely submerses it in liquid. The supercomputer was developed by the Japanese venture companies PEZY Computing and ExaScaler.

Shoubu became the first supercomputer to surpass the milestone of 7 gigaflops per watt (billions of operations per second per watt), a 33 per cent improvement over the best performer in the November 2014 ranking. "It is very exciting for us to be able to demonstrate our institute's commitment to building a sustainable future," says Motoyoshi Kurokawa of the RIKEN Advanced Center for Computing and Communication, who supervised the project's development. RIKEN is collaborating with the two venture companies to further optimize the system and to maximize the computer's capability to support realistic applications.

RIKEN's most powerful supercomputer, the K computer, also won first place in the July and November 2015 Graph 500 biannual ranking specifically designed to assess data-intensive processing. The victory was the result of a collaboration between RIKEN, Tokyo Institute of Technology, University College Dublin, Kyushu University and Fujitsu. "The K computer has consistently shown itself to be a very powerful instrument for data-intensive loads," says Kimihiko Hirao, director of the RIKEN Advanced Institute for Computational Science. "We plan to continue to use the computer's power to take on projects involving modeling processes that take place in the real world, contributing where possible to the improvement of society."

www.riken.jp/en/pr/topics/2015/20150804_1/
www.riken.jp/en/pr/topics/2015/20150715_1/

Research highlights



A map of the electron density of an algal chloroplast obtained using coherent x-ray diffraction imaging. The high-density area exhibits a C-shaped profile, which suggests that photosynthetic proteins are concentrated in the marginal regions of chloroplast.

BIOLOGY

X-ray vision reveals photosynthetic structures

Researchers demonstrate a powerful x-ray imaging technique for uncovering the secrets of cell structures and processes

Using a high-power x-ray laser, researchers at RIKEN, Tokyo University of Science and Keio University have visualized the site of photosynthesis inside the chloroplasts of a small red alga at an extremely high resolution[1].

Cell biologists seek to understand how cellular processes occur at various levels in time and space, such as the cascading processes whereby a single molecule evokes a response at the cellular level.

Existing techniques for such investigations are limited. Light microscopy of live cells has proved very useful for gaining such knowledge, but its resolution is restricted to approximately 200 nanometers. On the other hand, electron microscopy can realize much higher resolutions, but it requires sectioning thick samples.

In the current study, the researchers used coherent x-ray diffraction imaging (CXDI), a new technique that combines the advantages of light and electron microscopy. "The technique can be used to visualize internal structures of whole cells or organelles, which are too thick for electron microscopy and beyond the resolution of light microscopy," explains Yuki Takayama of the RIKEN SPring-8 Center, one of the lead authors of the study.

The research team used an x-ray free-electron laser (XFEL) to perform CXDI

of chloroplasts isolated from the unicellular red alga *Cyanidioschyzon merolae*. They chose this hot-spring-dwelling organism as they wanted to explore functions such as photosynthesis. "*C. merolae* is particularly suitable for studying cell functions because it is one of the smallest eukaryotes and has a simple cellular architecture," says Takayama.

While XFEL-CXDI is advantageous for visualizing native structures at high resolution, the intense x-ray beam destroys the sample after imaging, making it difficult to obtain sharp images. The team overcame this problem by imaging many chloroplasts cryo-fixed on a single substrate and then selecting diffraction patterns with high signals. This enabled them to reconstruct high-resolution images of the chloroplasts from the diffraction data.

They were able to visualize the distribution of electrons in the chloroplasts with a resolution of 70 nanometers—much higher than that of light microscopy. The electron-density maps exhibit a C-shaped structure (see image), which is similar in size and shape to those obtained in fluorescence images of stained chloroplast proteins. This agreement indicates that membranes rich in photosynthetic proteins are distributed in the marginal regions of chloroplasts.

The team intends to improve the resolution of their technique—possibly to better than 20 nanometers. "By visualizing whole *C. merolae* cells using this technique, we hope to understand how organelles cooperate in cell functions," Takayama says. ■

Reference

1. Takayama, Y., Inui, Y., Sekiguchi, Y., Kobayashi, A., Oroguchi, T., Yamamoto, M., Matsunaga, S. & Nakasako, M. Coherent x-ray diffraction imaging of chloroplasts from *Cyanidioschyzon merolae* by using x-ray free electron laser. *Plant & Cell Physiology* **56,** 1272–1286 (2015).

PHYSICS

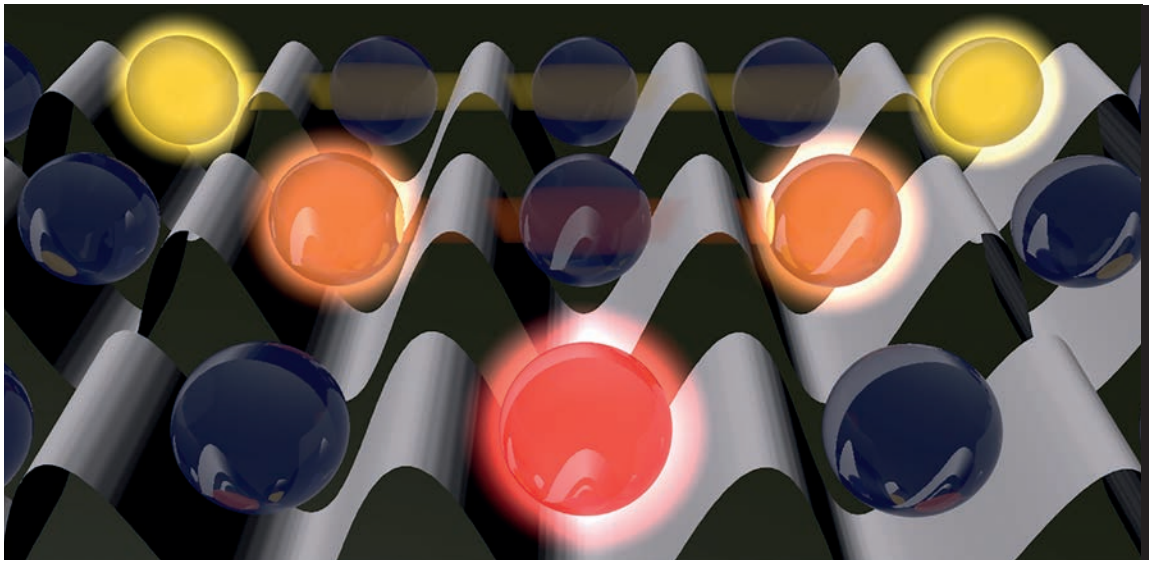
Entangled atoms

The observation of quantum entangled atoms has important implications for quantum information processing

The weird phenomenon of quantum entanglement has been observed between individual atoms in a laser-generated array of atoms for the first time[1].

Entanglement occurs when two or more quantum particles interact with each other in such a way that their quantum states become mutually dependent. It can be used to realize communication and computation capabilities that qualitatively surpass those achievable by classical physics. Entanglement can also give researchers a better understanding of quantum many-body systems, which can lead to the development of new materials and devices.

Now, Takeshi Fukuhara of the RIKEN Center for Emergent Matter Science, along with collaborators in Germany and the USA, has observed quantum entanglement in a line of equally spaced rubidium atoms prepared by loading extremely cold (several billionths of a kelvin) atoms into an 'optical lattice' generated by a set of standing laser waves. While entanglement has previously been detected in optical lattices based on average measurements for ensembles of atoms, this is the first time it has been seen between individual atoms.



Researchers initially excited one atom in a row of spin-down atoms to a spin-up state (red sphere) and then observed that entanglement propagated through the row as a function of time (orange and yellow spheres).

The atoms could have one of two possible spin states—spin up or spin down. Initially, the researchers prepared all the atoms in the spin-down state. They then promoted one atom to the spin-up state and used a quantum gas microscope to monitor the subsequent evolution of the system. Their measurements revealed a 'wave of entanglement' that traveled for up to six lattice sites (see image).

Fukuhara likens this experiment to giving a ball to a person standing at the center of a line of people. In the classical world, the person can pass the ball to the person on the right or the left. But in the quantum world, they can pass the one ball to both neighbors simultaneously. "In a similar way, we accessed the central atom and changed its spin state," explains Fukuhara. "This change (or spin excitation) is transferred to both adjacent atoms, like the quantum ball." By tracking the dynamics of how this excitation propagated at the single-atom level, the researchers confirmed that quantum entanglement is generated and transferred via these dynamics.

The researchers intend to investigate the dynamics of more-complex quantum many-body systems. "In the present study, we used a relatively simple system that can in principle be simulated using classical computers," explains Fukuhara. "A major goal of our research is to investigate entanglement and its dynamics in more-complex systems, which cannot be solved using classical computers." ■

Reference

1. Fukuhara, T., Hild, S., Zeiher, J., Schauß, P., Bloch, I., Endres, M. & Gross, C. Spatially resolved detection of a spin-entanglement wave in a Bose-Hubbard chain. *Physical Review Letters* **115,** 035302 (2015).

BIOLOGY | PRESS RELEASE

How innate immunity remembers

Epigenetic changes are implicated in how the innate immune system remembers past pathogens

The underlying mechanism for the memory of innate immunity has been uncovered by RIKEN researchers.

Acquired immunity was long thought to have memory, meaning that it could learn from new pathogens, whereas innate immunity was considered not to. However, things are not so clear cut. For example, plants and insects, which have only innate immunity, also seem to have immune memory. Innate immunity thus also appears to have memory, but researchers had been reluctant to accept this in the absence of a mechanism.

Now, researchers led by Keisuke Yoshida and Shunsuke Ishii of the RIKEN Molecular Genetics Laboratory have revealed the underlying mechanism for innate immunity memory—epigenetic changes induced by pathogen infections, mediated by a transcription factor called ATF7[1].

The research began from the discovery that the macrophages in ATF7 knockout mice appear similar to wild-type macrophages that have been activated by exposure to molecules that occur commonly in infections. The researchers had previously reported that ATF7-related transcription factors mediated epigenetic changes induced by heat shock or psychological stress that were maintained for long periods after exposure to stress. They thus

The hygiene hypothesis proposes that recent increases in allergies result from reduced exposure to unhygienic environments during infancy. Epigenetic changes induced by pathogen infections may explain the hypothesis.

speculated that infections by pathogens could induce epigenetic changes in macrophages via ATF7.

The team discovered that ATF7 silences the expression of a group of innate immune genes by binding to them, making the cell less responsive to infections. However, when a molecule found in the outer membrane of Gram-negative bacteria was administered to mice, ATF7 was phosphorylated, weakening its activity so that immune-related genes were no longer silenced. "Even three weeks after administration, the genes showed increased activation," says Ishii. "In mice, this leads to increased resistance to a Gram-positive bacteria."

This could shed light on the 'hygiene hypothesis'—the concept that infection by pathogens and exposure to an unhygienic environment during infancy reduces the risk of developing allergies later in life. "Though many researchers believe the hypothesis," says Ishii, "there is great uncertainty about how pathogen infection is memorized until adulthood. Our research provides a plausible explanation of how the changes are induced. It also means that affected genes can be used to diagnose allergies."

Another possible application is the choice of vaccine adjuvants—substances that activate innate immunity. The effects of adjuvants had generally been thought to end after a few days, but the present research showed that their effects can be maintained for longer. "These results could affect the selection method of adjuvants," says Ishii. "We hope they will contribute to the development of more efficient vaccines." ■

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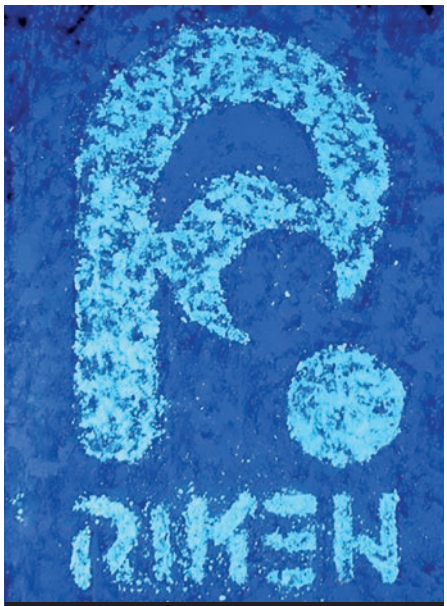
CHEMISTRY

Forcing a molecular light switch

A compound has been discovered whose fluorescence properties change dramatically on applying a mechanical force



RIKEN logo created by selectively grinding *cis*-ABPX01. This gives rise to regions with different fluorescence properties.

A chance observation led to RIKEN researchers discovering an organic compound whose fluorescence wavelength varies greatly when it is subjected to a mechanical force[1]. This property makes it an attractive material for various applications in security as well as medical imaging and therapy.

Shinichiro Kamino and Shuichi Enomoto of the RIKEN Center for Life Science Technologies, along with Atsuya Muranaka and colleagues from the RIKEN Center for Sustainable Resource Science and other scientists based in Japan, were studying some dyes when they noticed that a solid-state dye, *cis*-ABPX01, fluoresced at both near-infrared and blue frequencies. Subsequent spectroscopic and x-ray diffraction analysis indicated that this dual fluorescence stemmed from the two different crystal structures that the dye could adopt. The blue fluorescence came from a crystal arrangement of single molecules, whereas the near-infrared fluorescence originated from a configuration in which the repeating units consisted of two molecules joined together.

"This relationship between fluorescence and molecular structure inspired us to think that simple mechanical grinding might reduce the near-infrared fluorescence and enhance the blue fluorescence," Kamino explains. Sure enough, the researchers observed this predicted change in fluorescence when they ground the mixture of structures in a mortar. By selectively grinding certain regions, they could produce areas with different fluorescence properties (see image).

The change also proved to be readily reversible: exposing the ground material to dichloromethane vapor restored the near-infrared fluorescence while reducing the blue fluorescence. Furthermore, this reversible switching could be repeated several times.

While other molecules have been discovered whose fluorescence properties vary on applying a mechanical force, the shift in fluorescence wavelength between near-infrared and blue light observed for *cis*-ABPX01

is considerably larger than that for other compounds. This remarkable dual fluorescence raises the possibility that *cis*-ABPX01 could be used as a component in signaling systems for a wide range of industrial, biological and medical applications.

"We think that these molecules could be used to sense mechanical forces in cells and tissues," says Kamino. He explains that there is growing interest in the field known as mechanobiology, which looks at the role of mechanical forces in altering cellular activity and tissue behavior in biological systems.

The researchers plan to investigate using *cis*-ABPX01 and related molecules to detect medically significant changes in living systems. They are also interested in exploring applications such as security tags whose optical properties change when they are subjected to mechanical tampering and other applications in which it is important to detect mechanical forces. ■

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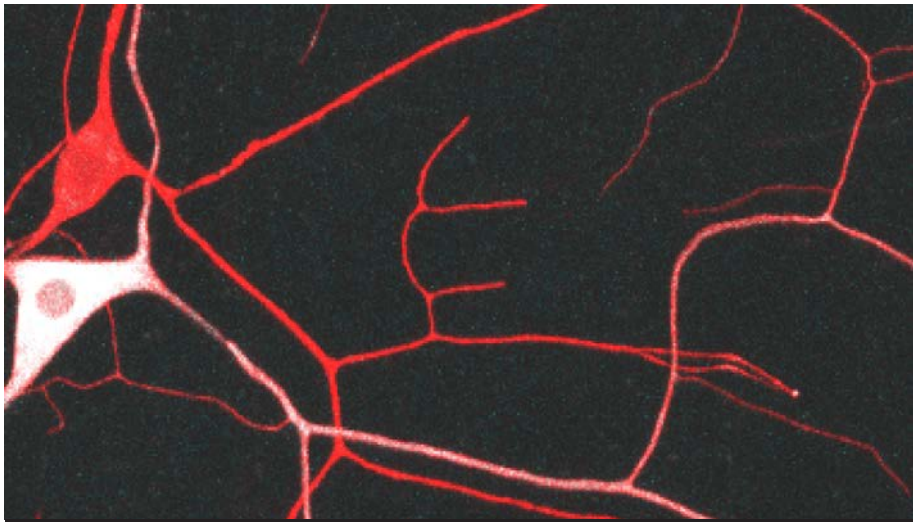
BIOLOGY | PRESS RELEASE

How neurons get their branching shapes

A protein is found to inhibit the branching of neuronal projections

For over a century, scientists have known that dendritic arbors—projections that neurons use to receive information from other neurons—differ in size and shape for different types of neurons. Now, researchers at the RIKEN Brain Science Institute have discovered a protein that helps shape dendritic arbors. In particular, their work reveals how the protein Centrosomin prevents dendrites from branching out[1].

Dendrites grow and branch as structural elements called microtubules push the ends out in specific directions. Microtubules are often likened to cellular scaffolding; they are built on site by growing out from one end. To determine how microtubule growth and dendritic branching are regulated, the researchers examined sensory neurons from *Drosophila* fruit flies.



Neurons with simple (red) and highly branched (white) dendritic arbors have different microtubule cytoskeleton properties.

They focused on a type of *Drosophila* sensory neuron that has very limited dendritic branching and expresses the transcription factor Abrupt. They began by determining that the expression of Abrupt leads to reduced arbors, whereas its absence results in more-complex arbors. The team next tested a group of candidate proteins in the pathway of molecular events initiated by Abrupt, looking for one that regulates microtubules. They found that loss of Centrosomin—a protein that makes microtubule-based structures necessary for cell division—resulted in more extensive dendritic branching, while its addition could block the increase in branching caused by a lack of Abrupt.

The researchers then discovered that by working with another protein called Pericentrin-like protein, Centrosomin could control where new microtubules form within the dendrites. When one end of a microtubule is attached to something, it does not push out new dendritic branches as it grows. However, the opposite is true when microtubules form at no particular site: new branches are likely to form as it grows. Further testing revealed that Centrosomin acts as a glue that fixes microtubules, particularly to Golgi bodies, which is why its presence leads to simpler branching.

"The shape and complexity of neuronal dendrite arbors are often disrupted in neurological diseases," notes Adrian Moore, who led

the research team. "It turns out that the two microtubule regulators we found in this study of *Drosophila* neurons—Centrosomin and Pericentrin-like protein—are encoded by genes mutated in some human brain disorders. Learning more about how neurons control the growth of dendrites will help us understand these diseases more completely, and we may discover how to initiate and direct neuron growth as therapy for diseases and after neuronal injury." ■

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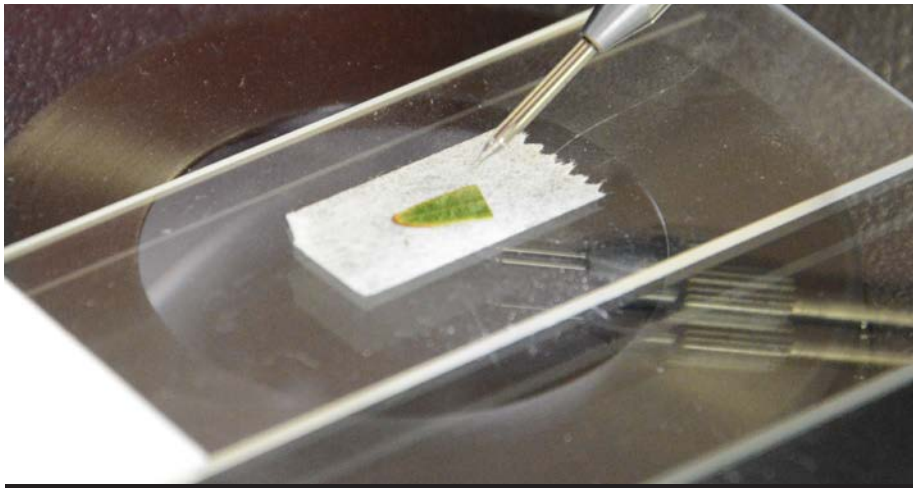
BIOLOGY | PRESS RELEASE

Getting a snapshot of cell molecules in a flash

A new analytic method allows the molecules present in a single plant cell to be determined in minutes

Scientists can now take a peek into a single plant cell and—within minutes—find out what small molecules are inside it, such as metabolites, hormones, nutrients and lipids, using a new method developed by RIKEN researchers[1].



A nanospray tip is used to extract the contents of a single plant cell, which are then analyzed by a mass spectrometer, providing a rapid analysis of the molecular contents of the cell.

Understanding exactly what is taking place inside a single cell is no easy task. For DNA, amplification techniques are available to make the task possible, but for other substances such as proteins and small molecules, scientists generally have to rely on statistics generated from measuring many different cells together. Unfortunately, this means that researchers cannot look at what is happening in each individual cell.

Now they can, thanks to a new method based on seven years of work at the RIKEN Quantitative Biology Center and Hiroshima University. In this method, the contents of the single target cell are directly sucked up by a metal-coated glass capillary called a nanospray tip under a stereo microscope (see image) and the contents are directly fed into the inlet of a mass spectrometer. Within minutes, the mass spectrometer detects hundreds or thousands of molecular peaks, which can then be matched to databases to determine the metabolites present in the plant cell under the specific conditions it was under when its contents were removed.

This method promises not only to speed up our ability to understand how molecules are distributed in cells in time and space, but also to transform how research is done in agricultural science. "Using this method we can compare the molecular peaks of cells which are in different stages of growth, different locations, or responding to different circumstances using statistical analyses of the mass spectrometry data." says Tsutomu Masujima, who leads RIKEN's Single Cell Project. "If, for example, we find that certain metabolites are increased in a specific strain, it implies that the enzyme or protein of this specific metabolic pathway may be the key to the specificity of this strain. It might also help us to identify new pathways that are important."

For the moment, the team is focusing on plant cells, but they are preparing a protocol for animal cells—which is much more difficult since the cells are smaller and softer—together with high-content screening applications.

Given the potential of the new method, the researchers have decided to publish the protocols for the benefit of the global research community. ■

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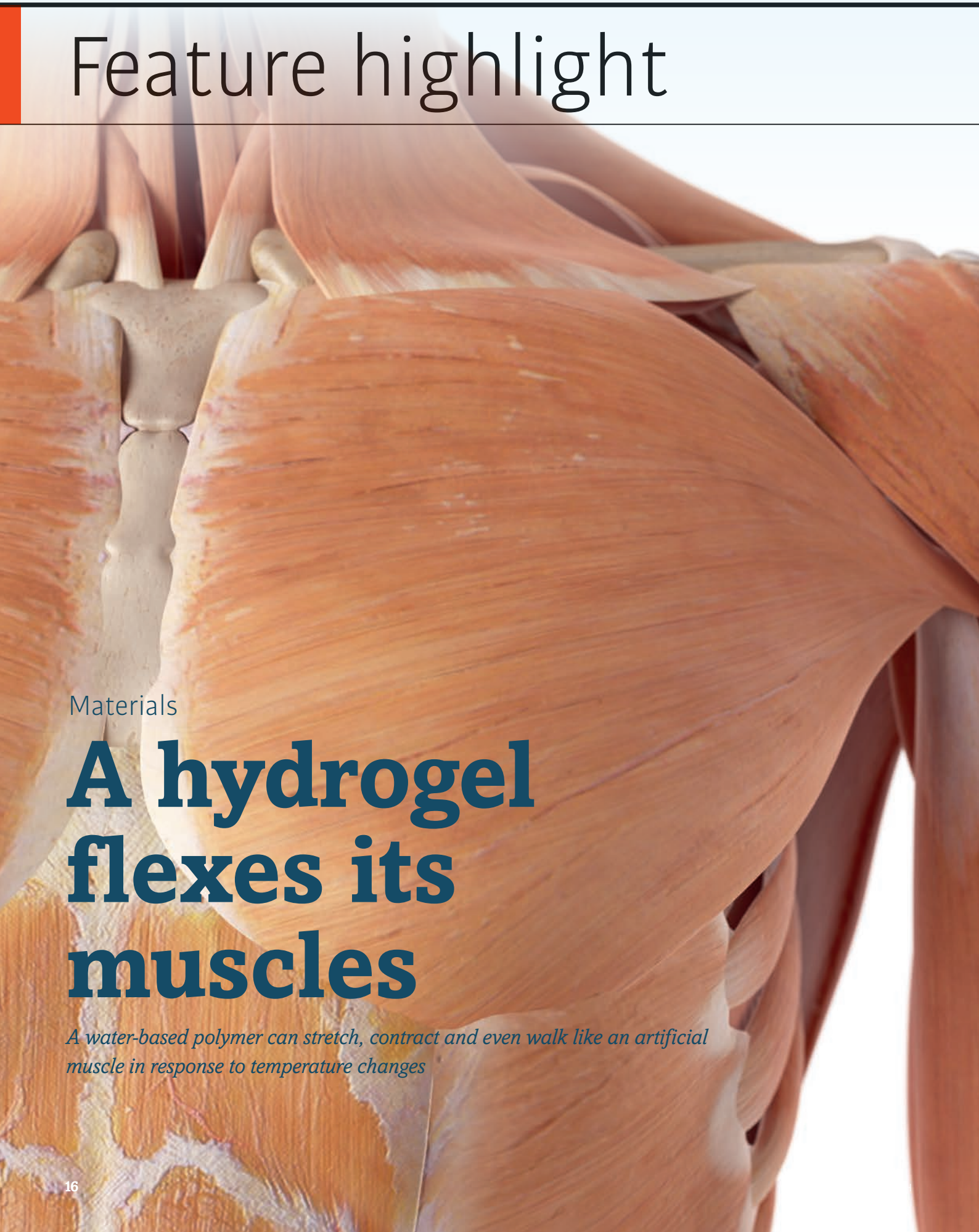

Materials

A hydrogel flexes its muscles

A water-based polymer can stretch, contract and even walk like an artificial muscle in response to temperature changes

Hydrogels are translucent, squishy polymers that resemble tissues by holding large amounts of water within an interlocked molecular network. A team led by RIKEN researchers has developed a way to cause these biocompatible materials to act like artificial muscles that rapidly expand and contract when heated and cooled[1]. To demonstrate the potential of this approach, the team fabricated an L-shaped prototype that changes shape and 'walks' across a surface when subjected to temperature changes.

While hydrogels have garnered interest for applications such as scaffolds for tissue growth, researchers have found it challenging to turn them into controllable actuators that simulate muscle movement. Most hydrogels swell or shrink in response to water absorption or desorption, but these deformations occur uniformly, whereas actual muscles deliver forces in specific directions. Furthermore, expelling and absorbing water from hydrogels is usually a slow process, and thus cannot match the fast responses of muscles.

Now, Yasuhiro Ishida from the RIKEN Center for Emergent Matter Science and his colleagues have designed a hydrogel whose properties differ considerably from those of other hydrogels. Their thermoresponsive material can lengthen in one direction and contract in another without taking up or releasing water, allowing this muscle-like device to operate rapidly in an open-air environment. The secret to this behavior lies in the tiny nanosheets embedded in the hydrogel that harness electrostatic repulsion to make the hydrogel perform like a coiled spring.

Between the sheets

The first kinds of hydrogels scientists encountered were natural materials such as collagen and gelatin that swell when placed in water. Chemists soon devised ways to cross-link synthetic polymers together to form similar water-attracting gels. Such synthetic hydrogels have the advantage that they can be tailored to specific applications; for example, contact lenses require hydrogels with a high oxygen permeability, whereas drug delivery systems need hydrogels that are sensitive to external stimuli.

Ishida and his colleagues have been investigating a novel addition to standard hydrogel recipes: tiny flakes of see-through, two-dimensional crystals known as nanosheets that can add electric charges to the usually inert materials. Previously, the team used clay nanosheets to produce hydrogels that quickly self-heal after being sliced with a knife. In this case, ionic charges on the nanosheets stimulate new cross-linking reactions when the damaged gel is pushed together.

In their latest study, the RIKEN researchers collaborated with Takayoshi Sasaki from Japan's National Institute for Materials Science, a pioneer in the field of layered nanomaterials, to study substances known as exfoliated titanate nanosheets. Composed of ultrathin

Yasuhiro Ishida
Yasuhiro Ishida received his Bachelor of Science and Master of Science degrees in 1996 and 1998, respectively, from the University of Tokyo, Japan. In 2001, he received his PhD degree under the direction of Professor Takuzo Aida at the same university, and went on to launch his academic career as a research associate and lecturer. In 2009, Ishida was appointed as a team leader at RIKEN. His research interests include the development of smart soft materials based on supramolecular chemistry and polymer chemistry.

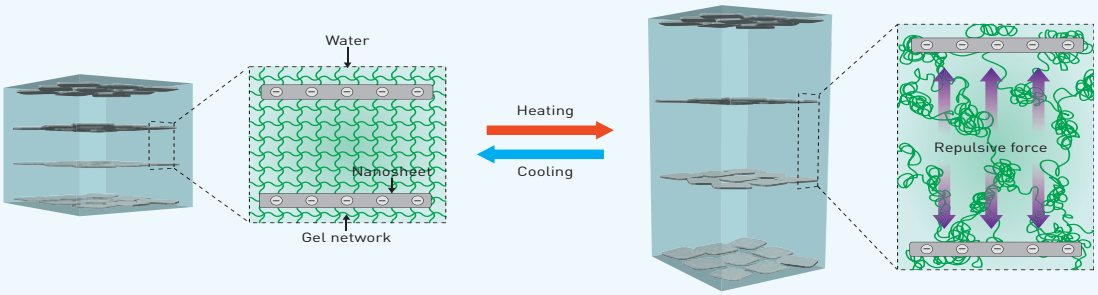

Figure 1: Researchers have created a muscle-like hydrogel by trapping face-stacked inorganic nanosheets within a polymer network. The repulsive force between the charged nanosheets becomes greater with increasing temperature, causing the material to elongate in one direction.

titanium–oxygen layers that are several micrometers long, titanate nanosheets have an extremely high density of negative surface charges and have found prior success as photocatalysts and biomolecular trapping agents.

A shocking twist

Intriguingly, the team discovered that when an aqueous dispersion of titanate nanosheets is hit with a strong magnetic field, the nanosheets align themselves into stacked layers (Fig. 1). This face-to-face arrangement produces a large electrostatic repulsion that holds the sheets apart—an unusual situation since man-made materials are usually held together by attractive interactions.

Harnessing this potential energy for hydrogel devices required finding a way to fix the magnetically induced structure in place. To achieve this, the researchers added the monomer *N*-isopropylacrylamide (NIPA) to an aqueous suspension of titanate nanosheets and exposed the mixture to magnetic fields while simultaneously irradiating it with ultraviolet radiation. The ultraviolet light initiates photopolymerization that cross-links the polymer of NIPA (PNIPA) with an organic binding agent, creating a hydrophilic gel that envelops the titanate nanosheet stacks and ensures that the face-to-face configuration remains after the magnetic field is switched off.

The molecular structure of PNIPA is known to be highly temperature sensitive. But when the team tested how a 15-millimeter-long, rod-shaped sample of the new hydrogel responded to water baths of various temperatures, they were shocked—in 1 second, the hydrogel elongated by over 70 per cent in warm 50-degree-Celsius water, while a cooler 15-degree-Celsius bath produced a similarly fast contraction.

"Every researcher in this field believed the opposite should happen—they thought PNIPA hydrogels should contract on heating and expand on cooling," explains Ishida. "When we saw our deformations, we were sure there was some new physics behind this phenomenon."

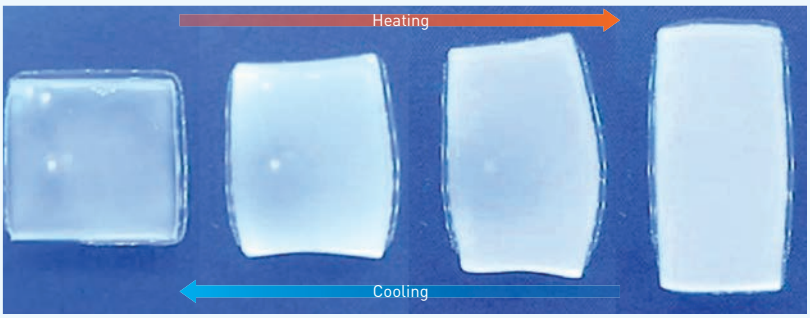

Figure 2: A square section of a new thermoresponsive hydrogel lengthens in one direction while simultaneously contracting in the other —kinematics similar to those seen in human muscles.

Finding the critical point

To better understand this system, the researchers examined a square hydrogel as the temperature was slowly raised from 25 to 45 degrees Celsius (Fig. 2). This analysis showed that once the polymer reached 32 degrees Celsius, it swiftly lengthened in one direction while simultaneously contracting in the other. The team deduced that above this critical temperature, the PNIPA releases free water held between the charged nanosheets. This process causes instant elongation without significantly changing the volume as the sheets move apart to reduce electrostatic repulsion. Cooling the gel produces the opposite effect.

"The three-dimensional network that supports our hydrogel behaves like a net composed of coiled springs," explains Ishida. "The nanosheets are trapped within it and can move without falling apart."

The researchers exploited their hydrogel's large and rapid thermally induced shape changes by designing an actuator that walks when exposed to alternating temperature cycles. Their L-shaped gel contains two 'feet' in contact with a horizontal, underwater surface. On heating, the back foot elongates, propelling the gel forward. A quick plunge in temperature then draws the feet back into position for another kick forward. "It's remarkable to watch the hydrogel walk," says Ishida. "It's as if it had a will of its own."

The team is currently working on improving this material's properties with a view to achieving their ultimate goal—a new generation of hydrogel-based artificial organs and muscles.

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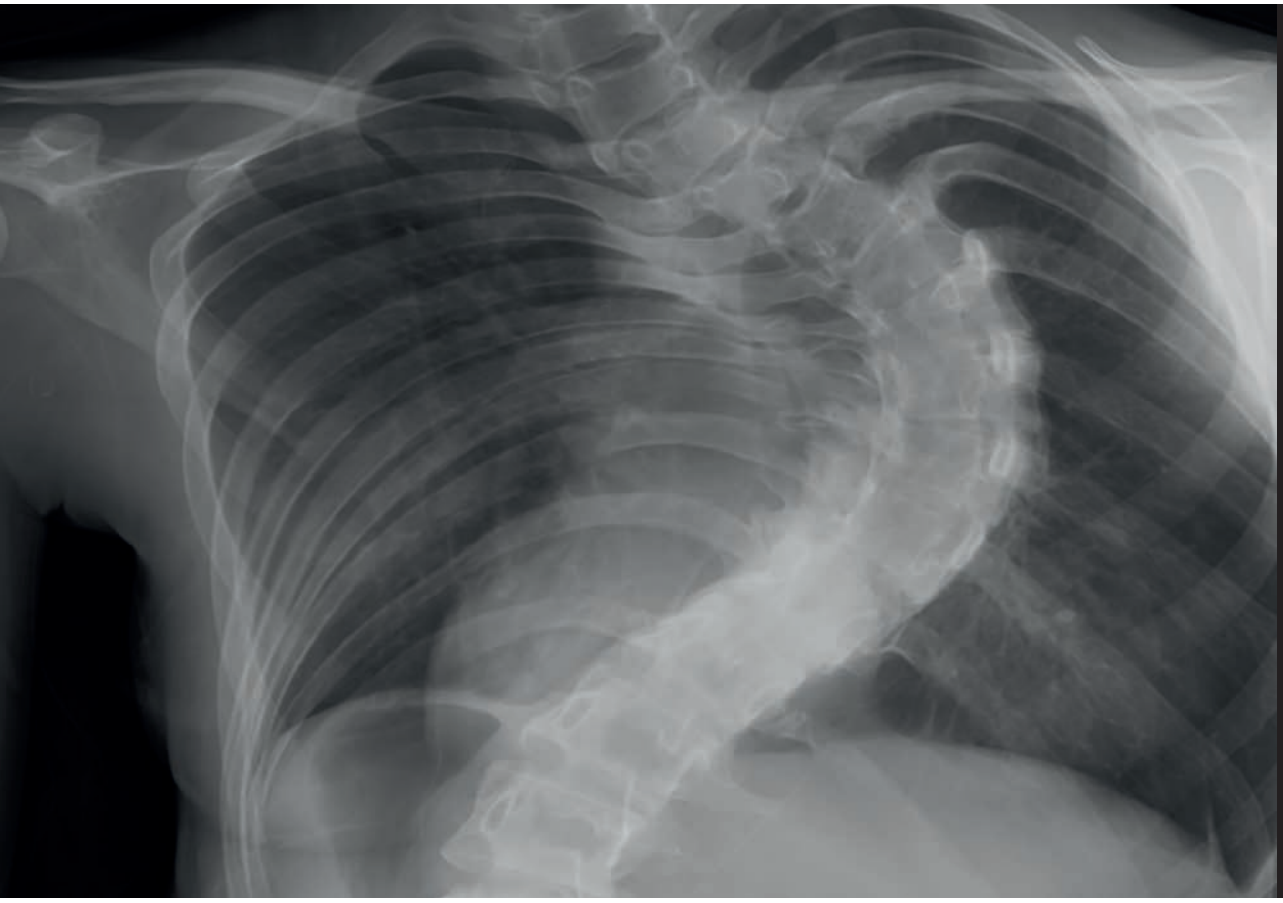

Researchers have uncovered the genetic causes of adolescent idiopathic scoliosis—a condition that gives rise to an abnormally curved spine.

BIOLOGY | PRESS RELEASE

The genetic roots of adolescent scoliosis

The genetic underpinnings of a mysterious condition causing abnormal spine curvature have been determined for the first time

Adolescent idiopathic scoliosis—a condition characterized by an abnormally curved spine (see image)—afflicts tens of millions of children worldwide, but did not have a known cause. Now, a gene linked to susceptibility to the condition has been discovered by scientists at the RIKEN Center for Integrative Medical Sciences[1]. They found that the gene is associated with increased expression of the gene *BNC2* (*basonuclin 2*), which is in turn regulated by another gene called *YY1*.

"Adolescent idiopathic scoliosis is a complex and mysterious disease with awkward spinal deformities that can be a nightmare for affected people," explains team leader Shiro Ikegawa. "We were excited to find a single nucleotide polymorphism (SNP) located on human chromosome nine that is significantly associated with the disease."

The discovery began with a genome-wide association study of more than 10,000 volunteers with and without scoliosis. This type of study looks for small differences in genes—SNPs—that occur more frequently in people with a certain disease. After confirming the association between a particular SNP in two additional independent populations, the scientists determined that it is located near the part of the DNA that codes for *BNC2*.

The team found that *BNC2* is most highly expressed in the uterus, spinal cord, bone and cartilage in humans. "This told us that we were on the right track," says Ikegawa. "And evidence

that the SNP variation associated with the disease led to higher levels of *BNC2* expression told us that this SNP has the potential to regulate expression of *BNC2*."

The team found that not only was *BNC2* expression triggered by the protein YY1, which binds to the DNA around the SNP, but that for subjects with the at-risk SNP variant, the amount of the BNC2 protein produced when YY1 was present was much greater than for subjects with the non-risk variant. The *BNC2* gene is highly conserved across diverse species and plays roles in various tissues. To test how overexpression of *BNC2* affects development, the team expressed it in zebrafish embryos and found that it resulted in severe body curvature that was positively correlated with the amount of BNC2.

These results and the abundance of BNC2 in human bones suggest that adolescents with the disease-associated SNP variant may begin to produce excess BNC2 at puberty if other genetic or environmental factors are present.

The next step is to understand how BNC2 causes scoliosis and why it is so much more prevalent in women than in men. ■

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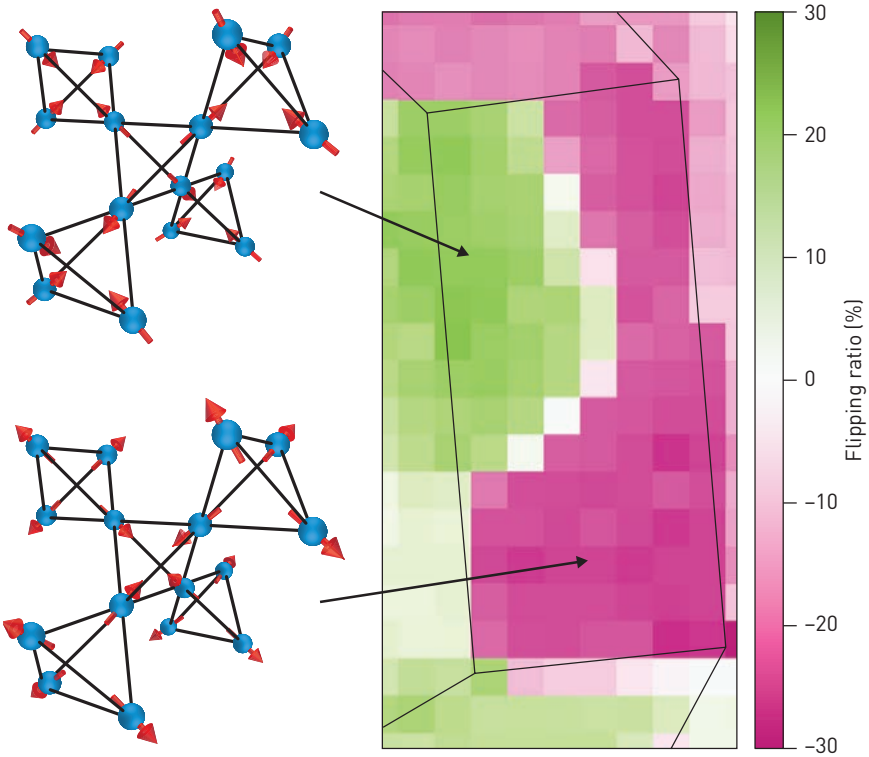
MATERIALS

A surprisingly simple magnetic flip

A simple technique makes it possible to control the spin directions of 'magnetic twins' hiding inside metallic crystals



A new technique that applies different combinations of magnets to an osmium-based crystal can 'flip' regions of magnetic spin from one orientation to another (pink and green shading).

A RIKEN-led research team has stumbled on a remarkably simple way to flip the magnetic orientation of mirror-image magnetic domains in the unusual magnetic material pyrochlore[1]. This flipping technique also makes it possible to observe these domains precisely in real space, providing a new tool for exploring spin-based device applications.

Pyrochlore crystals have attracted the attention of physicists because of their unusual magnetic potential. These crystals consist of tetrahedral crystal units, where the electron spins at the four vertices are in a constant state of magnetic 'frustration' that results in multiple magnetic ground states.

Recently, researchers found evidence of 'all-in–all-out' configurations in pyrochlore lattices, where all four spins either point toward the center of the tetrahedron or away from it (see image). This arrangement breaks the magnetic frustration and produces only two ground states that are related by time-reversal symmetry. However, distinguishing the two types of magnetic domains is tricky because the all-in–all-out magnetic structure is an antiferromagnetic arrangement that does not respond to typical electronic and magnetic probes.

Taka-hisa Arima and his colleagues from the RIKEN SPring-8 Center and RIKEN Center for Emergent Matter Science, in collaboration with researchers from across Japan, realized that one way to spot these elusive twin domains is to examine the resonant scattering signal from polarized x-rays. In this technique, the energy of the incident x-ray can be tuned to resonate with a specific site, element or electronic transition of a material, making the technique ideal for locating magnetic symmetry transitions. By measuring the changes induced by right- and left-handed circularly polarized light using a low-temperature microdiffraction technique, the team deduced a 'flipping ratio' that identifies the local magnetic domain orientations.

When the researchers measured the flipping ratios of pyrochlore crystals of cadmium-osmium oxide ($Cd_2Os_2O_7$) at the SPring-8 synchrotron radiation facility, they found that the domain structures were significantly affected just by cooling a sample near a permanent magnet. The existence of such a simple, reversible and controllable way to manipulate spins was a surprise. "Because the all-in–all-out structure cannot host any magnetization, almost no-one predicted that its magnetic domain could be controlled by a magnetic field," notes Arima.

The researchers also suggest that this technique can image and control any type of pyrochlore with an all-in–all-out structure—not just $Cd_2Os_2O_7$. "Recent theories predict that iridium oxide pyrochlore might have exotic electronic states along magnetic domain walls, as well as on its surface," explains Arima. "Our technique could provide quite useful information about these systems." ■

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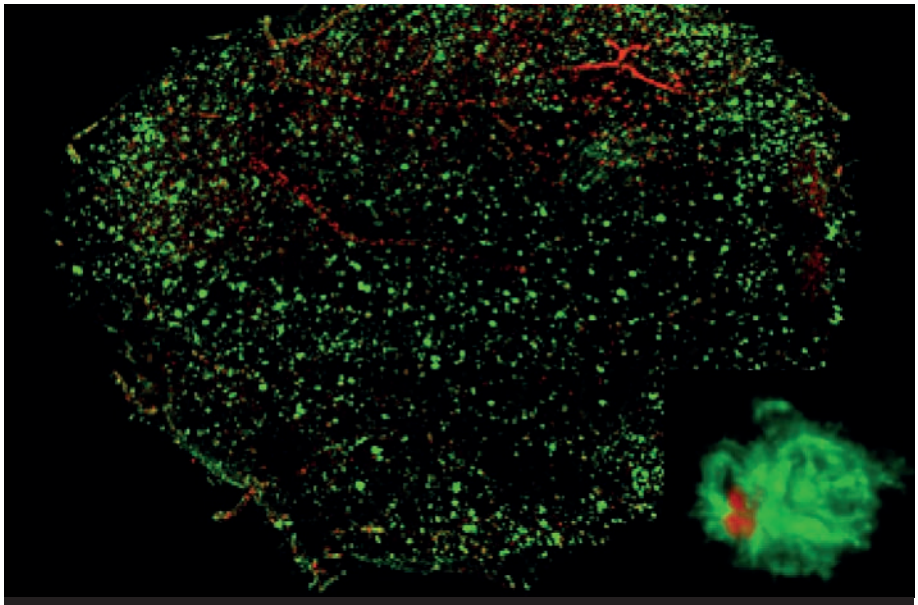
BIOLOGY | PRESS RELEASE

See-through brains ready for study

A new technique for making tissue transparent enables three-dimensional imaging to be performed without damaging structures



Multicolor high-resolution three-dimensional images of amyloid beta plaques in older mice from a genetic mouse model of Alzheimer's disease obtained using a method for preparing transparent tissue called ScaleS.

RIKEN researchers have developed a new technique for creating transparent tissue that can be used to image three-dimensional (3D) brain anatomy at very high resolutions, and they have used it to obtain new insights into Alzheimer's disease[1].

Generating see-through tissue—a process called optical clearing—is important because, when combined with advanced microscopy imaging techniques, it would allow the complex structural details of organs and cells to be revealed. Previous methods were limited because the transparency process can damage the structures under study.

"Our method, called Sca*l*eS, is a real and practical way to see through brain and body tissue," explains Atsushi Miyawaki of the RIKEN Brain Science Institute. "It enables accurate 3D structural information to be obtained that cannot be readily achieved through traditional 2D methods."

"The key ingredient of our new formula is sorbitol, a common sugar alcohol," reveals

Miyawaki. "By combining sorbitol in the right proportion with urea, we could create transparent brains with minimal tissue damage that can handle both fluorescent and immunohistochemical labeling techniques."

The new technique creates transparent brain samples that can be stored in Sca*l*eS solution for more than a year without damage. Internal structures maintain their original shape, and brains are firm enough to permit the micrometer-thick slicing necessary for more detailed analyses.

"In addition to allowing tissue to be viewable by light microscopy, a practical solution must also ensure accurate tissue preservation for effective electron microscopy," says Miyawaki.

Sca*l*eS provided an optimal combination of cleared tissue and fluorescence signals. Miyawaki believes that the quality and preservation of cellular structures viewed by electron microscopy is unparalleled.

The team has devised several variations of the Sca*l*e technique that can be used together. By combining Sca*l*eS with AbSca*l*e—a variation for immunolabeling—and ChemSca*l*e—a variation for fluorescent chemical compounds—they generated multicolor high-resolution 3D images of amyloid beta plaques in older mice from a genetic mouse model of Alzheimer's disease (see image).

The team used the technique to visualize in 3D the mysterious diffuse plaques seen in the postmortem brains of Alzheimer's patients that are typically undetectable by 2D imaging. Contrary to current assumptions, the diffuse plaques were not isolated, but were shown to be extensively associated with microglia—mobile cells that surround and protect neurons.

"Our technique will be useful not only for visualizing plaques in Alzheimer's disease, but also for examining normal neural circuits and pinpointing structural changes that characterize other brain diseases." ■

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BIOLOGY

Controlling the cargo of cells

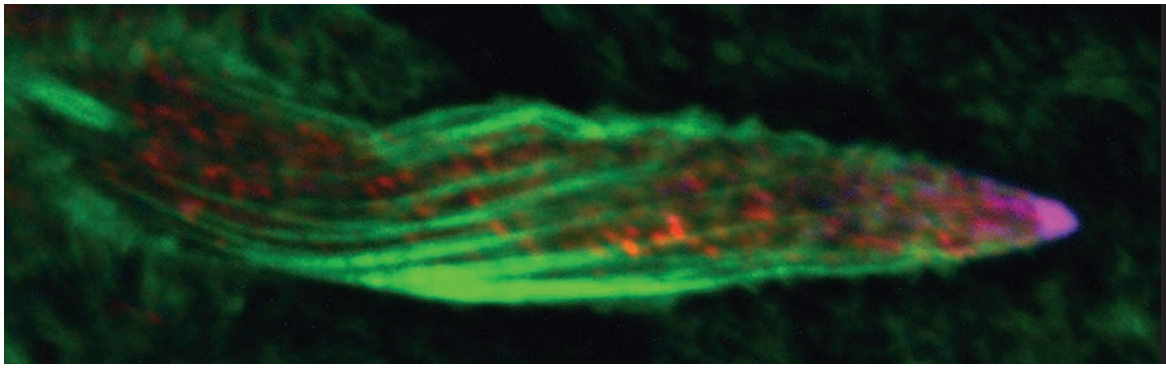
Insights into molecular transport and sorting systems in elongating cells may help prevent problems when things go wrong

Through studying the cells of flies, RIKEN researchers have clarified the mechanisms by which cell components are transported in one direction during cell elongation[1]. These findings are expected to have wide relevance in biology and medicine.

'Polarized' cell elongation, in which differences between opposite ends or 'poles' of a cell are set up and maintained, is a crucial stage in the development of many cells and organisms. This stage requires the coordinated transport and selective retention of proteins and other molecules, but the molecular mechanisms underpinning these processes are poorly understood. Unraveling these processes is important because disruption of the transport system within cells can cause various diseases, including some involving the degeneration of nerve cells.

Now, Tetsuhisa Otani and his colleagues at the RIKEN Center for Developmental Biology, along with Uri Abdu at Ben-Gurion University in Israel, studied the elongation of the surface bristles of *Drosophila* flies to gain insights that have broad application. "Bristle cells are an excellent model system for understanding polarized cellular organization," Otani explains, "and what we learn from this system might be extendable to other polarized cell types."

A protein called IKKε regulates the organization of the bristle tips, but until now it was not clear how this protein became localized at the end of bristle cells and how it was kept there. Otani and his colleagues found that a protein called Spindle-F grabs hold of the IKKε protein and anchors it on microtubules, which form a



Spindle-F proteins (stained red) and IKKε proteins (stained blue) are localized toward the tip of *Drosophila* bristle cells.

scaffolding within cells (see image). Spindle-F is itself bound to the 'motor protein' dynein that moves cargo along the microtubules. The Spindle-F acts as an adaptor protein between dynein on the microtubules and the IKKε molecules. The researchers also found that a protein called Jvl regulates the retention of Spindle-F at the bristle tips.

"Our results suggest a basic regulatory principle for dynein-dependent transport," says Otani, "and they have potential implications for understanding how disruption of this transport leads to neurodegenerative diseases."

Identifying the molecules involved and their interactions is only one step toward understanding complex transport processes. An important challenge is to uncover how the Jvl protein regulates the system. Otani considers that the role of the molecular motor protein dynein may hold the key to revealing how cells do not suffer 'lost luggage'—amazingly, all the required components somehow get transported to the correct places during the complex processes of cell development. He and his team intend to explore further the key aspects of how this is achieved. ■

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PHYSICS | PRESS RELEASE

Protons and antiprotons appear to be mirror images

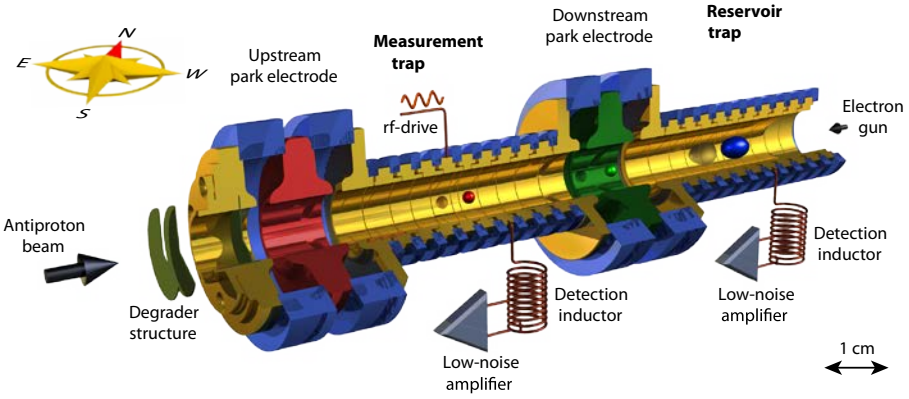
The standard model of particle physics lives to fight another day after surviving an exacting measurement of its applicability

In a stringent test of a fundamental property of the standard model of particle physics, known as CPT symmetry, researchers from the RIKEN-led BASE collaboration at CERN (European Organization for Nuclear Research) have made the most precise measurements yet of the charge-to-mass ratio of protons and their antimatter counterparts, antiprotons[1]. The study was conducted using CERN's Antiproton Decelerator, a facility that provides low-energy antiprotons for antimatter studies.

The experiment tested the principle of CPT invariance. This principle holds if a system remains unchanged on reversing three fundamental properties: charge (C), parity (P; a 180-degree flip in space) and time (T). CPT invariance is a central tenet of the standard model, and it implies that antimatter particles will be perfect mirror images of their matter counterparts, with only their charges reversed.

"This is an important issue," says Stefan Ulmer, who led the research, "because it might help us to understand why we live in a universe that has practically no antimatter, despite the fact that the Big Bang must have created both matter and antimatter. If we had found violations of CPT, matter and antimatter might have different properties—for example, antiprotons might decay faster than protons. But we have found, within quite strict limits, that the charge-to-mass ratios are the same."

The team used a scheme similar to that developed by the TRAP collaboration at CERN in the 1990s. They received antiprotons and negative hydrogen ions—as a proxy for protons—from the Antiproton Decelerator and trapped single antiproton–hydrogen ion pairs in a magnetic Penning trap (see image), decelerating them to ultralow energies. They then measured the cyclotron frequency of the pairs—a measurement that allows scientists to determine the charge-to-mass ratio—and



Schematic of a magnetic Penning trap at the Antiproton Decelerator in CERN (European Organization for Nuclear Research).

compared them to find how similar they were. They measured approximately 6,500 pairs over 35 days.

"We found that the charge-to-mass ratio is identical to within just 69 parts per trillion," says Ulmer. This measurement has four times higher energy resolution than previous measurements of proton–antiproton pairs, and further constrains the possibility of violations of CPT invariance. "Ultimately," he says, "we plan to achieve measurements that are at least ten or a hundred times more precise than the current standard."

According to BASE member Christian Smorra: "There are many reasons to believe in physics beyond the standard model, including the mystery of dark matter and, of course, the imbalance between matter and antimatter. These high-precision measurements put important new constraints and will help us to determine the direction of future research." ■

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BIOLOGY

Not all plant growth hormones are the same

A poorly understood signaling molecule appears to serve a different physiological role from its better-studied cousins

Plant growth is directed by molecules called auxins, which actively change their distribution within a plant in response to environmental cues such as light and gravity. Now, RIKEN researchers have discovered that one member of this hormone family does not undergo such active transport, and may serve a different function to other auxins[1].

Coleoptile (yellow part) in an etiolated young maize plant. The plant growth hormones indole-3-acetic acid (IAA) and phenylacetic acid (PAA) show similar distributions in the coleoptile, but unlike IAA, PAA does not employ active transport to realize this distribution.

The best-known auxin is indole-3-acetic acid (IAA), but there are other related compounds such as phenylacetic acid (PAA), whose roles are much less clear. "The study of IAA, which was identified as an auxin in the 1930s, has its roots in Charles Darwin's phototropism experiments of 1880," explains Hiroyuki Kasahara, from the RIKEN Center for Sustainable Resource Science, who led the study. "But it has been reported that higher plants also produce PAA as an auxin, even though it has relatively low biological activity compared with IAA." Previous studies indicated that, unlike IAA, plant tissues may not actively transport PAA. To resolve this question, Kasahara's team analyzed PAA production and distribution in various plants.

They found that PAA was produced more abundantly than IAA, and that the levels of this auxin varied considerably from tissue to tissue. The researchers also confirmed that PAA seems to activate some of the same growth-related biochemical pathways triggered by IAA, although to a considerably lesser extent. The auxin receptor complexes formed by PAA and IAA did not overlap completely, suggesting that the two auxins may exert different physiological effects, despite activating very similar subsets of genes.

Kasahara's team next examined the auxin distribution within the sheath surrounding maize seedling shoots, a structure known as the coleoptile (see image). While both IAA and PAA showed similar distributions—a gradient with the highest density at the coleoptile tip—only IAA appears to employ an active transport system to achieve this distribution. Likewise, changing the orientation of the maize shoots from vertical to horizontal led to a rapid redistribution of IAA within the coleoptile, whereas the PAA gradient remained largely unchanged.

Kasahara found this result striking. "People have considered polar transport to be a fundamental function of naturally occurring auxin," he says, "but PAA lacks this property."

His team identified some genes responsible for PAA production and found that they partially overlapped with the IAA-generating machinery. However, PAA production was not halted by mutations that thwart IAA synthesis, suggesting that distinct pathways may also be involved. "We are now trying to show a specific role of PAA in plants that might be evolutionarily conserved," says Kasahara. ■

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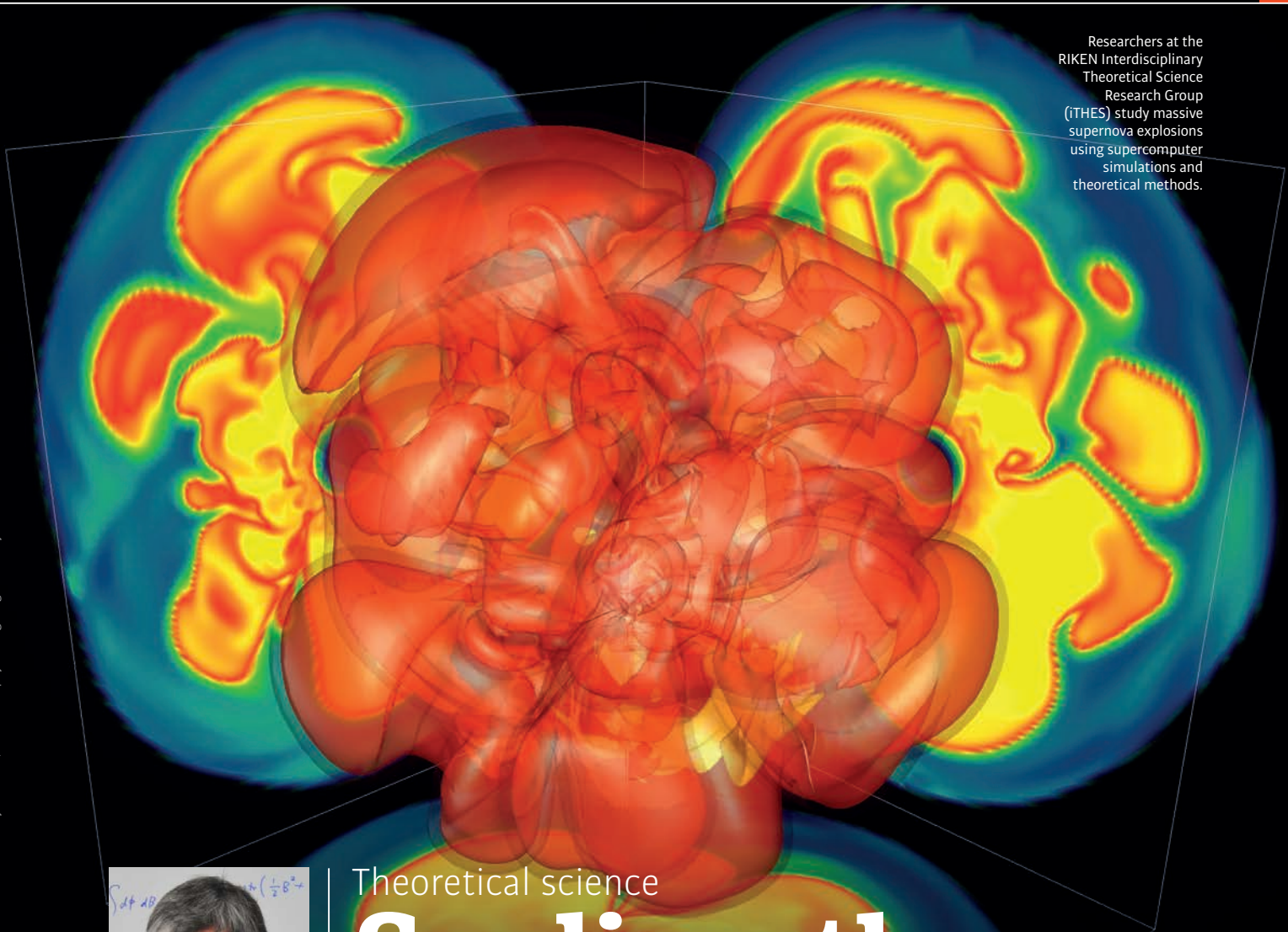
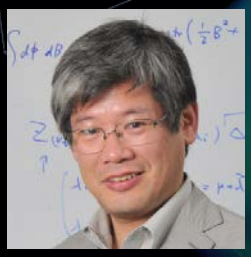


Researchers at the RIKEN Interdisciplinary Theoretical Science Research Group (iTHES) study massive supernova explosions using supercomputer simulations and theoretical methods.



Tetsuo Hatsuda is director of the RIKEN Interdisciplinary Theoretical Science Research Group

Hatsuda received his PhD in 1986 from Kyoto University and was a professor at the University of Tokyo until 2012, before moving to RIKEN. He is also deputy director of the RIKEN Nishina Center for Accelerator-Based Science.

Theoretical science

Scaling the Universe

Some of the most intractable scientific problems involve natural and physical processes that span many scales and disciplines, from atoms to organs, and from quarks to galaxies. Developing coherent theories that can describe these fundamental phenomena will be crucial to understanding the origins of the Universe and life on Earth. Researchers at iTHES are inventing solutions to link the vast with the tiny.

Creating a coherent model of stellar lifecycles is one of the biggest challenges facing science today. The wide range of spatial scales involved makes this very difficult. The RIKEN Interdisciplinary Theoretical Science Research Group (iTHES) was established in 2013 to develop theoretical and computational tools for integrating the many scales and disciplines of science. Ultimately, researchers at iTHES are helping to answer some of the most fundamental questions about the origins of the Universe and life on Earth.

A single supernova, for example, can burn as bright as all the stars in its host galaxy. It reaches temperatures and pressures high enough to synthesize heavy elements, jettisoning them out into the cosmos where they accumulate over billions of years into stars, planets and life as we know it. The age, geometry and components of the Universe can be measured by the brightness of a specific type of supernova that occurs when a white dwarf star explodes. More massive stars instead submit to gravitational forces and collapse into dense neutron stars, or further into black holes, while their outer layers explode as supernovae. These supernovae eject energized neutrinos, most of which will travel through the Universe forever.

From the furthest emission to the smallest elementary particle is a difference of scale many orders of magnitude wide. Scientists have traditionally studied discrete aspects of these physical processes by limiting their investigation to more manageable ranges. Researchers at iTHES, however, are utilizing powerful supercomputers as well as developing theoretical methods to help scientists cross the boundaries between the different scales.

> *Ultimately, researchers at iTHES are helping to answer some of the most fundamental questions about the origins of the Universe and life on Earth.*

Unifying theories

The concept of bringing together many disciplines to create a broader understanding is not a new one. In the early days of the scientific revolution, scholars moved freely across the natural sciences. As the depth of knowledge about the natural world increased, however, distinct disciplines branched off into specialized sub-disciplines.

In the past century, important new theories have brought us closer to the reunification of the sciences. One such idea is the mathematical concept called the renormalization group, which was developed to study macroscopic phenomena emerging from complex microscopic fluctuations. This approach involves breaking down a multiscale problem into a series of more manageable steps, one for each length scale. The strategy was first used in the 1950s to resolve the conflict between quantum field theory, which predicts that electrons have infinite charge, and the finite electron charge measured in experiments. Kenneth Wilson later conducted Nobel-prize-winning work using the renormalization group method to describe the critical point at which a material changes its state between, for example, ferromagnetic and paramagnetic phases. The approach has revealed similarities between many superficially distinct phenomena.

Similar theories are only just beginning to emerge in the biological sciences. Atsushi Mochizuki, who currently heads the Interdisciplinary Theoretical Biology Team at iTHES, introduced a concept called linkage logic to analyze the dynamics of very complex biological networks[1]. Gene expression, for example, involves the interaction of numerous species of DNA, RNA and proteins. To understand how these linkages work, researchers typically conduct experiments in which they manipulate the activity of one or a few molecules and then examine the effects. But regulatory networks are too large and complex to understand solely through approaches based on trial and error. Mochizuki thus developed a mathematical method for stripping the regulatory network down to its essence. Using linkage logic, he was

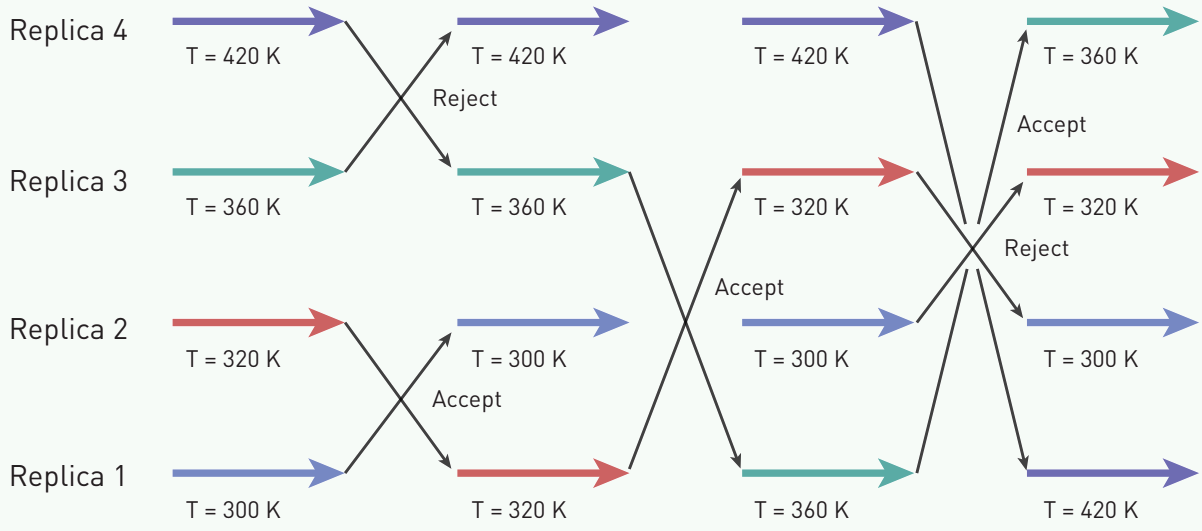


Figure 1: Researchers at iTHES have developed a technique called replica-exchange molecular dynamics that can expand the temperature range of protein simulations using parallel computing systems. The technique involves simultaneously simulating many copies of a molecular system at different temperatures. From time to time, the temperature between neighboring replicas are exchanged and this exchange is either accepted or rejected based on a statistical algorithm.

able to show that only 16 of 76 genes that control embryogenesis in the sea squirt *Ciona intestinalis* are needed to describe the dynamics of the process.

Existing devices that can make objects disappear from sight have a serious and critical problem—they can conceal the wearer from external onlookers, but also blind them to external onlookers.

Protein folding and invisibility cloaks

Every team at iTHES is developing, expanding and applying concepts like linkage logic that transcend different scales and help to explain complex phenomena.

The iTHES Interdisciplinary Condensed Matter Physics Team led by Franco Nori, for example, is studying quantum information processing devices that could be used to solve difficult problems in various areas of physics. Simulating the spin states of just 40 particles would require more computer memory than was used to store all of the information produced by humanity in 2007. Mathematical and statistical techniques like the renormalization group and linkage logic could help to curtail the exponential explosion of operations required to simulate such quantum systems, but only to a marginal degree. Quantum simulators and quantum computers, on the other hand, would be able to store these large amounts of information in a relatively small amount of physical space. Nori's team is studying various aspects of quantum nanoelectronics and information processing for next-generation computing systems.

Yuji Sugita, whose laboratory is a member of the Interdisciplinary Mathematical and Computational Collaboration Team at iTHES, is developing a method called replica-exchange molecular dynamics that can expand the temperature range of a target molecule's accessible conformational space in simulations.

The technique is especially suitable for use on parallel computing systems such as the K computer and has been used to study protein folding and stability. For this application, the technique allows identical copies of a molecular system such as a peptide protein in solution to be simulated simultaneously, at different temperatures. At specific time intervals, temperatures between two neighboring copies are exchanged based on the statistical algorithm known as the Metropolis criterion (Fig. 1). Adaptations of the original method have also been developed to exchange other parameters like pressure and surface tension. Sugita and a team of RIKEN researchers recently incorporated the method into a new molecular dynamics software called GENESIS, which was used to simulate the cytoplasm of the bacterium *Mycoplasma genitalium* at a resolution of more than 100,000,000 atoms.

Masato Taki, a particle physicist at the Interdisciplinary Fundamental Physics Team, iTHES, has collaborated with an engineer at Tokyo Institute of Technology to develop a mathematical theory for an invisibility cloak[2]. Existing devices that can make objects disappear from sight have a serious and critical problem—they can conceal the wearer from external onlookers, but also blind them to external onlookers. Magical cloaks like those worn by Harry Potter, however, allow for asymmetric visibility. To achieve this, Taki and his collaborators propose bending photons using an optical resonator lattice—a system that they have managed to successfully simulate. They are currently focused on engineering a metamaterial that can achieve this in real life.

From quarks to fish

Collaboration is crucial to the success of iTHES. And one of the best ways to encourage multidisciplinary collaboration is to create an environment that facilitates genuine and consistent engagement, especially among younger scientists.

Senior iTHES researchers are encouraged to step outside their comfort zones as an example to their younger colleagues. The group hosts a weekly Friday coffee meeting and organizes regular seminars, where academic and industry leaders are invited to speak about drug design, artificial intelligence, autonomous cars and Google's translating technology. iTHES hosts joint symposiums and workshops with institutes that share common research interests like the Simons Centre for the Study of Living Machines in India and the Core for Theoretical Science Research at Osaka University. The group is also in the process of formalizing agreements for cross-appointments with institutes abroad.

Returns on these investments in the form of cross-pollination of ideas are already being felt. Takashi Okada, a young postdoc with a background in particle physics, recently joined Mochizuki's theoretical biology laboratory to develop a mathematical theorem for identifying the response patterns of chemical reaction networks to perturbations of reaction enzymes. And particle theorist Noriaki Ogawa at the RIKEN Quantum Hadron Physics Laboratory splits his time between biology and physics. He is currently working with a diverse team to describe the regular arrangement and pattern formation of cells in fish retina that allows them to see red, blue, green and ultraviolet light. And finally, postdoctoral researcher Yuji Sakai, who used to spend his time theorizing about quarks and gluons is now fully committed to theoretical biology in Mochizuki's laboratory. He is trying to uncover the mystery of how a tightly coiled string of DNA, two meters long, peels apart to form identical copies without getting entangled. Sakai hopes to model this fundamental, yet routine, biological process of replication.

The Universe holds vast secrets that can only be answered by free and curious minds. iTHES hopes to help researchers transcend scientific boundaries to answer the big questions about the cosmos and human existence.

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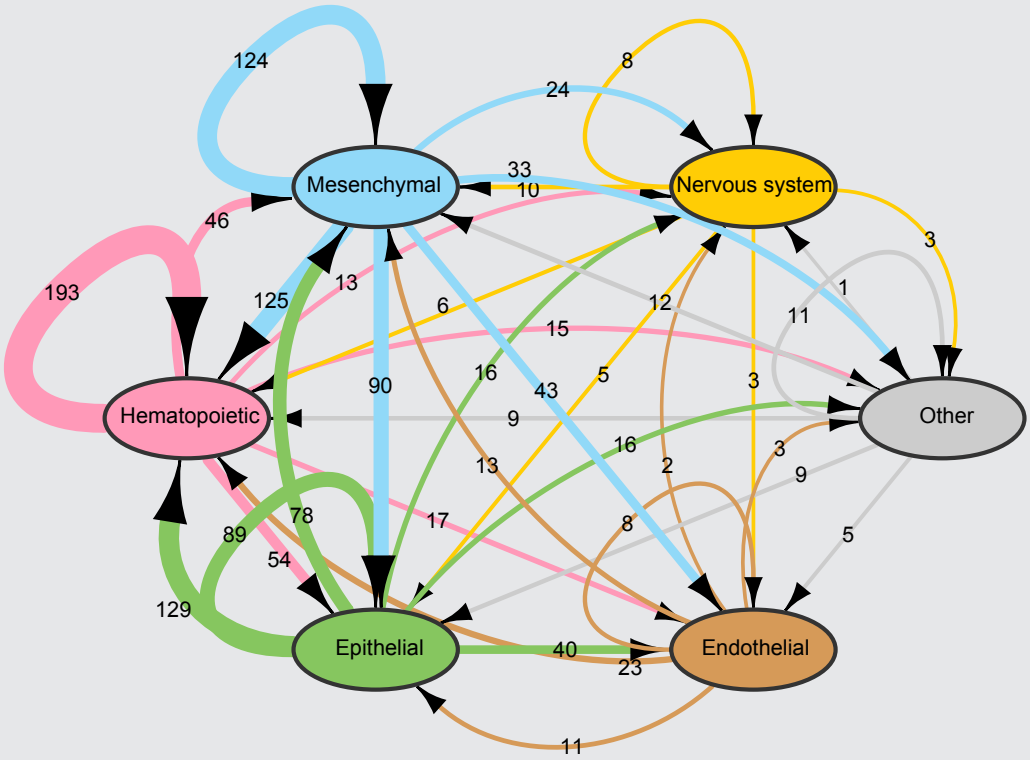

A schematic of a ligand–receptor network depicting how cells of different biological functions and origins (lineages) interact with each other.

BIOLOGY | PRESS RELEASE

Mapping cellular communication

Researchers map out how cells in the human body communicate with each other

In their struggle to survive and prosper, multicellular organisms rely on a complex cellular communication network. Now, an overall map explaining how the 40 trillion or so cells in the human body communicate with each other has been published by a team led by RIKEN scientists (see image)[1]. The team produced the map by systematically analyzing the relationships between ligands—substances such as insulin and interferon that embody messages between cells—and receptors—proteins on cell surfaces that receive these messages when bound by ligands.

The development of multicellular organisms from unicellular ancestors is one of the most profound evolutionary events. Intercellular communication coordinates activities between multiple cell types and facilitates organism-wide processes such as immune response, growth and homeostasis. Defects in cell-to-cell communication, including the dysregulation of autocrine signaling, are implied in the development of cancer, and autoimmune and metabolic diseases.

Despite the importance of this process, studies of intercellular communication between specialized cells in higher organisms such as mammals have generally focused on communication between just a few cell types and have been limited to small numbers of ligand–receptor pairs.

To address this, the team looked at gene-expression data measured by the cap analysis of gene expression (CAGE) method in

the RIKEN-led FANTOM5 project, using information from existing databases and many past publications, to generate the first large-scale draft map of primary interactions between cells.

The group, led by researchers from the RIKEN Center for Life Science Technologies, examined the expression of the 1,894 ligand–receptor pairs (based on 642 ligands and 589 receptors) that have been reported in the literature so far. They then drew up a large-scale map of cell-to-cell communication between 144 primary human cell types. They found that most cells express tens or even hundreds of ligands and receptors, creating a highly connected signaling network made up of cell types that can communicate with each other through multiple ligand–receptor paths.

This analysis provided new insights into how cells communicate. "One intriguing conclusion is that signals between cells of the same type are surprisingly common, accounting for approximately two-thirds of cell–cell partners," says Jordan Ramilowski, the first author of the study. "We also discovered that receptors generally seem to have evolved earlier than ligands."

"Elucidating how cells communicate in an organism can contribute to the development of medical treatments," explains Alistair Forrest, who led the project. "In particular, this data can be a key to discovering receptors that can be targets for therapies in various diseases." ■

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PHYSICS

Materials enter a new phase

Building materials one atomic layer at a time aids the search for exotic phases of matter

A new technique for identifying exotic states of matter in crystalline materials has been demonstrated by RIKEN researchers.

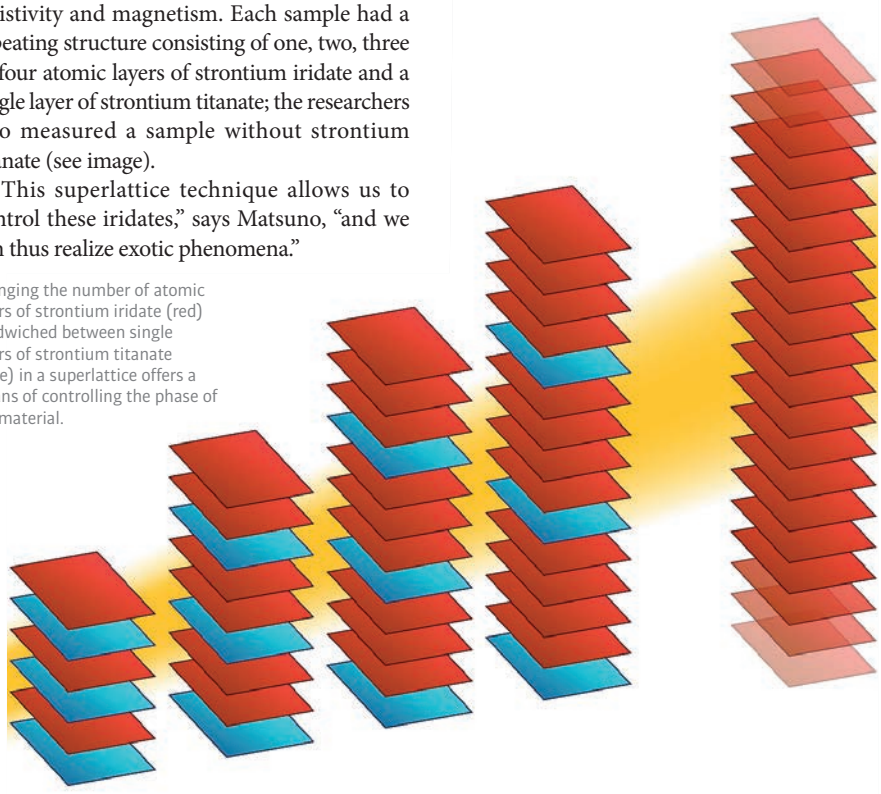
The electrons in some crystalline materials work together to create a host of unusual states, or phases. This collective behavior in these so-called correlated electron materials occurs because the electrons interact with each other via an intrinsic property known as spin, which is related to the rotation of the electrons about their axes. Although correlated electron materials have attracted much interest in the past decade, it has proved difficult to identify the exact mechanisms that give rise to specific phases and to determine what drives a material to switch from one phase to another.

Now, Jobu Matsuno from the RIKEN Advanced Science Institute and his colleagues have investigated these mechanisms in a class of materials called iridates[1]. These materials are interesting because their behavior is predominantly governed by two effects that are roughly equal in magnitude: the repulsive Coulomb force between electrons arising from their electric charge and the spin–orbit interaction, which arises because the spin of an electron interacts with its orbital motion. Theoretical analysis indicates that competition or cooperation between these two effects gives rise to a number of exotic phases in iridates.

To explore the influence of the spin–orbit interaction on the formation of these phases, Matsuno and his team created a series of iridate-based samples and measured their resistivity and magnetism. Each sample had a repeating structure consisting of one, two, three or four atomic layers of strontium iridate and a single layer of strontium titanate; the researchers also measured a sample without strontium titanate (see image).

"This superlattice technique allows us to control these iridates," says Matsuno, "and we can thus realize exotic phenomena."

The scientists found that the magnetic ordering temperature and the resistivity decreased with increasing number of strontium iridate layers. They also discovered that, in



Changing the number of atomic layers of strontium iridate (red) sandwiched between single layers of strontium titanate (blue) in a superlattice offers a means of controlling the phase of the material.

the sample containing three atomic layers of strontium iridate, a transition from a semimetal phase to an insulating state was closely linked to the appearance of magnetism.

These results indicate the potential for finding unusual states of matter using the superlattice approach. The team hopes to use the method to identify a phase known as a topological insulator—a recently discovered class of materials that usually have a large spin–orbit interaction. "Theorists say that some iridates might host an even more exotic state called a topological Mott insulator," Matsuno notes. ■

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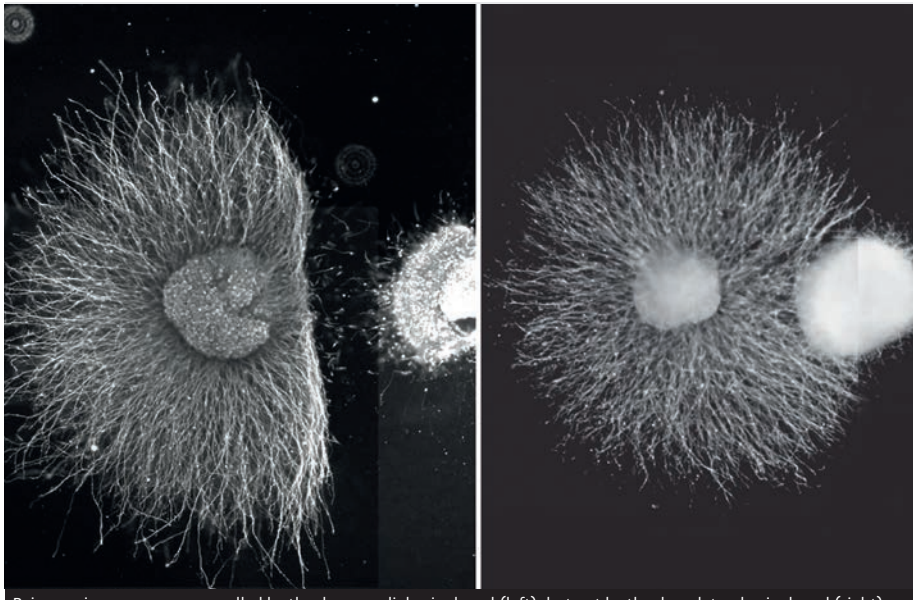
BIOLOGY | PRESS RELEASE

Lipids direct neuron organization in the spinal cord

Lipids released by neuron-supporting cells guide the arrangement of neurons in the spinal cord

Healing spinal cord damage is incredibly difficult because of the precise way in which neurons need to be reconnected. Now, RIKEN scientists have discovered that in addition to proteins, lipids are also necessary for guiding the long extensions of neurons known as axons. In particular, they discovered how a phospholipid released by cells in the nervous system that support neurons controls the positioning of sensory neurons within the spinal cord[1].

Axons are the roads that allow neural information to travel. During development, axon growth is guided by a patterned distribution of molecules that attract or repel them, forcing them to go in the proper direction.



Pain-sensing neurons are repelled by the dorsomedial spinal cord (left), but not by the dorsolateral spinal cord (right).

"While many proteins are known to direct axon growth and network formation," says Hiroyuki Kamiguchi of the RIKEN Brain Science Institute, "we discovered that glial cells have the ability to release membrane structural lipids in specific patterns that can then control axon migration and neuron organization." Specifically, the researchers found that a lipid called *lyso*-phosphatidyl-β-D-glucosideLysoPtdGlc (LysoPtdGlc) plays a major role in separating axons of pain- and position-sensing neurons.

Before reaching our brains, sensory information from our skin and muscles first goes to the spinal cord. Axons carrying this information enter the spinal cord together but soon go their separate ways: those responsible for feelings of pain travel along the side of the spinal cord, whereas those that let us know where our muscles are propagate closer to the midline.

When researchers labeled spinal cord sections from chicks with markers for LysoPtdGlc and the two different types of sensory neurons, they found that LysoPtdGlc was located only near the midline region where position-sensing axons are located. This led the team to hypothesize that when axons of pain-sensing neurons encounter LysoPtdGlc, they are repulsed from this midline area and forced to travel in the more lateral region of the spinal cord.

To test this, they looked at how cultured pain-sensing neurons responded to LysoPtdGlc

and found that concentration gradients of LysoPtdGlc repelled axons from the pain-sensing neurons (see image). This function of LysoPtdGlc was confirmed when blocking access to the lipid with an experimental antibody prevented pain-sensing neurons from being repelled.

The researchers then injected the antibody into the spinal cord of chick embryos. Their hypothesis proved to be correct: axons of pain-sensing neurons were no longer repelled, but instead migrated into the region in the spinal cord reserved for position-sensitive neurons.

"With these findings, we can begin to investigate whether this lipid-based signaling system can be a therapeutic target for spinal cord injury," says Kamiguchi. ■

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PHYSICS

Finding new magic

Nuclear physics experiments support the existence of a new, stable 'magic' number of atomic neutrons in a relatively unexplored region of exotic nuclei

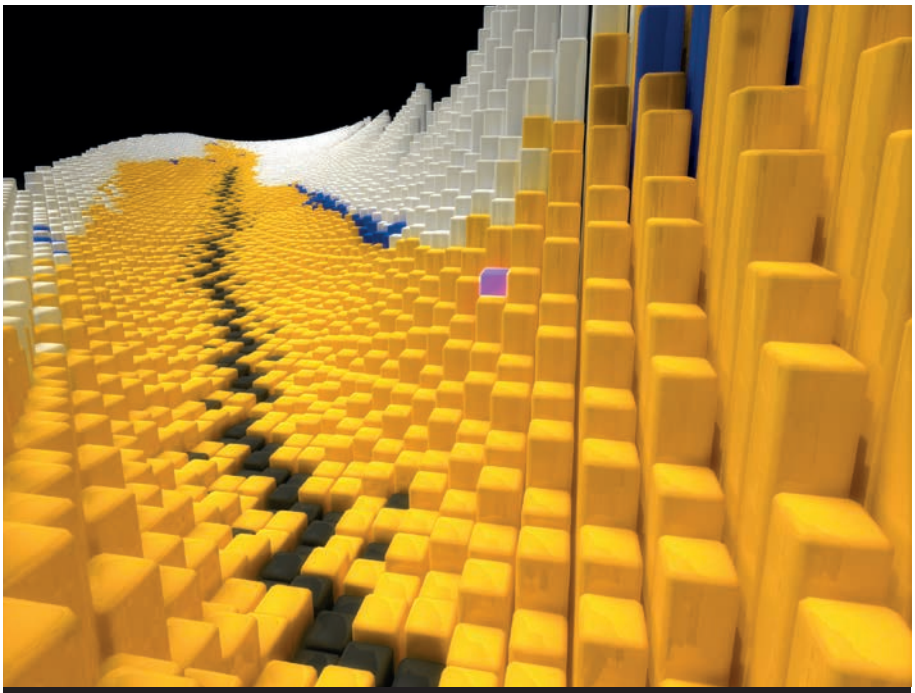
The unique capabilities of RIKEN's Radioactive Isotope Beam Factory (RIBF) have made it possible to verify that the isotope of argon with 32 neutrons is sufficiently stable to be considered 'magic'[1]. This finding supports the existence of a new magic neutron number of 32 for elements in the sub-20 atomic number range and demonstrates that there is much to explore in this region of light exotic nuclei.

The stability of a nucleus is determined by how many protons and neutrons it contains. Most naturally occurring light atoms have roughly equivalent numbers of protons and neutrons and are relatively stable. When there is a large imbalance in these numbers, the nucleus will be unstable and will exist for only a limited time before undergoing radioactive decay.

Isotopes with certain magic numbers of protons or neutrons are unusually stable, which physicists attribute to the complete filling or 'closure' of energy levels called nuclear shells. The set of standard magic numbers in long-lived nuclei—2, 8, 20, 28, 50, 82 and 126—is well established as an explanation for many observations in nature. However, things become more complicated for exotic isotopes that can be produced using particle accelerators.

"The evolution of nuclear shell structure in exotic nuclei is one of the major current topics in experimental and theoretical nuclear physics," explains David Steppenbeck, who led the research team. "A new neutron magic number of 32 has been reported in calcium and titanium, but this subshell closure has remained unexplored in very exotic nuclei such as argon."

Steppenbeck and his team, which included researchers from the RIKEN Nishina Center, the University of Tokyo and other institutions in Japan and Italy, studied in detail the isotope argon-50, which has 32 neutrons and 18 protons. Such a study is challenging because of the difficulty in generating sufficiently large numbers of these nuclei. "The unique intensities of the radioactive beams provided by the RIBF were essential to the current study," says Steppenbeck. "Although other facilities can produce argon-50 ions, their production rates are too low to perform a study like ours in realistic time frames."



A map showing the natural and exotic nuclei and their stabilities. The isotope argon-50 (pink), which has 32 neutrons and 18 protons, has been shown to contain a magic number of neutrons.

The team's results firmly establish the persistence of the neutron magic number 32 in systems with fewer than 20 protons (see image).

"The behavior of these neutron subshell closures in nuclei even farther from stability, such as sulfur and silicon, is also of great interest," notes Steppenbeck, "although investigating this exotic nuclear region will require further development of our current experimental tools." ■

Reference

1. Steppenbeck, D., Takeuchi, S., Aoi, N., Doornenbal, P., Matsushita, M., Wang, H., Utsuno, Y., Baba, H., Go, S., Lee, J. *et al.* Low-lying structure of ^{50}Ar and the $N = 32$ subshell closure. *Physical Review* Letters **114,** 252501 (2015).

BIOLOGY | PRESS RELEASE

Turning off the infanticide instinct

Two brain regions are shown to be responsible for determining whether a male mouse will attack or care for mice pups

Many bachelor mammals, including lions, mountain gorillas, and mice, attack and kill the offspring of other males—a form of infanticide—yet display parental behavior once they themselves become fathers, even if the offspring are not their own. Now, RIKEN researchers have discovered two small brain regions that control which of these very opposite behaviors a male mouse will exhibit.

Infanticide in the animal kingdom can often be attributed to an innate male reproductive strategy—by eliminating newborn pups fathered by other males, a virgin male mouse will free up a female for his own mating attempts. After mating and living with a pregnant female, a male mouse will switch behavior and begin taking care of the offspring—even if they are not his. To investigate how this switch occurs, the scientists examined brain activation patterns induced by parenting and infanticide.

They exposed adult male mice to mouse pups and recorded their pup-directed behavior—paternal or infanticidal. The researchers then measured the level of c-Fos—an indicator of recent neuronal activity—in nine forebrain regions to find out if activity in these brain regions was related to the behaviors. They found that c-Fos expression in the central part of the medial preoptic area (cMPOA) was associated with paternal behavior, whereas expression in the rhomboid nucleus of the bed nucleus of the stria terminalis (BSTrh) was associated with infanticidal behavior. In fact, past behavior could be determined simply by looking at the c-Fos expression patterns.

"We were surprised at how accurately c-Fos expression patterns in the cMPOA-BSTrh area could categorize these social behaviors," says Kumi Kuroda at the RIKEN Brain Science Institute. "Amazingly, retroactive classification was 95–97 per cent accurate."

The team then tested whether the cMPOA and BSTrh are necessary for the two behaviors. They found that lesioning the BSTrh inhibited infanticidal behavior in virgin males, whereas lesioning the cMPOA abolished parental behavior and actually induced fathers to attack newborn pups.

The team also conducted tests using wire mesh to prevent infanticide or parental acts. "c-Fos expression patterns in these regions were just as accurate at predicting attempted behaviors," explains Kuroda. "Along with the lesion results, this indicates that the cMPOA and BSTrh are responsible for the urge, or drive, behind these very different social acts."

Bachelor mountain gorillas will attack baby gorillas, whereas those that have been fathers will nurture them. The two brain regions responsible for these very different behaviors have been discovered in mice.

"We think that social experience with a female must lead to paternal behavior through enhanced activity in the cMPOA, but we need to determine how this happens," says Kuroda. "We also want to investigate the function of these brain regions in primates, and are currently doing so in common marmosets." ■

Reference

1. Tsuneoka, Y., Tokita, K., Yoshihara, C., Amano, T., Esposito, G., Huang, A. J., Yu, L. M. Y., Odaka, Y., Shinozuka, K., McHugh, T. J. & Kuroda, K. O. Distinct preoptic-BST nuclei dissociate paternal and infanticidal behavior in mice. *The EMBO Journal* **34,** 2652–2670 (2015).

BIOLOGY

Symbiotic bacterium in termite gut pinned down

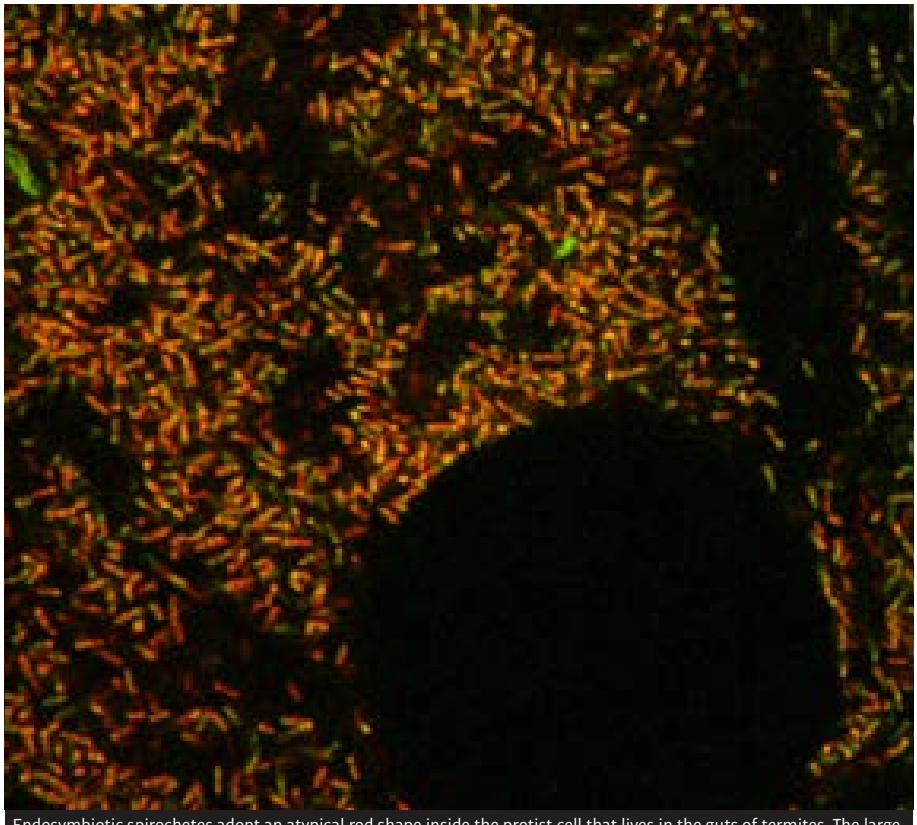
Researchers identify the bacterium that generates nutrients and energy for wood-feeding termites

The bacterial species responsible for producing energy and nutrients that fuel wood-feeding termites has been pinpointed by a research team led by RIKEN scientists[1].

Termites thrive on a diet of wood thanks to a remarkable biological coexistence involving two other organisms. A symbiotic protist that lives in the guts of these wood-eating insects breaks down cellulose found in plant cell walls. And inside each protist lives beneficial bacteria that assist the metabolic process.

"This symbiotic relationship makes efficient use of the abilities of both the bacteria and the protist and it substantially benefits the carbon, nitrogen and energy metabolism of the host termite," says Moriya Ohkuma, head of the microbe division at the RIKEN BioResource Center in Tsukuba.

Ohkuma and his colleagues discovered that the bacteria in this three-way symbiosis play two key roles: they transform the by-products of the protist's breakdown of cellulose into an energy source that termites can use, and they take nitrogen from the atmosphere and turn it into a nutrient. Previously, it was not known which bacteria were behind these functions nor the extent to which they contributed to the activities of the whole gut.



Endosymbiotic spirochetes adopt an atypical rod shape inside the protist cell that lives in the guts of termites. The large dark region is the nucleus of the protist cell.

The team measured the activities of these two key roles with careful fractionations of gut microbes. This enabled them to identify one bacterial species inside the protist's cells as the main contributor to gut activities; this species belonged to the spirochetes. Since culturing the microbes was not an option, the scientists did the next best thing: they sequenced all the genes from this bacterium.

The researchers discovered an unexpected 'twist'. With very few exceptions, spirochete species typically have spiral morphologies, but this one had a simple rod shape (see image). Furthermore, it lacked flagella, which are necessary for maintaining a spiral shape.

This microbe was responsible for most of the beneficial energy and nutrition production. The researchers analyzed the draft genome of this species and discovered several genes that explained its metabolic abilities.

This spirochete is closely related to other spirochetes found on the cell surface of the same protist in the termite gut. Ohkuma suspects that the inside species (the so-called endosymbiont) probably descended from its outside cousins.

He plans to compare the functions and genomes of these two close relatives to determine whether the energy and nutrient roles of the endosymbiont were newly acquired after the microbe colonized the protists' innards or whether these were ancient abilities. Further studies will shed light on how the genome has started to change during the evolution of symbiosis. ■

Reference

1. Ohkuma, M., Noda, S., Hattori, S., Iida, T., Yuki, M., Starns, D., Inoue, J.-i., Darby, A. C. & Hongoh, Y. Acetogenesis from H_2 plus CO_2 and nitrogen fixation by an endosymbiotic spirochete of a termite-gut cellulolytic protist. *Proceedings of the National Academy of Sciences USA* **112,** 10224–10230 (2015).

The power of hornet juice

RIKEN researchers turned an amino acid mixture produced by giant hornets into a commercially successful sports drink that improves athletic endurance and fat metabolism

Hornets travel long distances in search of insects to feed their larvae.

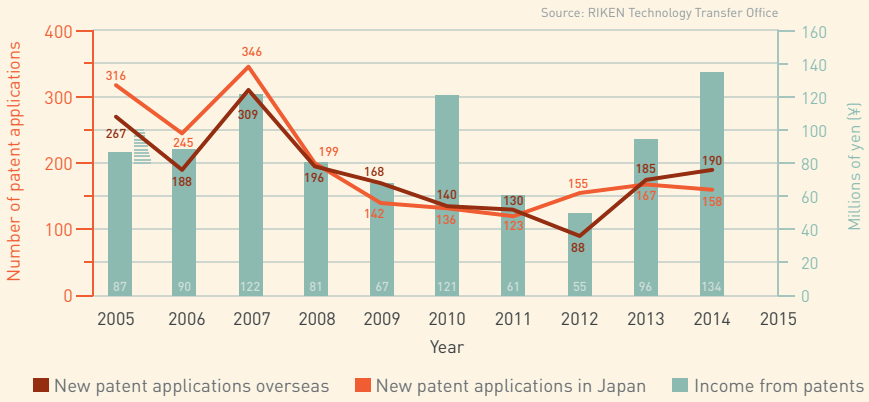
Takashi Abe was out hunting for giant hornets in the forests of Nagoya when he felt a venomous sting. Immediately, he called an ambulance that rushed him to the nearest hospital. Within minutes, his blood pressure had dropped dangerously low and he lost the strength to move his hands and legs. "All I could do was roll my eyes," recalls the RIKEN insect toxicologist. This terrifying experience in 1981 would lead him on a lifelong journey to invent a sports drink that is now consumed by millions of people a year, among them gold-medal Olympians.

Spit and stamina

The day after recovering from the sting, Abe decided to continue his research on the hornets (*Vespa mandarinia*) by studying their behavior more closely. Their feeding habits were particularly intriguing. Adult hornets fly almost 100 kilometers a day in pursuit of insects to feed their developing young. They grind fresh prey into tiny meatballs and exchange it with their larvae for saliva, which is the only source of food for the adult workers, whose narrow waist and short gut restricts their diet to liquids.

Abe wondered what was in the translucent spit that gave the adult hornets the energy to travel such distances. He tested saliva samples secreted by nesting larvae and found they contained 17 different amino acids. "It was a special composition, very different from the amino acids consumed as proteins in milk, meat or fish," he explains. He named the amino acid concoction '*Vespa* amino acid mixture', or '*V*AAM' for short, and filed the first of many patents in Japan and abroad.

Abe found that *V*AAM helped mice swim in a



turbulent pool. Mice fed *V*AAM swam for significantly longer than those fed either water, glucose or a different amino acid mixture. The same mice also had less lactate and more glucose in their bloodstreams, a finding that established the role of glucose homeostasis in endurance exercise.

The low lactate levels suggested that *V*AAM sped up fatty acid metabolism, one of two sources of the energy required for muscle activity. Abe repeated the swimming mouse experiment, this time testing for free fatty acids in the bloodstream and found that *V*AAM also accelerated fat oxidation. Over the years, he would conduct many more studies to characterize the exact metabolic effect of *V*AAM and its efficacy for reducing fatigue. "Amino acids are typically thought of as nutrients," he says, "but *V*AAM pointed to a different role for amino acids as transmitters of information to the body."

*V*AAM in a can

By the early 1990s, many major companies showed interest in commercializing *V*AAM as a sports drink for athletes. But problems with flavor and financial feasibility persisted. "Amino acids have a very bitter taste, like raw fish," explains Abe. Furthermore, except a select few amino acids like glycine, glutamic acid and lysine produced in industrial quantities and used in animal feed and food additives, many *V*AAM components were not available in large quantities.

A felicitous partnership with the food company Meiji Dairies and biotech giant Kyowa Hakko eventually resolved these problems. Kyowa developed efficient techniques for fermenting many more amino acids en masse and extracting others from bird feathers. Larval saliva contains a lot of trehalose, a natural sugar, so Abe decided to introduce the same sweetener to *V*AAM, which significantly improved its taste. More good news came when a Japanese company succeeded in cheaply manufacturing trehalose from starch.

> *Abe wondered what was in the translucent spit that gave the adult hornets the energy to travel such distances.*

The first product named VAAM (without the italicized 'V') was launched in Japan in September 1995. It contained 3 grams of the amino acid mixture diluted in a small silver can with a bold-colored logo. The brand became so successful that Meiji Dairies released a whole range of sister products, from VAAM Powder to VAAM Jelly Diet Special and Super VAAM. VAAM was everywhere—in drugstores, health food shops and convenience stores. Even Olympic athletes were drinking VAAM, culminating in the long-distance runner Naoko Takahashi's gold-medal win at the 2000 Sydney Olympics.

Success story

VAAM is one of several commercially successful products that have originated from RIKEN, including the detergent Attack that contains an enzyme discovered at the institution, says Ryosuke Furubayashi, deputy director of the Collaborations Division and head of the Technology Transfer Office that secures patent rights from RIKEN's research activities and transfers them to industry, as well as providing support and information for researchers about the demands of industry.

"Compared to ten years ago, RIKEN today is more focused on investing resources in inventions that are attractive to industry," he says. This means that while the number of patents filed has plateaued over the years, the amount of money RIKEN earns from these patents is increasing. Collaborations with industry have also matured to include the establishment of long-term joint research centers or laboratories customized to the needs of a specific company.

More than thirty years since his near-death experience, Abe is still trying to understand the unique properties of *V*AAM. Most recently, he has characterized its effect on gene expression. An intervention study on sedentary women above the age of sixty by researchers at the University of Tsukuba in 2008 suggests that VAAM could also benefit non-athletes and the elderly. Abe offers himself as an example of one of VAAM's most loyal consumers: "You should drink it too," he says. ■

Researchers from the Measurement Information Laboratory, Innovation Center, RIKEN Cluster for Industry Partnerships and the UEL Corporation have jointly developed a polygon editing software called POLYGONALmeister®.

Collaborations with industry

RIKEN's profound commitment to working with industry is realized in many ways, through its joint and sponsored laboratories, commissioned research, trainee internships and shared facilities, among others. It is most directly exemplified through its Baton Zone Programs, in which representatives of science and business work together.

These programs involve handing over knowledge from one partner to another. The process is managed by a dedicated group called the RIKEN Innovation Center, whose job it is to support the transfer of RIKEN's scientific achievements into commercial products through partnerships with private companies.

In the past year, collaborations between RIKEN and industry have led to the development of various products, including the sports drink VAAM (see the "Impact" article on page 34) and the polygon editing software POLYGONALmeister® (see image). Another important part of RIKEN's collaborations is the RIKEN Venture system, which enables researchers to commercialize new knowledge and technologies that emerge during the course of conducting basic research at RIKEN into products that benefit industry and people's everyday lives.

Technology transfer

New drugs, green technology, simple and efficient medical diagnoses, improved decorative and crop plants—these are only a few examples of the kinds of products developed at the RIKEN Innovation Centers, primarily based at the RIKEN Cluster for Industry Partnerships (CIP). Laboratories at the CIP work closely with industry to ensure that their research satisfies industry requirements and can be translated quickly into useful products.

The CIP includes two research programs for industry and society: the RIKEN Program for Drug Discovery and Medical Technology Platforms and the RIKEN Preventive Medicine and Diagnosis Innovation Program, both of which contribute to health research.

Shin-ichiro Fujii (left), a project leader at the RIKEN Program for Drug Discovery and Medical Technology Platforms, is working on cancer immunotherapy.

For more information, please visit:

RIKEN Cluster for Industry Partnerships: ***www.riken.jp/en/research/labs/rci***
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