WINTER 2016

RIKEN RESEARCH

SHOWCASING THE BEST OF JAPAN'S PREMIER RESEARCH ORGANIZATION
www.riken.jp/en/research/rikenresearch

LORD OF THE RINGS

A spinning disk 100 times bigger than Saturn's

A LIGHT TOUCH
Single photon excites multiple atoms

DEPTHS OF DEPRESSION
World-first scan reveals where the blues are born

BREAKING WITCHWEED'S SPELL
Saving plants from parasitic invasion

ALL-TIME HIGH
Paired clocks measure elevation with precision

RIKEN Nishina Center for Accelerator-Based Science

The RIKEN Ring Cyclotron, the oldest ring cyclotron at the RI Beam Factory, is used for nuclear physics experiments as well as to generate plant mutants.

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.

RIKEN Research is an online and print publication that highlights the best research published by RIKEN. This publication is a selection of the articles published by RIKEN at: www.riken.jp/en/research/rikenresearch
Please visit the website for recent updates and related articles. Articles showcase RIKEN's groundbreaking results and are written for a non-specialist audience.

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

RIKEN International Affairs Division
2-1, Hirosawa, Wako, Saitama,
351-0198, Japan
Tel: +81 48 462 1225
Fax: +81 48 463 3687
E-mail: rikenresearch@riken.jp

ISSN 1883-3519

RIKEN Research is published by RIKEN in collaboration with the Partnership and Custom Media unit of Nature Research, a division of Nature Japan KK.

RIKEN RESEARCH

RIKEN

www.riken.jp/en

3 **Editorial**

Competition and collaboration

4 **People**

Cooking chemical elements

Sarah Naimi
Spin Isospin Laboratory

Miniaturizing experiments

Yaxiaer Yalikun
Laboratory for Integrated Biodevice

6 **Briefs**

This season's memorable moments and milestones

Stem cell transplant / Italian researcher represents Japan / Time for business / G7 ministers visit RIKEN / New element nihonium / Life science symposium / RIKEN's new status / Strasbourg collaboration

10 **Research highlights**

10 A strange turn of events

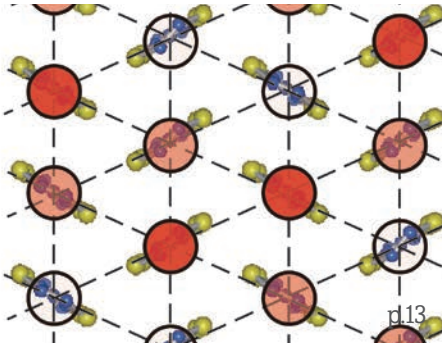
11 An unexpected autoimmune connection

12 Pinning down parasitic plants

13 'Snap freezing' produces different state

14 Split memories

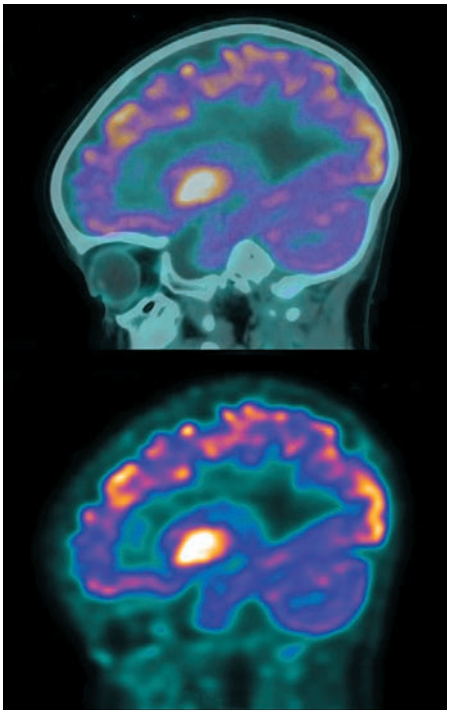
15 A protein pushes ahead of the curve



p.13

16 **Feature highlight**

Imaging the birth of new brain cells



19 Research highlights

19 Don't I know you from somewhere?

20 The search for disorder in order

21 Unleashing the potential of Chinese licorice

22 Single photon excites two atoms at once

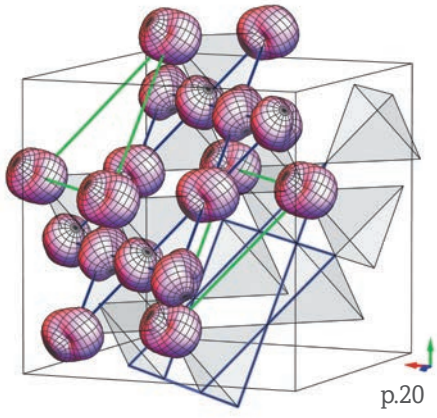
23 Probing energy transfer using light

24 Molecules behave differently in a crowd

25 The cosmic consequences of imperfection

26 Perspectives

Meeting our resident gut microbes



p.20

p.24

29 Research highlights

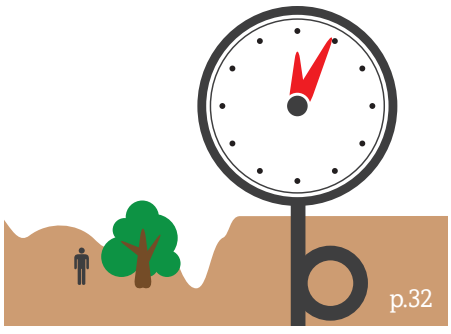
29 Illuminating plants' response to light

30 Fluorine offers solar power boost

31 New drug tackles cancer at the stem

32 The height of accuracy

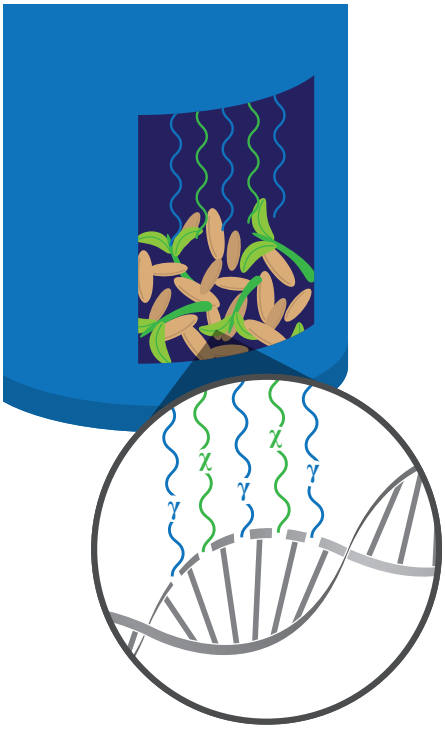
33 Sending bone growth down the spine



p.32

34 Impact

Delightful mutants



Competition and collaboration

Yoichiro Matsumoto
Executive Director, RIKEN

The Japanese government granted RIKEN the special status of Designated National Research and Development Institute on 1 October 2016. Under this new classification, RIKEN President Hiroshi Matsumoto has pledged to turn RIKEN into a global research partner. "RIKEN will strive to make dramatic breakthroughs in an environment of fierce international competition, plant the seeds of innovation, and contribute to solving pressing social problems. RIKEN will also seek to become a central institution exerting strong leadership in Japan's innovation system by forging ties with industry and universities and working with the government to realize its policies."

RIKEN has a long history of collaborating with business and academia. Masatoshi Okochi, our third president who led the institute for 25 years, was a strong proponent of forging internal and external ties. He founded companies to market RIKEN's inventions and launched the chief scientist system to encourage cooperation between researchers.

With humanity facing many threats to its survival, we need collaboration now more than ever. Strategic networks must be built between disciplines and organizations that have become entrenched in narrow, isolated approaches to science. To make this possible, research institutes must become more competitive in areas such as human and institutional resources, research funding, facilities, global openness, clarity of vision and management efficiency. An environment of intense and open competition will foster win–win relationships. But institutions must also be careful to take a long-term, inclusive and sustainable approach to research.

Global agendas such as the Sustainable Development Goals adopted by the United Nations are so complex and intertwined that no individual research institute can tackle them alone. This recognition has led to the establishment of numerous research networks in various fields all over the world. We need to move towards establishing a 'network of networks' that enables the highest level of cooperation among institutes on a global scale. Committed to this goal, RIKEN is transforming into a global hub for science and innovation.

Cover story: A gigantic ring system has been discovered spinning around a star 400 lightyears away.
Page 10

Keep up to date

Online
www.riken.jp/en

Facebook
facebook.com/RIKEN.english

Twitter
twitter.com/RIKEN_en

LinkedIn
linkedin.com/company/riken

Cooking chemical elements

Sarah Naimi

Research Scientist

Spin Isospin Laboratory
RIKEN Nishina Center for Accelerator-Based Science

Please describe your role at RIKEN.
I seek new and creative ways to answer fundamental questions in nuclear astrophysics. An auxiliary goal is to introduce a culture of using mass spectroscopy for nuclear physics research at RIKEN. Japan has only recently begun applying this technique to research in nuclear physics—largely through the initiatives of the RI Beam Factory (RIBF) at RIKEN. I have worked in the area for almost 10 years at large facilities in France, Switzerland and Germany.

Please briefly describe your current research.
I measure the masses of short-lived nuclei. RIBF's Rare-RI Ring—a storage ring at RIKEN for circulating radioactive isotopes—can measure the masses of these exotic nuclei in less than 1 millisecond, which is more than 100 times faster than the time it takes you to blink. Exotic isotopes played a major role in the formation of chemical elements found on Earth today. They were cooked in the most efficient pots in the Universe—the stars. Their properties, especially their masses, are very important ingredients for understanding how they were cooked. In a way, I am trying to understand the taste of life.

How did you become interested in your current field of research?
I always wanted to study astrophysics, but I didn't know how to contribute to this vast field. I first tried astrochemistry, and then I tested theory. Eventually, I landed in a nuclear physics laboratory while at the ISOLDE radioactive isotope beam factory in CERN (European Organization for Nuclear Research), Switzerland. I loved the experience and settled on experimental nuclear physics.

I am amazed by how the smallest bound object—the nucleus—and the energy it carries (which is nothing more than its mass, based on Einstein's famous equation $E = mc^2$) has brought life to the Universe.

As a teenager, I would spend long summer nights gazing at the stars in the clear Algerian sky.

What made you decide to become a scientist?
The stars have always fascinated me. As a teenager, I would spend long summer nights gazing at the stars in the clear Algerian sky. I was convinced that there was something up there related to our origin. Becoming a scientist was the only way to discover the mysteries of our Universe. It was the only thing I wanted to be.

What has been the most interesting discovery in your field in recent years?
The observation of ripples in space–time, known as gravitational waves. The discovery opens up the possibility of observing the gravitational waves produced by massive collisions between compact objects in the Universe, such as neutron stars. Gravitational waves can carry valuable information about the nucleosynthesis and nuclear properties of chemical elements produced during these collisions. Such observations will revolutionize our understanding of nuclear astrophysics.

What is the best thing about working at RIKEN?
RIKEN is a very stimulating environment. It also has very high expectations in terms of research output, which pushes researchers to venture into the unknown. At the same time, we receive a lot of support for our explorations.

How do you balance your personal and professional lives?
I remind myself everyday of my goals: I want to enjoy life while also engaging in bold research. ■

Miniaturizing experiments

Yaxiaer Yalikun

Postdoctoral Researcher

Laboratory for Integrated Biodevice
RIKEN Quantitative Biology Center

Please describe your role at RIKEN.
I mainly support other teams at the RIKEN Quantitative Biology Center by developing microfabrication technologies and microfluidic devices that can be used in biology, chemistry and medicine. I also come up with solutions for enhancing the efficiency of experiments, minimizing the size of conventional experimental systems, and designing mini labs on tiny chips.

Please briefly describe your current research.
I develop technologies for handling, manipulating and stimulating small objects, solutions, and single cells. These technologies enable an entire experimental setup to be placed close to where the samples are prepared, which reduces cost, enables miniaturization and cuts analysis time. Our research requires background knowledge in many disciplines, including chemistry, biochemistry, mechanics and materials engineering.

How did you become interested in your current research field?
I majored in mechanical engineering at university and worked as an electrical engineer in my previous job. I later discovered that all of the technologies I had mastered can be applied to the development of microelectromechanical systems, which can be used to explore fundamental biological phenomena.

What excites you the most about your current research?
I play an indispensable role in the scientific journeys of many research teams. Nothing is more fulfilling than seeing the devices I fabricate being used to observe things we could never see before.

> *Having set a world record once, I now want all of my research endeavors to set new world records.*

What has been one of your most important research achievements?
I established a method using femtosecond-laser processing to fabricate a flexible microfluidic chip, which is only 12 micrometers thick and made entirely out of glass. The method reduces the thickness limitation of fabricating flexible glass devices, which can be used for targeted drug delivery to the brain, implantable medical devices, wearable technologies and high-resolution imaging of small biological objects such as bacteria and proteins. Having set a world record once, I now want all of my research endeavors to set new world records.

How has being at RIKEN helped your research?
The researchers in my laboratory are leading experts in a wide range of fields. I learn something new from them every day and have started to think from different viewpoints.

What is the best thing about working at RIKEN?
I can decide what areas I want to focus on and chase my own dreams. Only in RIKEN it is possible for me to come up with an idea, discuss it with world-class researchers and then immediately get to work on realizing it in state-of-the-art facilities. We even have access to an electron-beam lithography system that can draw 10-nanometer thin lines and patterns on a chip.

What do you wish you had known before coming to RIKEN?
I am originally from Xinjiang Uyghur Autonomous Region in China. I joined RIKEN in May 2015 and in April 2017 will become a special postdoctoral researcher. I just wish I had moved here several years earlier.

Please tell us about your professional goals.
I want to continue to do what I enjoy —offering critical technologies and devices for discovering new and unknown principles in biological systems. ■

Careers at RIKEN

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

Monkeys see eye to eye

Scientists at RIKEN have successfully transplanted cells from one monkey into the eyes of many other monkeys. The method could become a standard treatment for retinal diseases within five years if it proves to be effective in human clinical trials, said researchers at the RIKEN Center for Developmental Biology.

Stem cells derived from mature cells are known as induced pluripotent stem (iPS) cells. These cells can be grown into healthy tissue in the lab, and offer a promising material for replacing damaged tissue. One practical way of applying the technology for quick patient tissue repair would be to establish large banks of healthy tissue derived from iPS cells. But this presents a problem—when transplanting cells derived from one individual to another, the recipient's immune system often recognizes the cells as foreign and attacks them.

The RIKEN researchers overcame this hurdle by grouping donor and recipient cells by their major histocompatibility complexes (MHC)—proteins found on the surfaces of immune cells. Monkeys with matched MHCs accepted the transplanted cells for at least six months, whereas monkeys with unmatched MHCs rejected the cells relatively quickly. The study was published in the journal *Stem Cell Reports* in September 2016.

www.riken.jp/en/pr/press/2016/20160916_2/

RIKEN geneticist Piero Carninci has become the first non-Japanese ambassador for research and academia in Japan.

Italian researcher to be face of Japan

Piero Carninci, Italian geneticist and director of the Division of Genomic Technologies at the RIKEN Center for Life Science Technologies, has become the first non-Japanese spokesperson for research and academia in Japan. The Japan National Tourist Organization appointed Carninci under its MICE (Meeting, Incentive, Conference, Event) Ambassador Programme, which selects influential individuals "to act as the face of the nation, contributing to publicity efforts and helping to attract more international conferences to Japan."

"It's an honor to have been selected to this position, and I hope to use my network inside and outside RIKEN to attract researchers to Japan and contribute to its further internationalization," said Carninci. He joins a group of 38 Japanese representatives, who include Hiroshi Komiyama, chairman of the Mitsubishi Research Institute and president emeritus of the University of Tokyo, and Takashi Onishi, president of the Science Council of Japan.

www.riken.jp/en/pr/topics/2016/20160830_1

Time for business in research

Representatives from 19 research institutes in 11 countries committed to strengthening collaboration with industry and reached a consensus on the importance of vision-driven innovation at a summit in Kyoto on 1 October 2016. The event was organized by RIKEN and the National Institute of Advanced Industrial Science and Technology in Japan.

"I'm impressed by the strong desire of national research institutes to work with industry, sharing their vision of a future society," said Katsumi Emura, executive vice president and chief technology officer of the Japanese information technology company NEC.

The summit was held ahead of the Science and Technology in Society forum in Kyoto, which brought together close to 1,200 world leaders in academia, government, business and media to address global challenges, such as food security, antibiotic resistance and low-carbon growth.

www.riken.jp/en/pr/topics/2016/20161013_2

G7 health ministers visit RIKEN

Health ministers from the Group of Seven (G7) leading industrial countries toured the RIKEN Kobe campus on 12 September 2016, where they peered into microscopes and encountered a supercomputer. The delegates visited the RIKEN Center for Developmental Biology and attended a talk by Masayo Takahashi, who described her pioneering clinical work using induced pluripotent stem cells to treat age-related macular degeneration. Takahashi brought samples of the cells for the ministers to examine under the microscope.

At the RIKEN Advanced Institute for Computational Science (AICS), the guests visited the K computer—the most powerful machine in the world for processing large amounts of data. They also heard from AICS Director Kimihiko Hirao on the K computer's latest scientific achievements, which include simulating the formation and evolution of dark-matter halos over a period of 13 billion years.

The ministers were in Kobe to attend the G7 Kobe Health Minister's Meeting, where they pledged to support universal health coverage, improved responses to health crises, and joint activities on dementia.

www.riken.jp/en/pr/topics/2016/20160912_1

Nihonium takes its place at the table

From left: Hideto Enyo, director of the RIKEN Nishina Center for Accelerator-Based Science, Kosuke Morita, group director of the Research Group for Superheavy Element, Kouji Morimoto, team leader of the Superheavy Element Device Development Team, and RIKEN President Hiroshi Matsumoto.

Time to update your periodic tables. The International Union of Pure and Applied Chemistry (IUPAC) has officially approved the names and symbols for four new elements, including element 113 discovered at RIKEN. The superheavy element can now be called nihonium (Nh), after the Japanese word *Nihon*, which means 'Japan' or 'land of the rising sun'. The IUPAC announcement was made on 30 November 2016, following a five-month period of public review.

Element 113 is the first element discovered in Asia to earn a permanent seat at the periodic table—"an intellectual legacy that will be passed down to future generations for the benefit of humankind," said Kosuke Morita following the announcement. Morita is group director at the RIKEN Nishina Center for Accelerator-Based Science, whose team discovered the element. "We feel truly honored and wish to express our deepest gratitude to all those who have given us support over the years."

Morita's team began looking for element 113 in September 2003, using RIKEN's heavy-ion linear accelerator (RILAC) at the RI Beam Factory (RIBF). The group successfully synthesized element 113 in July 2004, and then again in April 2005 and August 2012. On 31 December 2015, the IUPAC officially recognized the researcher's discovery and awarded them the right to name the new element.

www.riken.jp/en/pr/topics/2016/20161130_1

Life science symposium

More than 120 researchers gathered in Stockholm to attend an annual symposium

organized by the RIKEN Center for Life Science Technologies, the Karolinska Institutet and SciLifeLab. This year's conference was titled Frontiers in Life Science Technologies—Decoding Health and Disease and covered research in the areas of cellular imaging, molecular regulation, genomics, structural biology and drug discovery. Director of SciLifeLab, Olli Kallioniemi (pictured), greeted the participants. Presentations were divided into three key themes related to improving our understanding of disease: imaging, RNA and stem cell lines (see related article on page 16).

The day was packed with talks from researchers representing the three institutes, including Yasuyoshi Watanabe, director of the CLST, whose team has developed more than 250 molecular probes for tracking the movement of biomarkers of disease using positron emission tomography. The probes could help to visualize how drugs affect biological processes. Tadaharu Tsumoto, director of the Japan Society for the Promotion of Science, Stockholm Office, also informed the audience on opportunities for research exchange between Japan and Nordic countries.

www.clst.riken.jp/en/topics/event/160929seminar

RIKEN's new status

The Japanese government has granted three institutes a new status of Designated National Research and Development Institute: RIKEN, the National Institute for Materials Science (NIMS) and the National Institute of Advanced Industrial Science and Technology (AIST).

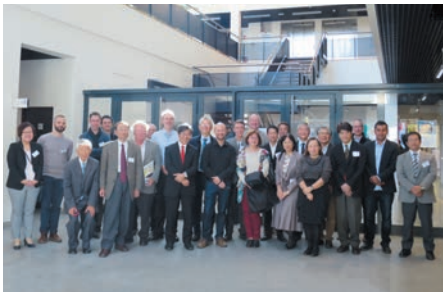
"The people of Japan have recognized us as an institution expected to make world-leading breakthroughs," said RIKEN President Hiroshi Matsumoto at a press conference organized in Kyoto on 1 October 2016 to announce the decision. "It is a heavy responsibility, and one that we must do our best to fulfill."

Matsumoto continued to describe three plans for fullfilling RIKEN's obligations under the new designation. "RIKEN will strive to make dramatic breakthroughs in an environment of fierce international competition, plant the seeds of innovation, and contribute to solving pressing social problems. RIKEN will also seek to become a central institution exerting strong leadership in Japan's innovation system by forging ties with industry and universities and working with the government to realize its policies."

Japan's Minister of State for Science and Technology Policy Yosuke Tsuruho also attended the event. "I have strong expectations that your institutes will make achievements that will put Japan at the forefront of global innovation," he told representatives of RIKEN, NIMS and AIST.

www.riken.jp/en

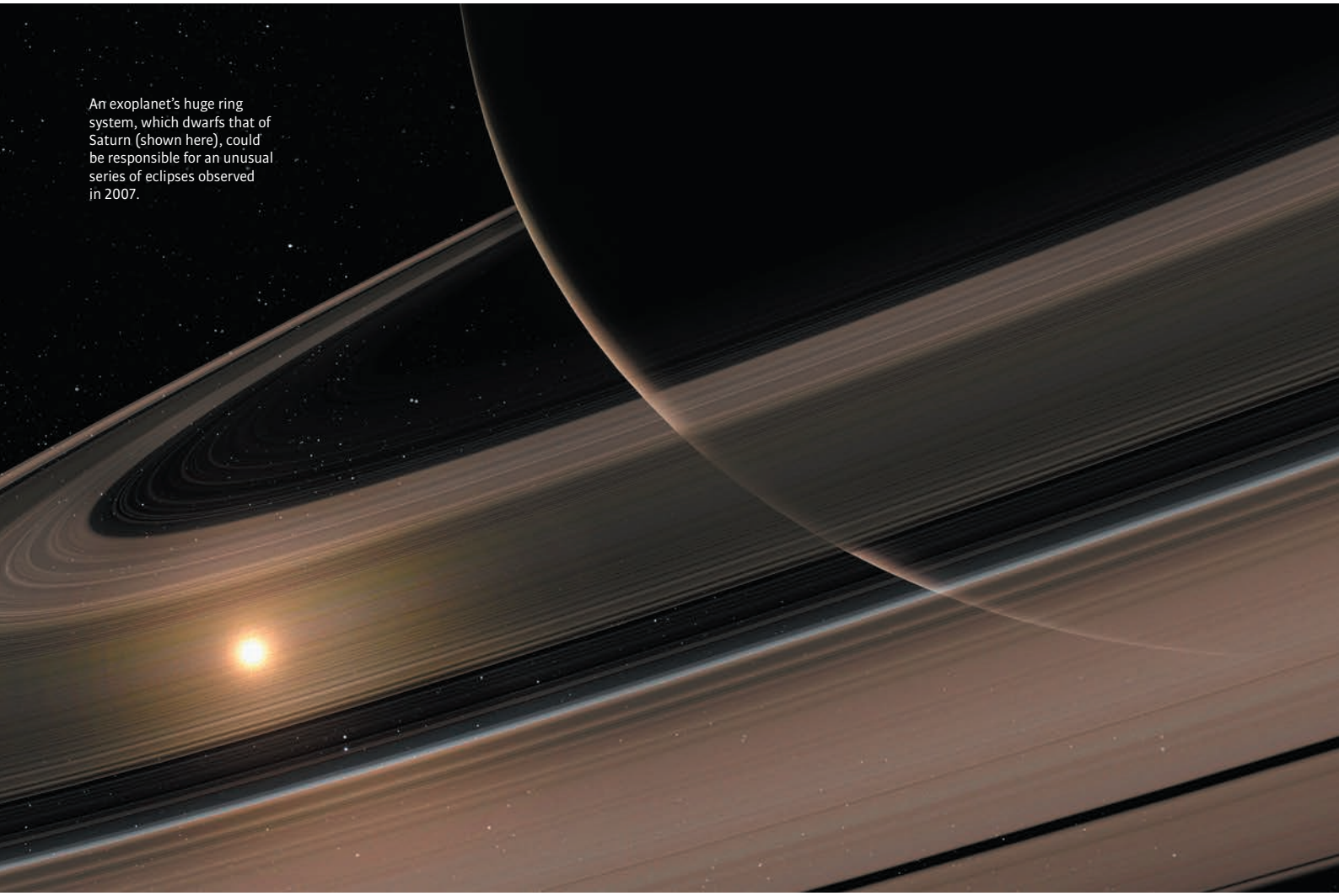
RIKEN–Strasbourg collaboration



The University of Strasbourg–RIKEN 20th Anniversary Workshop: Biology–Chemistry–Physics was held in Strasbourg, France, on 27–28 October 2016. The workshop commemorated two decades of partnership between the two institutes, beginning in 1996 when RIKEN signed a research agreement with Louis Pasteur University, one of three universities that were refounded as the University of Strasbourg in 2009. At the workshop, six researchers from each institute gave presentations in the fields of biology, chemistry and physics, with an aim to further promoting broad cooperation. The workshop attracted a diverse audience, young and old, who engaged in enthusiastic discussions.

www.riken.jp/en/pr/opics/2016/20161104_3

From left: RIKEN President Hiroshi Matsumoto, National Institute of Advanced Industrial Science and Technology President Ryoji Chubachi, Minister of State for Science and Technology Policy Yosuke Tsuruho, former minister Aiko Shimajiri, and National Institute for Materials Science President Kazuhito Hashimoto.



An exoplanet's huge ring system, which dwarfs that of Saturn (shown here), could be responsible for an unusual series of eclipses observed in 2007.

PHYSICS

A strange turn of events

The giant ring system of an exoplanet could survive for thousands of years if the rings rotate in the opposite direction of the planet orbiting its star

The giant ring system of an exoplanet could persist for more than 100,000 years, according to new calculations, but only if the rings orbit in the opposite direction to that of the planet around the star[1].

Steven Rieder of the RIKEN Advanced Institute for Computational Science and Matthew Kenworthy of Leiden University in the Netherlands studied a young, Sun-like star, which displayed a strange series of eclipses in 2007. The star began dimming irregularly over a two-month period, with sudden and dramatic changes in its brightness occasionally happening over a few hours. After a month, the brightness of the star faded by more than a factor of 20, before gradually coming back to full brightness by the end of the period.

Kenworthy had previously proposed a possible explanation for these eclipses—the star could be orbited by a planet with a gigantic ring system. The planet's rings would dwarf those of Saturn (see image), being more than 100 times larger.

But some scientists were skeptical of this explanation since the orbit of the exoplanet is highly elliptical so that the exoplanet is sometimes very distant from the star, whereas at other times it is close to it. During these close approaches, the gravity of the star would be expected to disrupt the rings.

In the present study, Rieder and Kenworthy performed simulations to see whether such a massive ring system can be stable for a long time. The results revealed that the system can persist for more than

10,000 orbits of 11 years, with one proviso: "The system is only stable when the rings rotate opposite to how the planet orbits the star," says Rieder.

Rings that turn in this sense are uncommon. Rieder and Kenworthy suspect that some catastrophe has reversed the direction of rotation of either the rings or the planet. "It might be far-fetched: massive rings that rotate in opposite direction, but we have now calculated that a 'normal' ring system cannot survive," says Rieder.

While there could be other explanations for the observations, they are unlikely. For example, the eclipses of the star could be caused by a free-floating object rather than by a ring system. "However, the chance of that is minimal," says Rieder. "Also, the velocity measured with previous observations may not be right, but that would be very strange, because those measurements are very accurate."

The astronomers now intend to investigate how the ring structure could form and how it changes over time. ■

Reference

1. Rieder, S. & Kenworthy, M. A. Constraints on the size and dynamics of the J1407b ring system. *Astronomy & Astrophysics* advance online publication, 21 November 2016 (doi: 10.1051/0004-6361/201629567).

MEDICINE

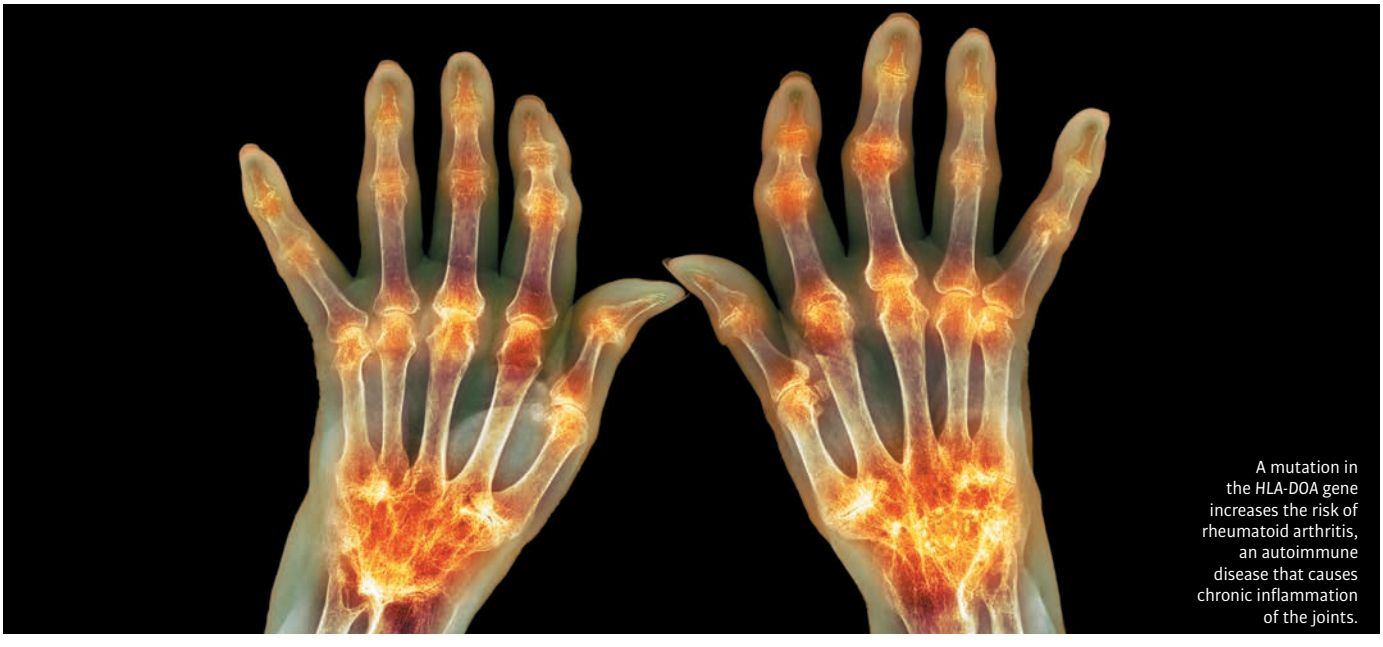
An unexpected autoimmune connection

A gene not considered a risk factor for disease has been linked to rheumatoid arthritis

A large study of Japanese people has identified a new gene variant linked to rheumatoid arthritis—a finding that could help reveal the root causes of the autoimmune disease[1].

Rheumatoid arthritis, which afflicts up to 37 million people globally, occurs when the body's immune system mistakenly attacks joints. The condition typically results in swollen and painful joints (see image), among other symptoms.

The variant found in the study is located in the human leukocyte antigen (HLA) system—a group of genes that regulates many immune functions. But unlike most HLA variants previously shown to raise the risk of rheumatoid arthritis, the newly-discovered one is in a 'non-classical' HLA gene—one that generally has much less genetic diversity than the 'classical' HLA genes that have been associated with many diseases.



A mutation in the *HLA-DOA* gene increases the risk of rheumatoid arthritis, an autoimmune disease that causes chronic inflammation of the joints.

"This is one of the first studies to demonstrate a contribution of a non-classical HLA gene to the risk of human diseases," says Yukinori Okada, a statistical geneticist at the RIKEN Center for Integrative Medical Sciences, who led the study.

Okada and his colleagues used four large data sets of people of Japanese ancestry to look across the HLA region in search of disease-linked variants. Their study included 23,731 healthy controls and 6,244 rheumatoid arthritis sufferers, most of whom had a severe form of the disease marked by the presence of autoantibodies known as ACPAs.

As previously found in studies of Europeans, common variations in the classical gene *HLA-DRB1* also showed the most significant disease risk in the Japanese population. Independently, however, a mutation in the non-classical HLA gene HLA-DOA seemed to strongly influence disease development in patients with the severe, ACPA-positive form of rheumatoid arthritis.

The researchers validated this finding in large cohorts of more than 7,000 individuals from East Asia and 23,000 people from Europe. They found that, among Europeans, the disease-linked version of *HLA-DOA* was often inherited in tandem with the disease-linked version of *HLA-DRB1*. In contrast, the two variants were mostly independently transmitted among Japanese individuals (other East Asians fell somewhere in between).

Okada's team also investigated the molecular mechanism by which the mutation in *HLA-DOA* impacts the development of rheumatoid arthritis. Unlike the variant in *HLA-DRB1*, which alters an amino acid in the protein encoded by the gene, the variant in *HLA-DOA* did not change the protein sequence. Instead, a gene expression analysis revealed that the mutation reduced the expression levels of *HLA-DOA*, a gene normally expressed at high levels in immune-related cells. ■

Reference

1. Okada, Y., Suzuki, A., Ikari, K., Terao, C., Kochi, Y., Ohmura, K., Higasa, K., Akiyama, M., Ashikawa, K., Kanai, M. *et al.* Contribution of a non-classical HLA gene, *HLA-DOA*, to the risk of rheumatoid arthritis. *American Journal of Human Genetics* **99,** 366–374 (2016).

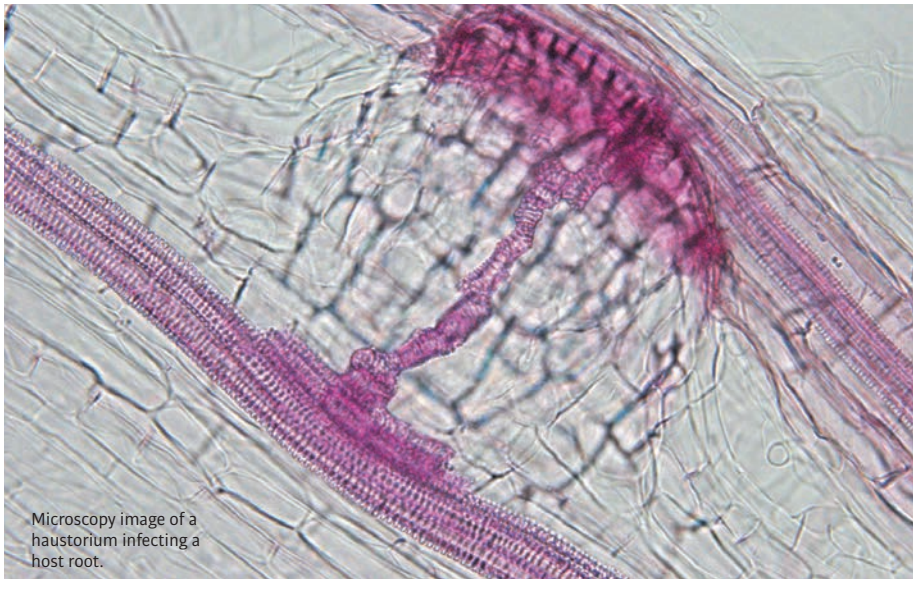
BIOLOGY

Pinning down parasitic plants

A key player in the infection mechanism of parasitic plants has been identified

RIKEN researchers have gained a deeper understanding of how specialized structures develop that allow parasitic plants to feed off other plants. They expect this will lead to more effective control of these parasites and bring improvements to agriculture.

Through an invasive organ known as a haustorium, the parasitic plant *Striga* siphons nutrients from the roots of crops such as sorghum, maize and rice, significantly reducing yields in Africa and Asia. Researchers at RIKEN have discovered that auxin, a well-known plant hormone, plays a crucial role in haustorium formation[1].



Microscopy image of a haustorium infecting a host root.

Striga is difficult to study in the lab because it requires a host plant. For the past decade, Ken Shirasu of the RIKEN Center for Sustainable Resource Science and his team have investigated the closely related plant *Phtheirospermum japonicum* as a proxy for *Striga*. "Nobody else was working with it," Shirasu says. "We had to set up everything, from the transformation system to the genomics tools."

The team is now reaping the rewards of that work. Using a combination of RNA sequencing and microarrays, they assessed which genes were expressed in *P. japonicum* following a chemical treatment to induce haustorium formation. They identified 327 genes with altered expression at different times after the treatment, generating a series of snapshots of the genetic steps in haustorium development.

Several of these genes are related to plant hormones, particularly auxin. While earlier

experiments with auxin inhibitors had implicated auxin in haustorium formation, it was unclear whether this was an indirect effect of interfering with auxin, which is involved in most aspects of plant development. The new data revealed that an auxin biosynthesis gene, *YUC3*, is activated in specific cells starting six hours after the treatment.

"When I saw that *YUC3* is uniquely expressed in the cells that detected the host plant, I thought 'Wow! This must be a very important gene,'" says Shirasu. To confirm the importance of *YUC3*, the team engineered plants in which the gene was deactivated. These plants often failed to form haustoria and infect host plants. By contrast, plants with increased *YUC3* formed a haustorium-like organ even when hosts were absent.

Although these results will not immediately help with controlling *Striga* infections, they will help scientists investigate the mechanism directing this process. "This is an anchor point from which we can explore which genes induce *YUC3* and which genes are induced by it," says Shirasu. The team has identified candidate genes acting downstream of *YUC3*, including genes that may be involved in controlling the host. ■

Reference

1. Ishida, J. K., Wakatake, T., Yoshida, S., Takebayashi, Y., Kasahara, H., Wafula, E., dePamphilis, C. W., Namba, S. & Shirasu, K. Local auxin biosynthesis mediated by a YUCCA flavin monooxygenases regulates haustorium development in the parasitic plant *Phtheirospermum japonicum*. *The Plant Cell* **28,** 1795–1814 (2016).

PHYSICS

'Snap freezing' produces different state

The state that a correlated-electron material adopts on cooling depends on how rapidly it is cooled

A liquid slowly cooled past its melting point forms a solid with a well-ordered, crystalline structure. On the other hand, if the same liquid is suddenly plunged below its melting point, its atoms or molecules are frozen in the same random arrangement they had when a liquid. Such solids are known as glasses.

Two RIKEN scientists have now found that a similar effect also occurs in correlated electron materials—intriguing materials that exhibit exotic properties because their electrons interact with each other via their spin and charge. Fumitaka Kagawa of the RIKEN Center for Emergent Matter Science and his colleague Hiroshi Oike discovered that the low-temperature state of such materials depends on how rapidly they are cooled[1].

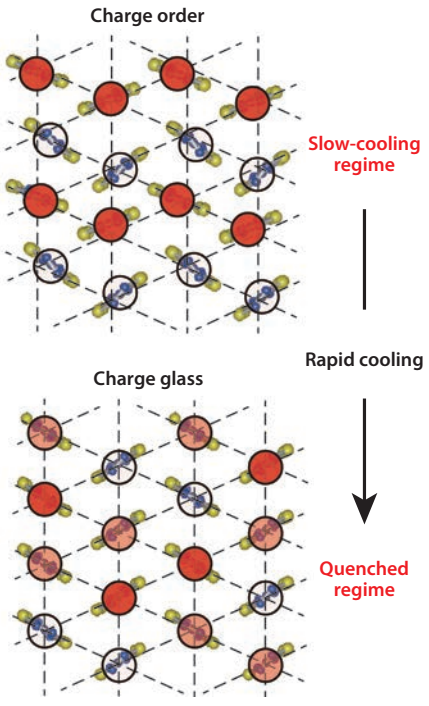
If a correlated electron material is cooled slowly, it will eventually settle into an arrangement that minimizes its energy, appropriately named the ground state. This is roughly analogous to the well-ordered periodic structure of a crystal.

However, Kagawa and Oike discovered that if the cooling rate is faster than a certain value, several correlated-electron materials do not enter the ground state but instead are frozen into a different charge or spin state—a bit like a glass.

For example, a material known as θ-$(BEDT\text{-}TTF)_2RbZn(SCN)_4$ usually exists in a state known as a charge order or charge crystal at temperatures below 200 kelvin. However, if it is cooled fast enough, it becomes a 'quenched charge glass', which has very different electronic properties (see image).

This finding goes against the received wisdom in the field. "Many condensed-matter researchers think that the low-temperature state of a material is uniquely determined in terms of energy and so the material always enters the ground state," says Kagawa. "We show that this common belief is not necessarily true."

The reason why this effect has not been noticed until now is because in most experiments on correlated electron materials, samples are slowly cooled at no more than a leisurely half a kelvin per second. But new cooling techniques that use laser or current pulses can cool at hundreds or even thousands of kelvins a second.



The low-temperature state of a correlated-electron material depends on whether it is cooled slowly (top) or rapidly (bottom).

The researchers believe that this phenomenon could be used in devices known as phase-change memories, which store data by using these states of matter to represent the ones and zeros used in computing. "We aim to develop a new class of high-speed nonvolatile memories that are based on the ground state and the quenched state," says Kagawa. ■

Reference

1. Kagawa, F. & Oike, H. Quenching of charge and spin degrees of freedom in condensed matter. *Advanced Materials* advance online publication, 21 June 2016 (doi: 10.1002/adma.201601979).

BIOLOGY

Split memories

Pleasant and unpleasant memories stored in neurons with distinct genetic identities

Like broccoli and French fries on a toddler's plate, the brain also keeps nice and nasty information in separate places. Pleasant experiences, tastes and smells are confined to the back of the basolateral amygdala (BLA)—an important memory center—whereas unpleasant ones are stored at the front.

RIKEN researchers have discovered that these opposing neurons in the amygdala are not only physically separated but also genetically distinct and that they influence positive and negative behaviors[1]. A team led by Susumu Tonegawa at the RIKEN–MIT Center for Neural Circuit Genetics has found that the anterior and posterior BLA neurons can disrupt expected behaviors if they are turned on or off by light pulses.

Positive and negative memories are stored in different places in the brain that activate two different genes.

To identify the 'negative' and 'positive' neurons, the researchers exposed male mice to either footshocks or the company of a female mouse. These experiences left their mark in the amygdala in the form of higher expression levels of the gene *c-Fos*, a marker of neural activity. Genetic profiling of the activated neurons revealed two genetic markers —*Rpso2* for negative neurons and *Ppp1r1b* for positive neurons. *Rspo2* was observed almost exclusively in the anterior BLA, while expression of the *Ppp1r1b* gene was concentrated in the posterior BLA. These spatially distinct positive and negative neurons differed in size, shape and electrical properties. The positive neurons were activated when mice were exposed to pleasant smells and water rewards, whereas the negative neurons became active in response to pain and unpleasant smells.

The anterior and posterior neurons not only responded to the value of rewards, but also were crucial for associated negative and positive behaviors, respectively. Mice were trained to respond to footshocks by immobilizing (negative behavior) or to perform a light-cued nose poke to receive water (positive behavior). The researchers could weaken these behaviors by simply targeting either the anterior or posterior BLA with precise light bursts during training.

The positive and negative neurons could even interfere with how well mice learned the associated positive and negative behaviors. The expected freezing behavior was reduced by light stimulation of positive neurons during footshock training, while activation of negative neurons impaired the mice in the water reward task. These neurons could effectively overcome the positive or negative meaning of powerful external stimuli like water or shocks; this is achieved through mutual inhibitory neural signaling between the anterior, or negative, and posterior, or positive, BLA. The BLA thus plays an important role in associating negative and positive stimuli with appropriate behaviors. ■

Reference

1. Kim, J., Pignatelli, M., Xu, S., Itohara, S. & Tonegawa, S. Antagonistic negative and positive neurons of the basolateral amygdala. *Nature Neuroscience* advance online publication, 17 October 2016 (doi: 10.1038/nn.4414).

BIOLOGY

A protein pushes ahead of the curve

A cell-signaling enzyme coordinates the three-dimensional reorganization of cell membranes

Exactly how cells ingest large molecules may be explained by the discovery of a new role for a known protein by RIKEN researchers[1].

The scientists began by conducting a comprehensive search for proteins that can cause cell surfaces to curve, which is important because the shape of a cell is integral to its function. Curving proteins work by binding to the fatty molecules that make up the membrane, known as lipids. The more proteins attach to lipids, the greater the curvature of the cell surface.

The team combined membranes made of different types of lipids with proteins from various mouse tissues and homed in on a protein that caused the lipid membranes to bend into tube-like structures.

To the researchers' surprise, the isolated protein turned out to be phospholipase Cβ1 (PLCβ1), an enzyme found in the brain known to be involved in various cellular signaling processes, including those that drive cell division and maturation. Interacting with the cell membrane, however, the protein seemed to play a different role.

When coupled with a lipid called phosphatidylethanolamine (PE), PLCβ1 appeared to initiate the formation of indented structures in the cell membrane known as caveolae. These indents contribute to processes such as signaling or the internalization of biomolecules from outside the cell. Electron microscopy showed that cells with sharply reduced levels of PLCβ1 produced notably fewer caveolae, and those that did form, exhibited abnormal structures (see image).

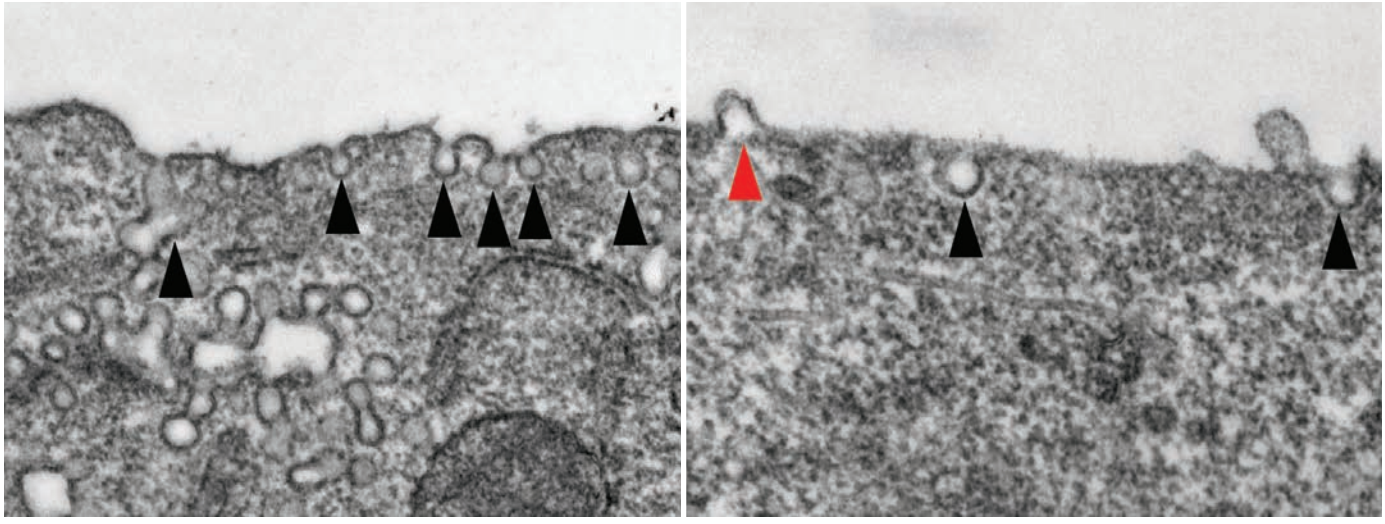
That PE was involved also came as a surprise to Toshihide Kobayashi of the RIKEN Lipid Biology Laboratory and colleagues. "This is a well-conserved lipid, from bacteria to mammals," says Kobayashi, "but previously, it was not known to be a molecule that PLCβ1 acts on." Subsequent experiments showed that the protein and lipid interact via a structural element that bears a close resemblance to elements found in other curvature-inducing proteins.

PLCβ1 is best known as an enzyme that produces two signaling molecules called inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate (PIP_2). But Kobayashi's team determined that mutant versions of the protein lacking this enzymatic function were still able to bind to membranes and initiate tubule formation.

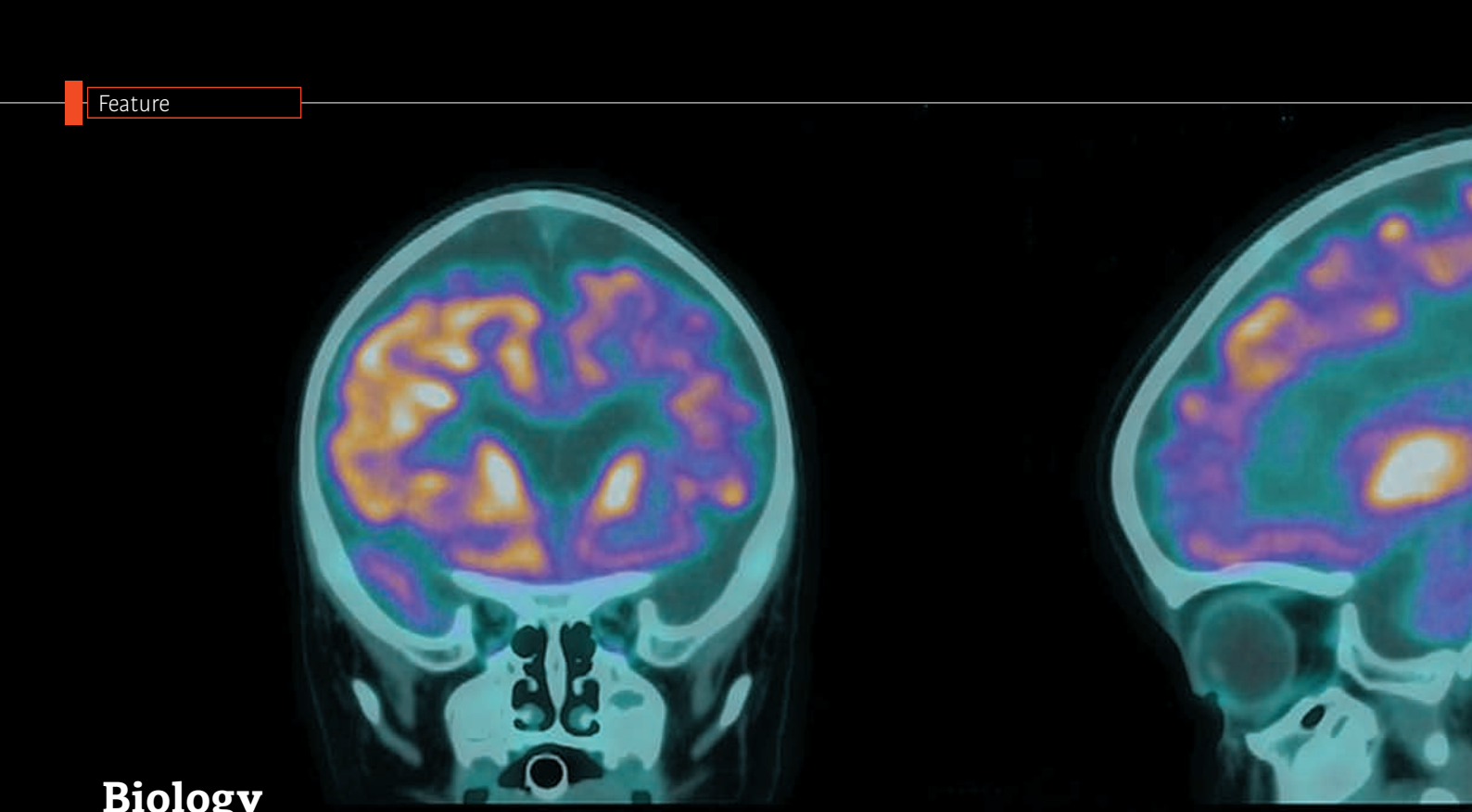
Kobayashi is currently trying to determine whether the enzyme's activity might somehow still be involved in the membrane-bending process. One hypothesis is that it drastically alters the physical shape of lipids near PE by changing PIP_2 to DAG. "This could induce a big alteration in the membrane curvature," says Kobayashi. It is also likely that the enzyme's activity does not play a role in PLCβ1-induced membrane curvature. Clarifying the precise contribution of PLCβ1 is a top priority for the team moving forward. ■

Reference

1. Inaba, T., Kishimoto, T., Murate, M., Tajima, T., Sakai, S., Abe, M., Makino, A., Tomishige, N., Ishitsuka, R., Ikeda, Y. *et al.* Phospholipase Cβ1 induces membrane tubulation and is involved in caveolae formation. *Proceedings of the National Academy of Sciences USA* **113,** 7834–7839 (2016).



Electron microscopy images show that relative to normal cells (left), cells deficient in the protein phospholipase Cβ1 (right) produce considerably fewer caveolae (black arrowheads) and exhibit atypical membrane bulging (red arrowhead).



Biology

Imaging the birth of new brain cells

PET pictures of new neurons in the brain promise to advance our understanding of depression and the effects of antidepressants

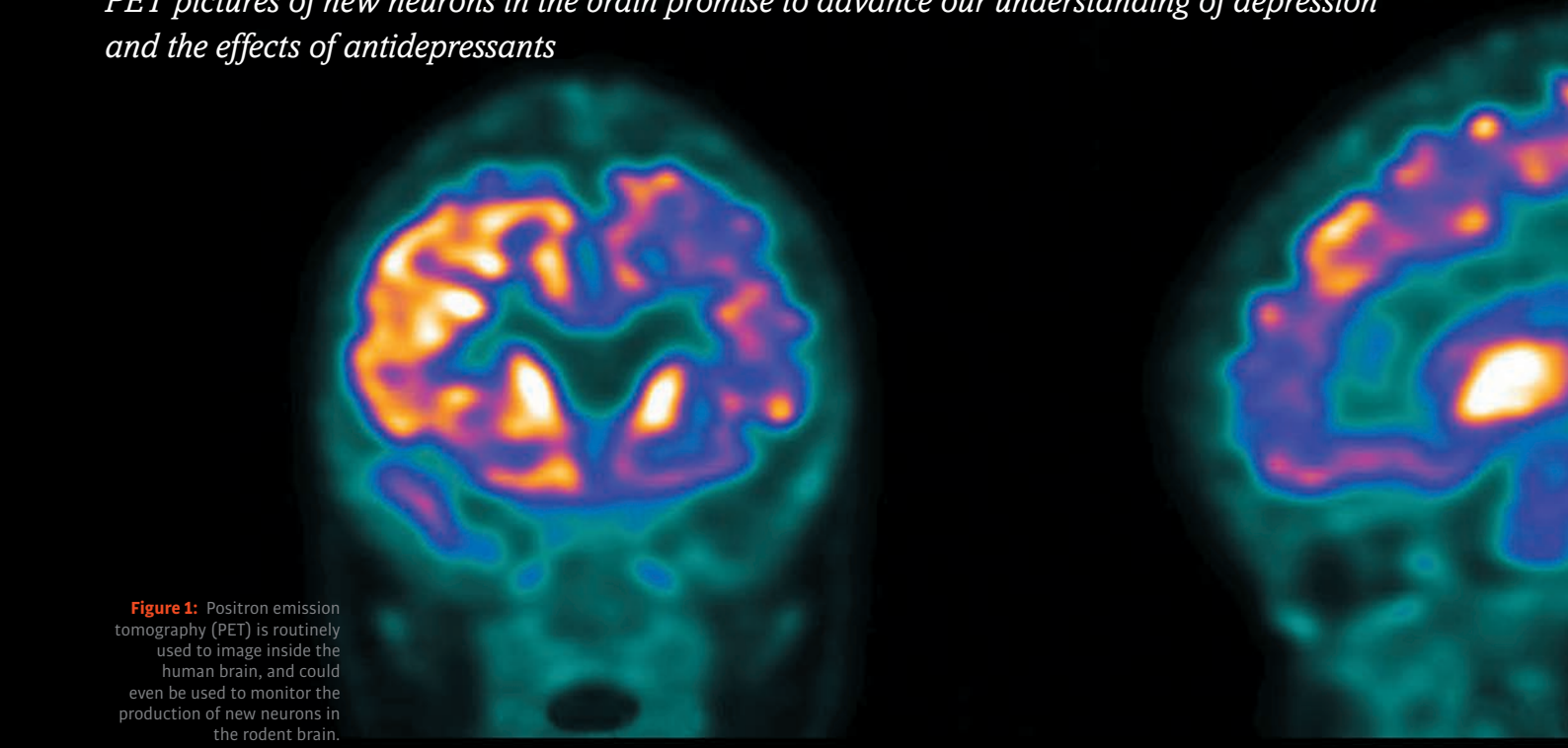
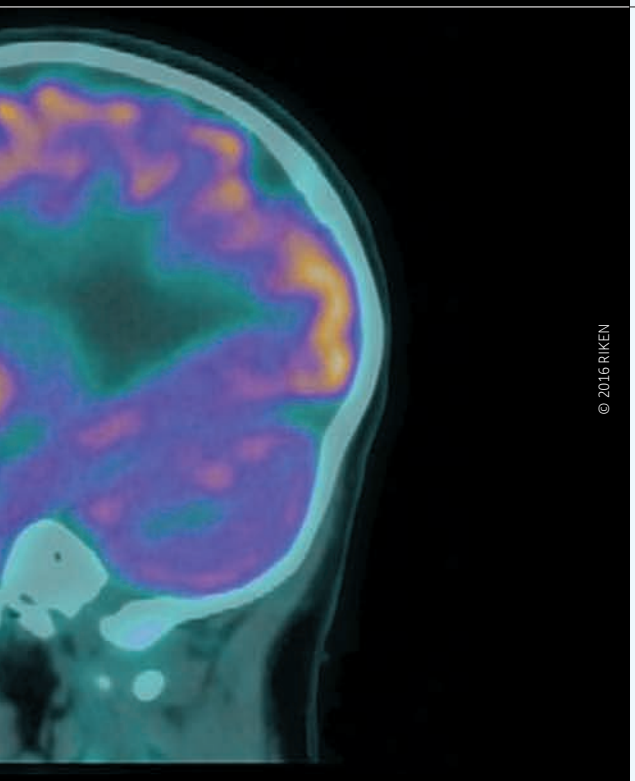
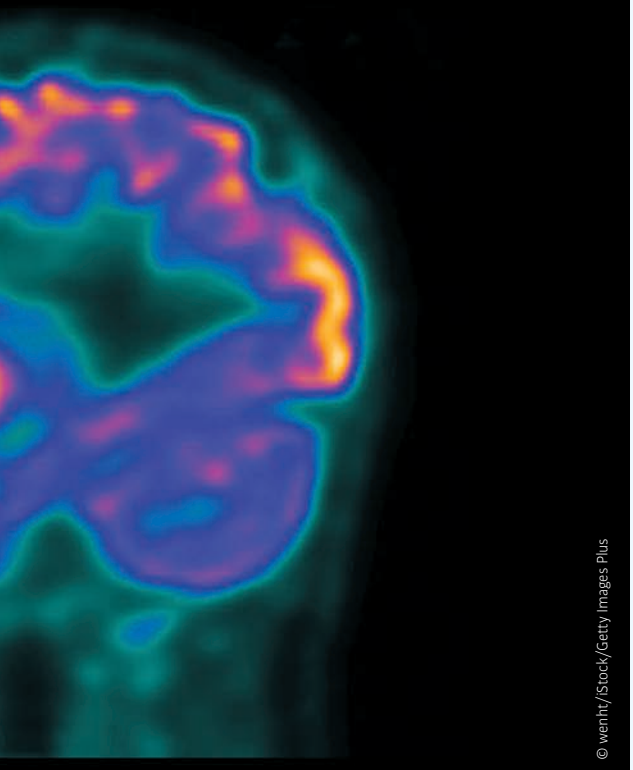


Figure 1: Positron emission tomography (PET) is routinely used to image inside the human brain, and could even be used to monitor the production of new neurons in the rodent brain.





The adult brain was long regarded as being unable to grow new neurons—you were stuck with what you had. We know now that this view is wrong and that the human brain continues to produce new neurons throughout our lives.

At least two distinct populations of neural stem cells continue to divide and produce new neurons throughout life in a process known as neurogenesis. One group births immature neurons that migrate to the olfactory bulb at the front of the brain, where they contribute to the sense of smell. The other generates neurons that enter the dentate gyrus of the hippocampus, a deep-brain structure critical for learning and memory.

Scientists have previously found a link between reduction in the neural volume of the hippocampus and depression. They have also discovered that antidepressants known as selective serotonin reuptake inhibitors, which include fluoxetine (Prozac), stimulate the growth of new cells in this region of the brain. Both these pieces of evidence suggest that adult neurogenesis in the hippocampus may play an important role in depression.

Yosky Kataoka, Yasuhisa Tamura and their colleagues at RIKEN have developed an enhanced molecular imaging technique that is so sensitive it can detect small fluctuations in the rate at which neurons are birthed in the brain[1]. The researchers anticipate that this technique could lead to new ways to diagnose depression and monitor the effects of antidepressants.

Tracing neural growth

Monitoring neurogenesis noninvasively is very challenging. While magnetic resonance imaging (MRI) can be used to observe neurogenesis in rats, MRI tracers have to be injected directly into the brain fluid, making the procedure risky and difficult to perform.

Another imaging technique that has been used to visualize and measure the rate of adult neurogenesis in the rodent hippocampus is positron emission tomography (PET) (Fig. 1). PET involves injecting small amounts of a radioactive tracer, which binds to a specific target molecule in tissues and organs, and then detecting the gamma rays it emits using a scanner. This enables researchers to monitor the distribution of the target molecule in an organ.

Scientists have used a PET tracer called 3'-deoxy-3'-[^{18}F]fluoro-L-thymidine ([^{18}F]FLT) that gets trapped in newborn brain cells to try to measure adult neurogenesis. Until now, though, they have been unsuccessful in these attempts because there was little difference in signal strength between regions with and without cell growth.

Yasuhisa Tamura of the RIKEN Center for Life Science Technologies and his colleagues suspected that the problem with [^{18}F]FLT was arising because membrane proteins called drug transporters actively

Yasuhisa Tamura
Yasuhisa Tamura obtained his PhD from Kansai Medical University in 2004. After working as a postdoctoral researcher for two years at the same university, he joined the former RIKEN Molecular Imaging Research Program (MIRP) as a research scientist in 2006. In 2012, Tamura became a senior scientist at the Cellular Function Imaging Team, RIKEN Center for Life Science Technologies (CLST). Since 2014, he has been concurrently working as a research scientist at the RIKEN CLST-JEOL Collaboration Center. His current research focuses on the role of NG2 progenitor cells in the brain as well as the peripheral tissues.

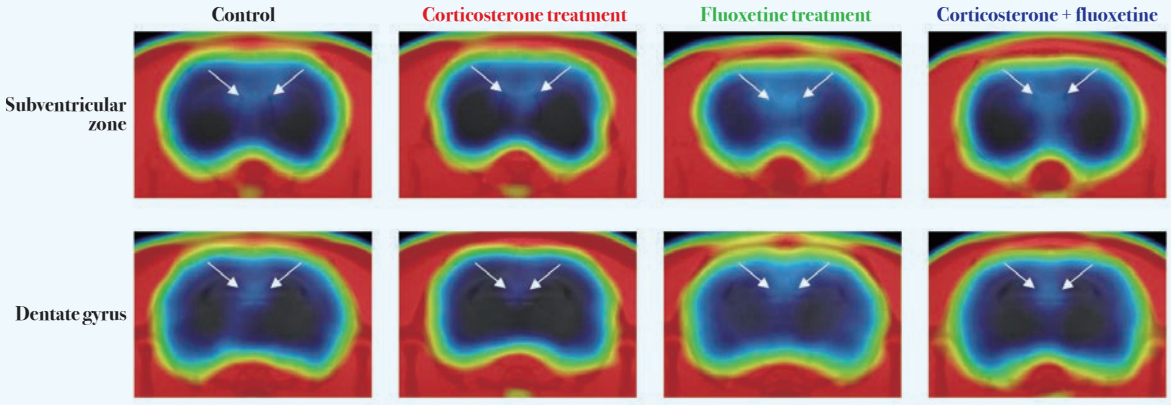


Figure 2: PET images show decreased neurogenesis in the dentate gyrus of rats treated with the stress hormone corticosterone (second from left), which is restored by treatment with the antidepressant fluoxetine (far right).

pump the tracer out of the brain. So to counteract this and boost the technique's sensitivity, they tried injecting rats with a drug prior to injecting the animals with the radioactive tracer. The team chose probenecid, a drug typically used to treat gout, because it inhibits drug transporters at the blood–brain barrier.

The color of depression

For periods of up to a month, the researchers gave the stress hormone corticosterone, which suppresses neurogenesis and induces depression-like behaviors, to one group of rats. To another, they gave both corticosterone and the antidepressant drug fluoxetine.

When they scanned the animals' brains, they discovered that the radioactivity was strong enough to allow them to distinguish between reductions in neurogenesis caused by corticosterone and recovery induced by treatment with the antidepressant (Fig. 2). Treating the animals with probenecid increased the uptake of the radioactive tracer by the brain, thus making the radioactive signal given off by newborn cells stronger.

To confirm their results, Tamura and his colleagues dissected the rats' brains and tagged newborn cells in slices of hippocampal tissue with fluorescent antibodies. They determined the number of tagged cells by examining the samples under a microscope. In line with the brain-scanning results, this analysis revealed that rats treated with corticosterone for one month had about 45 per cent fewer newborn neurons in the dentate gyrus than untreated control animals. In contrast, rats given both corticosterone and fluoxetine showed no such difference.

These results demonstrated that the enhanced PET imaging technique can visualize adult neurogenesis in the brains of live animals and that it is sensitive enough to detect dynamic alterations in the rate of the process caused by treatment with antidepressants.

Of mice and men

Although antidepressants stimulate adult neurogenesis in rodents, it is not known whether this is how they alleviate the symptoms of depression in humans.

Tamura says the enhanced imaging method developed by his team could help to resolve these questions and may eventually help clinicians not only to diagnose depression, but also to evaluate the effectiveness of antidepressant treatments.

"This is a very interesting finding because it has been a long-time dream to find a noninvasive test that can give objective evidence of depression and simultaneously show whether drugs are working in a given patient," says Kataoka, who led the team. "We have shown that it is possible, at least in experimental animals, to use PET to show the presence of depression and the effectiveness of drugs."

The team is keen to apply their imaging technique to humans. "Since it is known that these same brain regions are involved in depression in the human brain, we would like to try this technique in the clinic and see whether it turns out to be effective in humans as well," explains Kataoka.

This should not be too challenging to do. "Both probenecid and the PET tracer are already applicable to humans, so we can directly translate the work to humans. We are now testing it in non-human primates," says Tamura.

Reference

1. Tamura, Y., Takahashi, K., Takata, K., Eguchi, A., Yamato, M., Kume, S., Nakano, M., Watanabe, Y. & Kataoka, Y. Noninvasive evaluation of cellular proliferative activity in brain neurogenic regions in rats under depression and treatment by enhanced [^{18}F]FLT-PET imaging. *The Journal of Neuroscience* **36,** 8123–8131 (2016).

When a mouse is placed in a cage with a familiar mouse and an unfamiliar one, it will spend more time with the unfamiliar one—a behavioural trait that has helped pinpoint where social memories are stored in the mouse brain.

BIOLOGY

Don't I know you from somewhere?

Recollections of social encounters are stored in a different region of the brain than other memories

The location in the brain where social memories are stored has been identified by RIKEN researchers. Such memories are essential for establishing relationships, but it had been unclear if they are stored separately from other memories[1].

A team led by director of the RIKEN Brain Science Institute and the RIKEN–MIT Center, Susumu Tonegawa, determined the site of social memories in mice by exploiting natural rodent tendencies. Because mice naturally show more interest in the unfamiliar, a test mouse placed in a cage with two other mice—one familiar and one unfamiliar—will spend more time sniffing the new mouse. However, if the memory of the familiar mouse is disrupted, the test mouse will sniff both mice for about the same amount of time.

With this setup, the researchers used a combination of optogenetics and behavioral observation to test whether social memories are stored in hippocampal region CA1, a region known to be involved in memory storage. They discovered that optogenetic inhibition of the CA1 ventral subregion, but not the dorsal subregion, caused mice to act as if they did not remember the familiar mouse.

A closer look at the ventral CA1 showed that neurons active during the initial social interaction were connected to three regions, but preference for the unfamiliar mouse was disrupted only when output to one of them—the nucleus accumbens—was

inhibited. Interestingly, the same manipulation did not disrupt preference for unfamiliar objects, indicating that the circuit was specific for social memories. Additional tests confirmed this ventral CA1–nucleus accumbens circuit as the site of social memory storage.

Social memories do not seem to last as long as other memories, and through another set of experiments, the researchers determined what happens when mice naturally 'forget' the familiar mouse. After 24 hours, mice usually act as if they have never met the familiar mouse. However, when neurons storing the memory were optogenetically reactivated during the social discrimination task, more than 24 hours after the first meeting, mice suddenly lost interest in the familiar mouse, acting just as they did only 30 minutes after the initial meeting. This demonstrates that the memories still existed, but that, for some reason, the familiar mouse was unable to retrieve them after a certain time.

"Our findings provide the fundamental brain mechanisms underlying the ability to recognize individuals," notes Tonegawa. "Because some brain disorders like autism involve impairment in social interactions, further research along this line could contribute to development of new therapies for these disorders." ■

Reference

1. Okuyama, T., Kitamura, T., Roy, D. S., Itohara, S. & Tonegawa, S. Ventral CA1 neurons store social memory. *Science* **353,** 1536–1541 (2016).

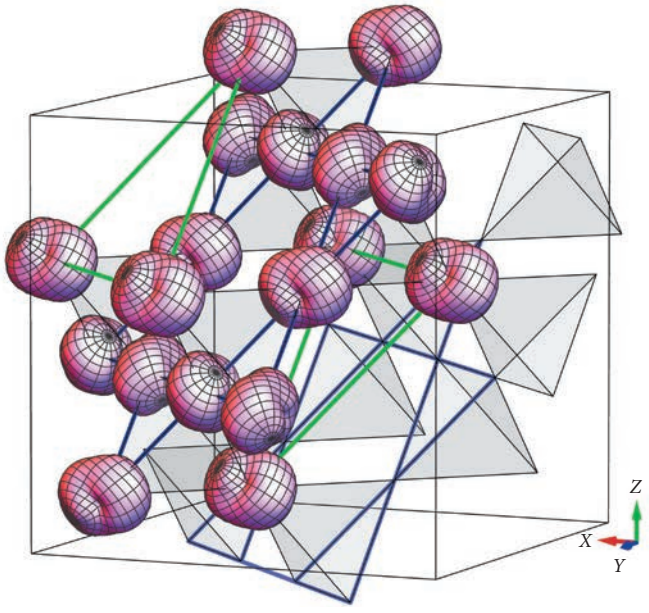
PHYSICS

The search for disorder in order

A signature of an exotic state of matter that remains disordered even at very low temperatures has been experimentally identified in terbium titanium oxide

Quantum effects are normally seen only at the atomic level, but certain materials, known as quantum matter, can show macroscopic properties that are direct manifestations of quantum effects. An exotic form of quantum matter that is characterized by its disorder has now been confirmed by RIKEN scientists[1].

Magnetism arises through a property of electrons known as spin, which can point either up or down. Materials exhibit magnetic properties when their spins become ordered. For example, neighboring spins in an antiferromagnet are always in opposite directions. But if the atoms in a material have a triangular arrangement, this becomes impossible and so they cannot stabilize into any coherent order of spins, a phenomenon known as frustrated magnetism.



Schematic representation of the hidden ordered arrangement of electric quadrupole moments in the compound terbium titanium oxide.

Frustrated magnets are exciting materials for physicists because they are predicted to host a wide range of unusual states of quantum matter. One example is a quantum spin liquid in which the spins are disordered—like the molecules in water—even at very low temperatures.

The compound terbium titanium oxide $Tb_2Ti_2O_7$ has been actively researched ever since the discovery in 1999 that it can become a spin liquid. More than 100 experiments have been conducted on it and various theoretical models have been proposed, but the true nature of this material is still unclear.

"Some experimental groups reported that the spins were not in an ordered state but were dynamically fluctuating. And so they might be in a spin liquid state, but the nature of this state has remained elusive," explains Shigeki Onoda from the RIKEN Center for Emergent

Matter Science. "In contrast, other groups working on different samples reported that a transition occurs from a high-temperature paramagnetic state to a low-temperature state that presumably has a hidden ordering of spins."

Onoda and his co-workers synthesized a series of high-purity single crystals of $Tb_2Ti_2O_7$ in which a small fraction of the titanium atoms had been replaced with more terbium atoms, a change in composition that nudged the materials slightly across the border between the spin-liquid regime and the hidden order regime.

They then performed thermodynamic, magnetization and neutron-scattering measurements together with theoretical calculations and obtained very good agreement between the two.

The team's analysis confirmed the existence of hidden order states and indicates it occurs due to interactions between the terbium ions, creating an ordered arrangement of so-called electric quadrupole moments (see image). Such a state is theoretically expected to border a quantum spin liquid.

"We intend to explore the spin liquid state more and hopefully confirm its existence experimentally," Onoda says. ■

Reference

1. Takatsu, H., Onoda, S., Kittaka, S., Kasahara, A., Kono, Y., Sakakibara, T., Kato, Y., Fåk, B., Ollivier, J., Lynn, J. W. *et al.* Quadrupole order in the frustrated pyrochlore $Tb_{2+x}Ti_{2-x}O_{7+y}$. *Physical Review Letters* **116,** 217201 (2016).

MEDICINE

Unleashing the potential of Chinese licorice

The secrets of Chinese licorice, a widely-used herbal ingredient in traditional Chinese medicine, have been unlocked through the sequencing of its genome

The genome of Chinese licorice, a natural sweetener and an important plant for traditional Chinese medicine, has been decoded by a team of RIKEN researchers.

Chinese licorice, or *Glycyrrhiza uralensis*, which is closely related to the plant used to make licorice candy, is an important component of traditional Chinese, or *kampo*, medicine. "It is incorporated in approximately 70 per cent of the 200 major traditional Chinese medicine formulations used in Japan," notes Kazuki Saito of the RIKEN Center for Sustainable Resource Science, who led the team. "Considering that 90 per cent of Japanese physicians prescribe *kampo* medicine in their practices, it is easy to see the importance of this plant."

Chinese licorice, or *Glycyrrhiza uralensis*, is an important ingredient in traditional Chinese medicine. Decoding its genome could lead to increased production of its useful compounds.

The team chose to examine the genome of Chinese licorice partly because it is known to contain the highest concentration of glycyrrhizin, a compound associated with the medical properties of the plant, which include anti-inflammatory, anti-cancer, anti-allergic and anti-viral activities.

By using a combination of short- and long-read sequencing and by comparing the genome with published sequences of other legume species, the researchers predicted that the plant's genome coded just over 34,000 proteins, which is somewhat higher than the 20,000 encoded by the human genome. The team focused on two genetic regions—one that codes saponins, which are important plant compounds that include glycyrrhizin, and another that produces isoflavonoids, which are also used as medicinal components.

The team found that genes in licorice and related plants are strongly conserved. This indicates that legumes use a small number of genes to create 'scaffolds' that allow an enormous diversity of compounds to be produced.

"Chinese licorice is an important and heavily consumed medicinal plant," says Keiichi Mochida, the first author of the paper. "We hope our work will make it possible to carry out molecular breeding to create strains that will grow sustainably in Japan and that will produce large concentrations of useful compounds such as glycyrrhizin."

"We very much hope that our draft genome sequence will facilitate the identification, isolation and editing of useful genes to improve the agronomic and medicinal traits of licorice through molecular breeding," says Saito. "There remains much to learn about the immense diversity of plant metabolism, and this research will contribute to further progress in that direction."

The group plans to examine differences between the genome of *G. uralensis* and other licorice species in order to deepen their understanding of the production of useful compounds. ■

Reference

1. Mochida, K., Sakurai, T., Seki, H., Yoshida, T., Takahagi, K., Sawai, S., Uchiyama, H., Muranaka, T. & Saito, K. Draft genome assembly and annotation of *Glycyrrhiza uralensis*, a medicinal legume. *The Plant Journal* advance online publication, 24 October 2016 (doi: 10.1111/tpj.13385).

PHYSICS

Single photon excites two atoms at once

Calculations reveal how one particle of light can excite two atoms simultaneously

Our understanding of quantum optics has received a boost by the discovery, by RIKEN researchers, of a way to excite two atoms with only one light particle, or photon (see image)[1].

Franco Nori from the RIKEN Center for Emergent Matter Science leads a team exploring how light confined in small mirrored cavities makes atoms behave strikingly differently from free atoms. In particular, the electromagnetic forces generated by the light in a cavity induce the atom's electrons to oscillate with the same frequency as the confined light. By tuning this so-called resonant frequency, scientists can trap atoms in high-energy excited states useful for quantum computing devices.

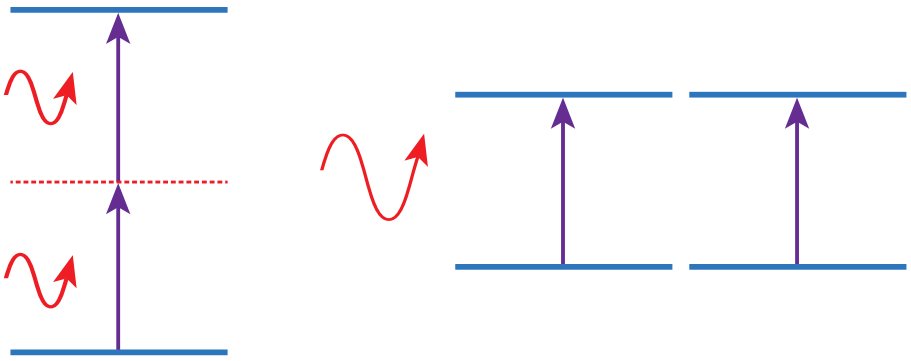
Intrigued by the possibility of using this ultrastrong coupling between the electromagnetic field and the atom's electrons to excite multiple atoms with a single photon, Nori and colleagues in Italy modeled two identical atoms in a cavity. To their surprise, the calculations predicted that if the cavity's resonant frequency is exactly twice the atom's transition frequency to a higher energy state, each atom can take half the photon's energy and jump to the higher energy state. The same effect can occur with three atoms when the resonant frequency is triple the atomic transition frequency.

Furthermore, the researchers discovered that the entire process should be reversible—multiple excited atoms in the cavity can relax by releasing a single photon.

Nori notes that the mechanism behind this process derives from the peculiar properties of light in a vacuum. "In effect, the system temporarily 'borrows' a second photon from the cavity," he explains. "These 'virtual' photons are generated from chance fluctuations in the cavity's vacuum. They appear and disappear all the time."

The virtual photon helps the two-atom–one-photon system reach a new quantum state that combines two situations—one where both atoms are in their ground states with a photon in the cavity and another where the two atoms are excited and no photon is present. "Eventually, the system emerges from this blend of two states into purely the excited one," Nori adds.

The researchers anticipate that this predicted phenomenon might be realized experimentally with superconducting 'artificial atoms' that have precisely engineered energy levels.



In two-photon absorption, two photons excite a single atom or molecule (left). Theoretical models show that one photon can excite two or more atoms or molecules (right). Photons represented by red arrows; atoms represented within blue lines.

"This process might lead to useful spectroscopic and diagnostic tools, but the details will take time," says Nori. Furthermore, excitation of two atoms by a single photon could produce quantum states in which more than two particles are entangled, which would be useful for quantum cryptography and computation.

Reference
1. Garziano, L., Macrì, V., Stassi, R., Di Stefano, O., Nori, F. & Savasta, S. One photon can simultaneously excite two or more atoms. *Physical Review Letters* **117,** 043601 (2016).

CHEMISTRY

Probing energy transfer using light

A new method for scrutinizing the energy transfer between molecules promises to improve our understanding of photosynthesis and further the development of energy harvesters

An all-RIKEN team has developed a powerful method for probing energy transfer, which could assist the development of energy-harvesting devices and unlock the secrets of photosynthesis.

The process of photosynthesis, by which plants convert sunlight into chemical energy, involves a minutely choreographed transfer of energy between molecules. But exactly how the energy is moved is not fully understood because it is highly challenging to observe these transfers, which occur extremely rapidly and on a very small scale.

Scientists have previously attempted to map how molecules become excited and pass on these excitations to other molecules. But they used optical spectroscopy, which relies on regular light waves and cannot see phenomena smaller than several hundred nanometers. It thus cannot detect many of the transfers, which occur at a nanometer scale.

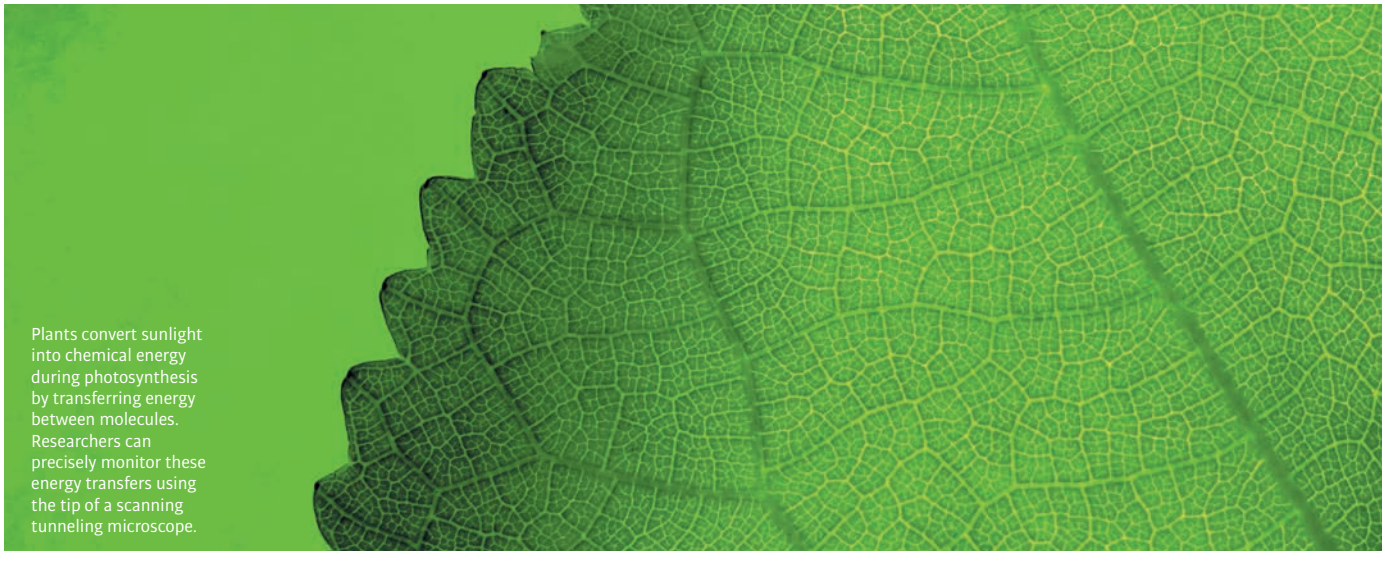
Now, Yousoo Kim and colleagues at the RIKEN Surface and Interface Science Laboratory have developed a powerful observational method to catch these transfers in action. It employs absorption and emission spectroscopy combined with a scanning tunneling microscope (STM).

The researchers used this method to observe energy transfer between two molecules—free-base phthalocyanine (H2Pc) and magnesium phthalocyanine (MgPc)—which emit fluorescent light at different energies. When they precisely stimulated the MgPc molecule alone with electrons from the STM tip, they detected luminescence signals from a nearby H2Pc molecule, clearly indicating that energy transfer from MgPc to H2Pc had occurred.

The team showed that the mechanism behind the process is resonance energy transfer, a form of transfer where energy is transmitted by resonance, in the same way that tuning forks will begin to vibrate together when they are tuned to the same frequency, rather than charge transfer from one molecule to another through shared electrons.

The researchers also demonstrated the possibility of using a single-molecule valve device for energy transfer based on a transition between different chemical forms, known as tautomers, of H2Pc, which could be seen as the energy transfer switched on and off like a blinking light.

"Using this technique, we have shown that it is possible to pinpoint how energy is transferred between molecules," says Kim. "This work could be used to design new



Plants convert sunlight into chemical energy during photosynthesis by transferring energy between molecules. Researchers can precisely monitor these energy transfers using the tip of a scanning tunneling microscope.

energy-harvesting devices such as photovoltaic cells, which also rely on energy transfer."

These insights into energy transfers will not only deepen our understanding of how energy is converted at the nanoscale level, but also open a path to create excitonic circuits with molecular architectures on solid surfaces. ■

Reference

1. Imada, H., Miwa, K., Imai-Imada, M., Kawahara, S., Kimura, K. & Kim, Y. Real-space investigation of energy transfer in heterogeneous molecular dimers, *Nature* **538,** 364–367 (2016).

BIOLOGY

Molecules behave differently in a crowd

A huge simulation on the K computer reveals that molecules follow different rules in a bacterial cell than in a test tube

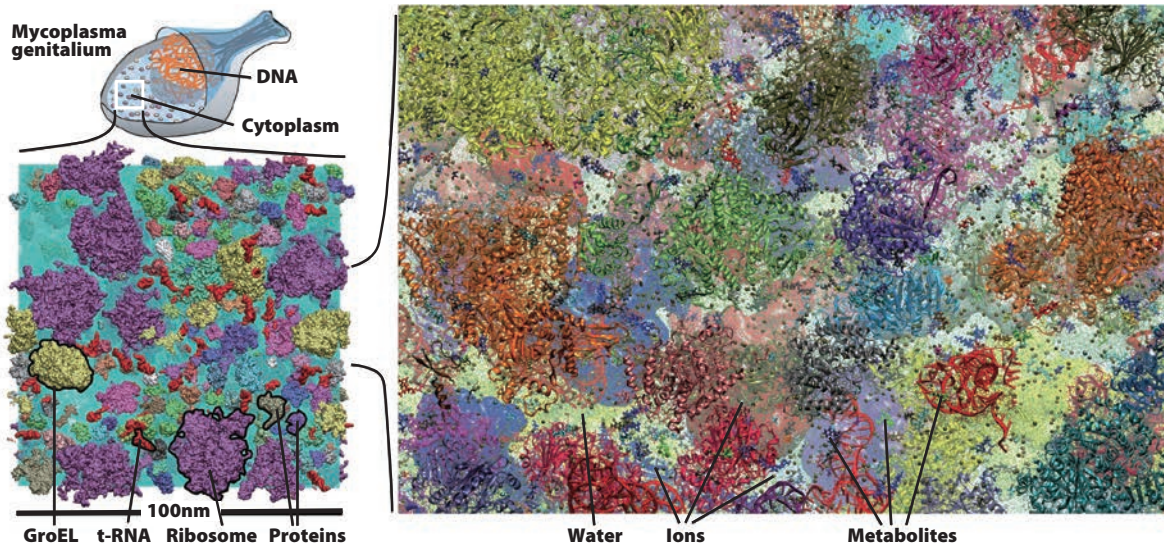
By drawing on the power of Japan's K supercomputer, researchers from RIKEN and Michigan State University have made intriguing observations about the relationships between molecules in the extremely crowded interior of a bacterial cell[1].

Studies performed in test tubes—*in vitro studies*—have given scientists vital insights into how molecules interact with one another. However, little is known about how molecules interact inside cells because the crowded environment can cause them to behave differently than in test tubes. The team modeled the inside of the smallest known bacteria—*Mycoplasma genitalium*—which is about 400 nanometers long. They dynamically modeled approximately a trillion atoms in the cell, making this one of the largest molecular dynamic simulations (see image). Even harnessing the enormous power of 65,536 processing cores of the K computer, the calculations took several months.

The results cast doubt on the prevailing assumption that in the crowded cellular environment, interactions between molecules are primarily governed by a phenomenon known as the volume exclusion effect. This effect implies that molecules monopolize a certain volume of a solvent—in this case water—in the solution around them, keeping other molecules from occupying that space. In contrast, the simulation found that other interactions, such as the electrostatic interactions between charged molecules, play a major role.

"This work has shown that there are major differences between *in vitro* conditions and the *in vivo* conditions in the cell," says Isseki Yu of the RIKEN Interdisciplinary Theoretical Science Research Group (iTHES). "We have found evidence for interactions beyond the volume exclusion effect, including protein–protein interactions and electrostatic interactions with ions and metabolites. These need to be taken into account when interpreting *in vitro* studies."

"This research has brought us one step closer to the dream of simulating a complete cell at the molecular scale," says Yuji Sugita, who led the project and is affiliated with iTHES, the RIKEN Theoretical Molecular Science Laboratory, the RIKEN Advanced Institute for Computational Science, and the RIKEN Quantitative Biology Center. "It will also contribute to drug development, as previous studies usually looked at interactions between proteins and a single candidate compound within water. Now, we will



A computer simulation of parts of a cell from the bacteria *Mycoplasma genitalium* shows how molecules behave in the extremely crowded environment of the cell. A close up of the model (right) reveals atom-level details.

be able to also analyze the interactions between the candidate compound and other molecules within the crowded cellular environment."

"This work is a large step forward toward the modeling of an entire cell in atomistic detail," says Michael Feig, who co-led the project at Michigan State University. "It will ultimately allow us to connect what we know at the molecular level with biological function at the cellular level." ■

Reference

1. Yu, I., Mori, T., Ando, T., Harada, R., Jung, J., Sugita, Y. & Feig, M. Biomolecular interactions modulate macromolecular structure and dynamics in atomistic model of a bacterial cytoplasm. *eLife* **5,** e19274 (2016).

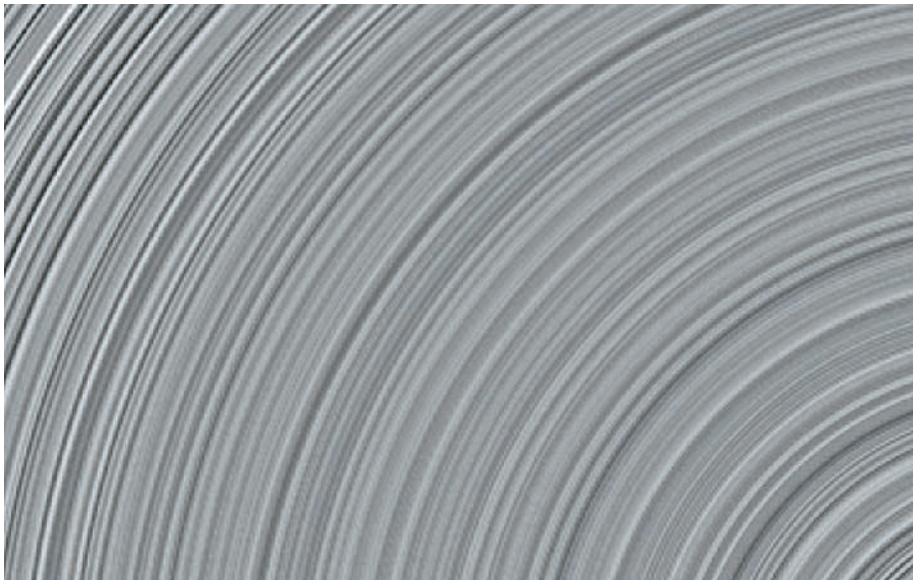
PHYSICS

The cosmic consequences of imperfection

Almost imperceptible imperfections in camera sensor technology will not hold back the search for cosmic dark matter and dark energy

A quest to find the enigmatic dark matter and energy thought to pervade the Universe will not be hampered by small imperfections in the devices used to detect them, researchers from the RIKEN Nishina Center for Accelerator-Based Science have ascertained[1].

The detectors are part of the Large Synoptic Survey Telescope (LSST) under construction in Chile, which will take super-precise snapshots of the entire visible sky every few days, for ten years. "There was much discussion and concern about how these detector issues could disturb the surveys," says Yuki Okura, who led the research.



'Tree ring' imperfections in the digital camera sensors of the next-generation Large Synoptic Survey Telescope (LSST) being constructed in Chile will have a negligible effect on scientific output.

The expansion of the Universe began with the Big Bang and later began to accelerate. But one of the biggest conundrums in cosmology is that the expansion should be slowing based on the amount of cosmic matter we can see. Astrophysicists now agree that the reason for this discrepancy lies in what we cannot see —dark energy, which is estimated to account for 70 per cent of the energy of the Universe.

Since dark matter and dark energy do not emit light of any kind, they have to be detected indirectly from the way that dark matter's mass bends light, an effect known as gravitational lensing. "This changes the apparent shape of distant galaxies, which provides information about the dark matter along the light path," says Okura.

By capturing extremely precise images of the billions of galaxies in the visible sky over time, the LSST will enable scientists to observe, for the first time, these vanishingly weak distortions in light emission caused by dark matter. But if the LSST is to be effective, every other possible source of distortion, including in the telescope's design and fabrication, will need to be accounted for.

"We wanted to be sure that the shape changes we detect are accurate and not the result of defects in the digital sensors that detect the light," explains Okura.

The sensors used to capture the digital images are manufactured from silicon wafers, which have 'tree ring' variations (see image) due to the way the silicon is grown. And the technique used to etch pixels on the sensor also produces periodic imperfections.

"Starting from measurements of the sensors in the laboratory, we calculated the image shape changes due to these imperfections and the effect they would have on our measurement of dark matter and dark energy," says Okura. "Fortunately, we were able to show that the effect is small enough to be neglected." ■

Reference

1. Okura, Y., Petri, A., May, M., Plazas, A. A. & Tamagawa, T. Consequences of CCD imperfections for cosmology determined by weak lensing surveys: From laboratory measurements to cosmological parameter bias. *The Astrophysical Journal* **825,** 61–70 (2016).

Gut microbiome

Meeting our resident gut microbes

Over the past decades, researchers at RIKEN have advanced our understanding of the role in health and disease played by bacteria in the human gut. Microbiologist Yoshimi Benno has used cell culture and genomic tools to describe the relationship between diet, the gut microbiota, and wellbeing. Immunologist Hiroshi Ohno has extended the analysis to an investigation of how gut bacteria communicate with immune cells. Combining Benno's focus on bacterial composition with Ohno's research into their function has led to many important insights.

Yoshimi Benno is head of the Benno Laboratory at the RIKEN Innovation Center
Benno's research interests are in microbial taxonomy and the gut microbiota, with a specific focus on finding new ways to culture bacteria and investigate the effect of functional foods, probiotics and prebiotics on gut bacterial communities.

Herbivores have demanding eating habits. Cows spend a third of their lives grazing. Koalas devote their few waking hours to chewing on eucalyptus leaves. And giant pandas can consume more than 12 kilos of bamboo a day. All of them rely on microbes in their guts to break down the long sugar molecules and digest their cellulose-rich meals.

Humans are no different. Our intestines host trillions of bacteria that produce essential nutrients from the foods we eat. Microbiologists have long been interested in these indigenous microbes, and, until two decades ago, the only way to study them was to isolate and grow the bacteria on a dish. Researchers at RIKEN have discovered hundreds of new bacterial species in this way, through more than 60 years of work in the area using innovative breeding techniques to simulate the internal gut environment, for example by tightly sealing fecal samples in glass jars.

But, culturing these bacteria has proven trickier than expected. Their nutrient regimens are often unknown, and growing them on conventional broths would be like putting a koala on a chocolate diet. Just as humans rely on bacterial products to survive, so do the bacteria themselves. At RIKEN, we developed a membrane filter technique that used the products of one group of bacteria to grow another group, which has introduced us to several more previously unidentified gut bacteria[1].

> *Of the species represented in the human intestines—as many as a thousand—more than half are still believed to be uncultured.*

Nevertheless, of the species represented in the human intestines—as many as a thousand—more than half are still believed to be uncultured.

Culture free

In the mid-1990s, researchers began exploring ways to study these bacteria without having to culture them. These molecular methods offered a more holistic picture of the gut microbiota. We developed one such process at RIKEN for comparing the diversity of microbial populations between people, called terminal restriction fragment length polymorphism (T-RFLP). The technique involves fluorescently tagging and multiplying all of the bacterial DNA in a sample. Special enzymes then chop the DNA at identically coded regions, and the strands are sorted. Fragments with similar sequences and lengths typically belong to bacteria of the same species. We first used this technique to observe significant differences in the fecal microbiota of patients with inflamed and ulcerated intestines compared to healthy individuals.

More advanced, high-throughput gene sequencing technologies later emerged to greatly speed up the process of discovery.

Then in 2006, American biologist Jeffrey Gordon published a paper

in *Nature* that blew us all away. The paper revealed that obese and lean mice and humans had different microbial compositions. I couldn't believe it. How could such a small community confined to tubular networks in the lower abdomen control the state of the entire body? Might there be more to their powers than previously imagined? Several years later, I found evidence of microbial influence not just on obesity, but on hormone levels in the brain and on aging. Sanitized mice raised in germ-free conditions produced twice as much dopamine compared to those injected with germs from conventional mice. We even found differences in the microbial balance of elderly individuals living in urban towns and those from villages famous for their longevity.

Crystal bowel

Alongside these studies, many researchers also wanted to find out how they could control, and potentially alter, microbial diversity. Diet was an obvious conduit. In the mid-1980s, a colleague and I made ourselves the subjects of such experiments by living on an entirely carnivorous diet. Every day, for 40 days, we consumed 1.5 kilograms of beef, and then sustained ourselves for the next two weeks on vegetables. We observed a distinct change in the bacterial cultures grown from our fecal samples.

Molecular studies extended the depth and breadth of such analyses. Our lab has collaborated with several companies to test the effects of different foods on gut microbial diversity, and human health. For example, mice fed a mixture of the amino acid arginine and friendly *Bifidobacterium* produced more polyamine compounds in their colons. In the long-term, they experienced less inflammation and age-related memory loss, and lived longer than mice fed a regular diet.

But food isn't the sole determinant of microbial diversity. Age, gender, body mass index, exercise, and smoking habits all play a role. My lab conducted a T-RFLP analysis on close to 4,000 stool samples donated by Japanese who have attended my public lectures over the years, and identified 8 distinct microbial profiles, from the seaweed lover to the elderly female. In 2014, I collaborated with Yu Sawai, who launched a company that provides paying customers with stool reports. Preventing disease could soon be as simple as tracking our bowel movements.

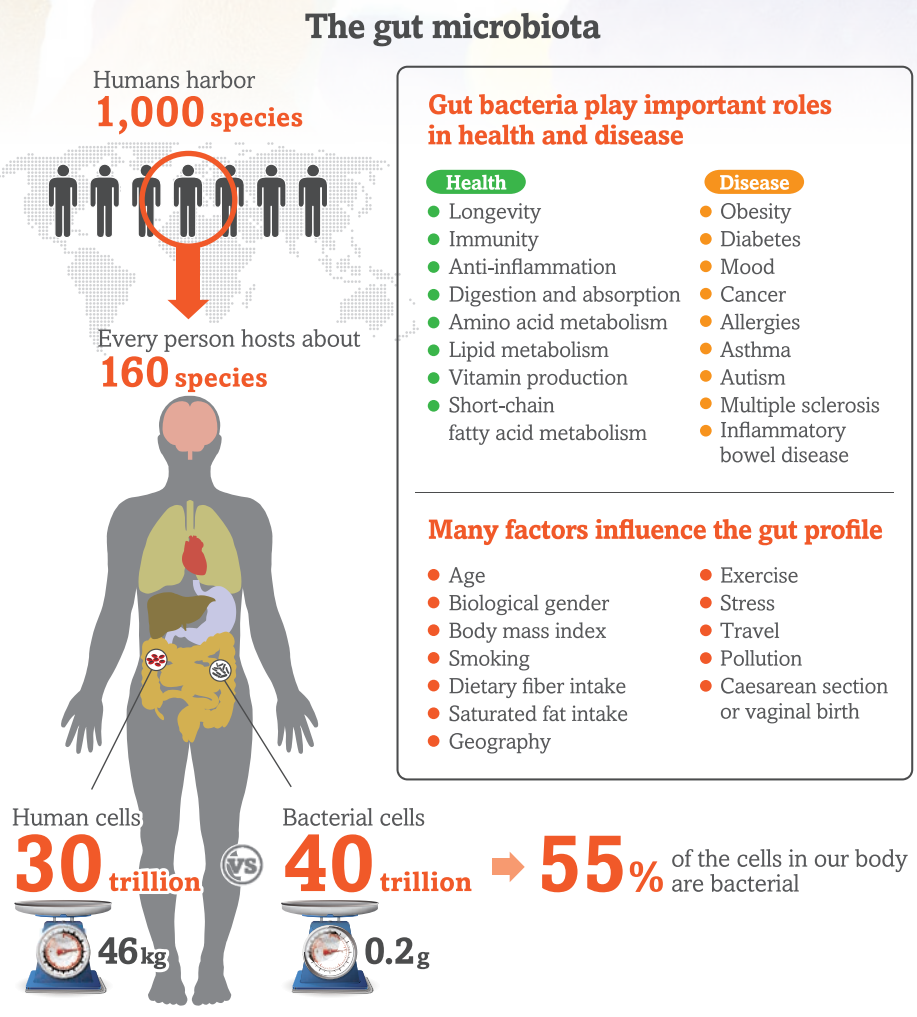


Figure 1: The gut microbiota play an important role in health and disease.

Hiroshi Ohno is group director of the Laboratory for Intestinal Ecosystem at the RIKEN Center for Integrative Medical Science

Ohno's primary research interests involve the relationship between the host and gut microbiota, with a focus on intestinal epithelial M cells, and the role of gut microbiota in the intestinal immune system as well as in host health and disease.

When the world's geneticists finished sequencing the human genome in 2003, they went looking for their next big project. Some codified the oceans, others took to transcribing chimpanzees, and a few strung together Neanderthal DNA from fossilized bones. RIKEN chose to be more introspective. In a pivotal meeting between director of the former RIKEN Genomic Sciences Center, Akiyoshi Wada, and microbiologist Tomotari Mitsuoka, the two decided to focus their sequencing efforts on the human gut microbiota. I was invited to the meeting and, as an immunologist specializing in the intestines, was greatly in favor of the decision.

By the early 2000s, microbiologists working with gut microbes had a general sense of what they did, especially in their capacity as nutrient couriers. But the specifics were hazy when it came to their protective role. Immunologists knew, for example, that germ-free animals were more susceptible to infections, which meant that the immune system was somehow working together with the gut bacteria. Genomics offered a way to capture the conversation.

Shotgun sequencing

The most popular genomics technique is known as shotgun sequencing. Typically, ultrasonic waves fired at an entire population of microorganisms taken from a sample slice the bacterial DNA sequences at random locations, and the fragments are sequenced and analyzed to produce a distinct genomic profile of the population.

By profiling samples from different individuals, researchers can begin to distinguish between healthy and unhealthy communities. The guts of individuals with diabetes, obesity or inflammatory bowel disease look very different from those of healthy individuals. Some studies have even found that transplanting fecal microbes from a healthy person to, for example, someone infected with the pathogenic bacteria *Clostridium difficile*, can restore the microbial balance to cure the disease.

> *The immune system maintains a cautious friendship with its resident microbes, the way one would with a tantrum-prone child.*

The immune system maintains a cautious friendship with its resident microbes, the way one would with a tantrum-prone child—generally a source of enjoyment until a sudden flare-up brings immeasurable grief. Shotgun studies were able to introduce the different parties involved in the relationship, but I wanted to find the interpreter. In 2009, we conclusively pinned that role down to M cells lining the gastrointestinal tract, distinguished by their lack of a brush-like fringe typical of most epithelial cells (Fig. 2).

To initiate an immune response, M cells engorge bacteria and spit them out the other end. We used a microarray-based approach to screen for genetic material specifically expressed by M cells, and discovered an mRNA called *Gp2*. Without Gp2 protein activity, mouse models could not defend themselves against harmful *Salmonella* Typhimurium.

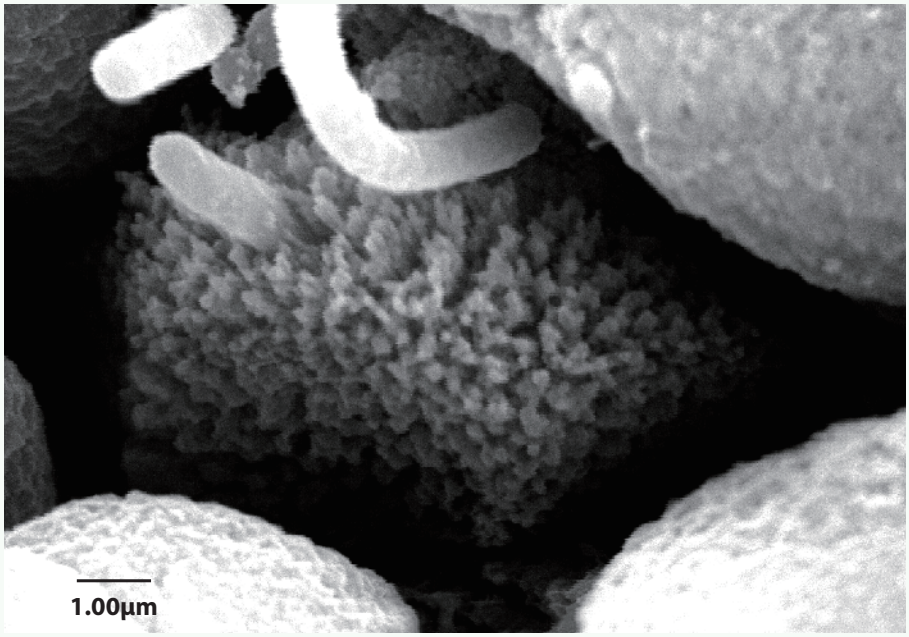


Figure 2: Specialized cells in the gut epithelium known as M cells (center), which appear more sunken than their adjacent cells, interact closely with filamentous bacteria (curled rods).

Microbial defense

Eventually even genomic studies reached their scientific limits. They had introduced immunologists to the multiple characters on the scene, but we wanted to understand the language of communication. To make sense of the internal noise, our lab at RIKEN consolidated the many different exhaustive studies of biological products, including catalogues of DNA and RNA, but also directories of proteins, lipids and metabolites.

Using this multi-omics approach, we discovered that a short-chain fatty acid, acetate, produced by beneficial *Bifidobacterium* is not only an essential source of energy, but also protects hosts from deadly infections. We later also found that another fatty acid, butyrate, produced by the faithful bacteria *Clostridia* can accelerate the differentiation of immune cells that suppress inflammatory and allergic responses[2]. These molecules transmit information that regulates our immune system.

We are applying similar comprehensive techniques, even combining clinical data, to identify the conveyors of other signals. I am leading a major initiative launched by the Japanese government in 2016 to invest millions of yen in research projects studying the crosstalk between the microbiome and host organisms. Ultimately we hope to eavesdrop on all the chatter in the human gut, between bacteria and immune cells as well as among the bacteria themselves.

References

1. Shimizu, H. & Benno, Y. Membrane filter method to study the effects of *Lactobacillus acidophilus* and *Bifidobacterium longum* on fecal microbiota. *Microbiology & Immunology* **59,** 643–652 (2015).

2. Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi,Y., Uetake, C., Kato, K., Kato, T. *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504,** 446–450 (2013).

For additional references, visit the online version of this article at:

www.riken.jp/en/research/rikenresearch/perspectives/8282

A fluorescence micrograph of *Arabidopsis*, which flowers when the days grow longer. This response to light, together with others, is mediated by photoreceptors known as cryptochromes.

BIOLOGY

Illuminating plants' response to light

Scientists have uncovered the mechanism by which plants respond to light levels

The mechanism that switches on a key photoreceptor in plants, allowing them to remain responsive to light, has been uncovered by an international team[1].

In addition to using light to produce energy via photosynthesis, plants exhibit other important responses to light. For example, they grow at night using energy stored during the day, and long-day plants begin to flower when the days grow longer and the nights shorter.

Blue light plays a critical role in activating plants' responses in natural light environments. It does this through the action of blue-light photoreceptors known as cryptochromes as well as other photoreceptors (see image). But the mechanism through which this response is turned on and off had remained elusive.

Some scientists have suggested that cryptochromes are activated and deactivated through photoreduction—a similar process to that used in photosynthesis, in which energy is moved across molecules by the transfer of electrons.

To determine whether this was the case, the researchers screened transgenic lines of *Arabidopsis*—a model grass used in plant genetics—to find lines that expressed phenotypes similar to a mutant strain that does not respond properly to blue light. They identified lines that overexpress a protein called BIC1, which corresponded to the mutant phenotype. The team determined that this

protein blocks the action of cryptochrome 2, a key photoreceptor.

The scientists also uncovered the mechanism by which this takes place. They found that it does not rely on photoreduction; rather, cryptochrome 2 undergoes a change in its three-dimensional structure—taking a dimer form when exposed to blue light. This, the active form, disappeared in the presence of the BIC1 protein.

"We have shown that there is a desensitization mechanism, where the photoactivated photoreceptor is regulated in blue light to avoid excess response," explains Minami Matsui of the RIKEN Biomass Engineering Research Division. "This is important as it allows plants to maintain the homeostasis of their blue light responsiveness in order to adapt to the fluctuating light environment in nature."

The study will help scientists develop better plants. "Through this work, we hope to learn how we can use the action of BIC1 to develop plants with better biomass characteristics." Matsui adds that the finding could have implications for animals as well: "This work is also important because animal cryptochromes also form homodimers, and this can help us gain clues into how the circadian rhythm is maintained in animals," adds Matsui. ■

Reference

1. Wang, Q., Zuo, Z., Wang, X., Gu, L., Yoshizumi, T., Yang, Z., Yang, L., Liu, Q., Liu, W., Han, Y.-J. *et al.* Photoactivation and inactivation mechanisms of *Arabidopsis cryptochrome 2*. *Science* **354,** 343–347 (2016).

CHEMISTRY

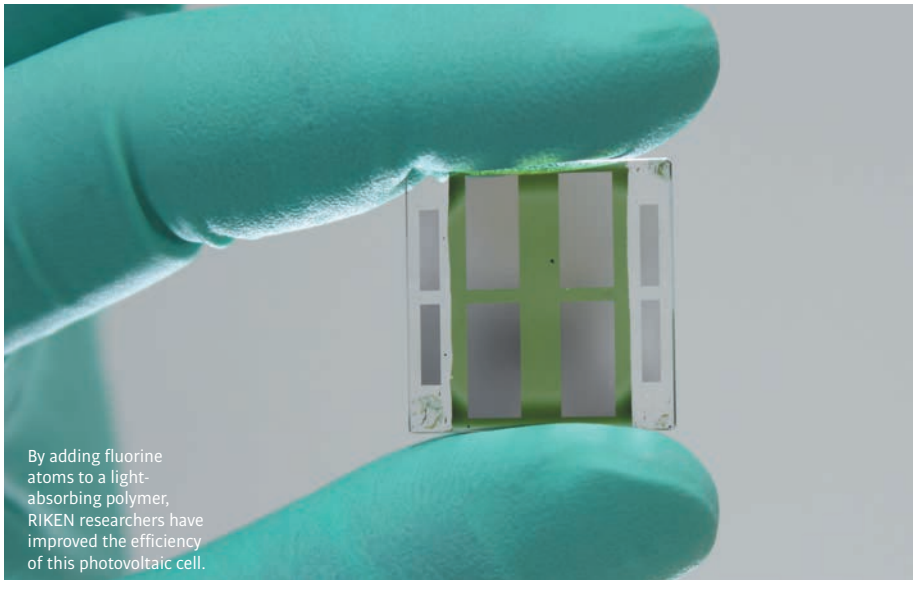
Fluorine offers solar power boost

Tweaking the chemical composition of polymer solar cells improves efficiency and voltage

Adding fluorine atoms to light-harvesting polymers could help to improve their performance in flexible solar cells, researchers at RIKEN have found[1].

Polymer solar cells use semiconducting polymers to absorb light, which kicks electrons in these molecules from the ground state to an excited state with a higher energy. An electron-accepting material then channels the excited electrons toward an electrode, thereby generating an electrical current.

Semiconducting polymers have the advantages of being lightweight, flexible and semi-transparent, and potentially they could be used in low-cost photovoltaic panels. But the best polymer solar cells convert only about 10 per cent of the light that falls on them into electrical power—roughly half the efficiency of conventional solar cells, which are based on inflexible silicon.



By adding fluorine atoms to a light-absorbing polymer, RIKEN researchers have improved the efficiency of this photovoltaic cell.

Now, Itaru Osaka of the RIKEN Center for Emergent Matter Science and his colleagues have shown that incorporating fluorine atoms into a photoactive polymer offers potential improvements. They studied a polymer that contains two types of chemical groups—naphthobisthiadiazole (NTz), and thiophene units. The scientists added two fluorine atoms to some of those thiophene units to make a new polymer dubbed PNTz4TF2 and four fluorine atoms to form PNTz4TF4.

The team found that adding fluorine lowered the energy level of molecular orbitals in the polymers. This increased the energy difference between them and the electron acceptor, resulting in a higher output voltage and reduced energy losses in the system in both cases.

For PNTz4TF2, attractions between fluorine atoms and other atoms in the polymer also made the polymer strands more rigid, helping to produce a more crystalline material, which

allowed more rapid movement of electrical charge through it.

The team then blended each polymer with an electron acceptor called PC71BM, which contains a ball of carbon atoms known as a fullerene. In the case of PNTz4TF2, a 230-nanometer-thick layer of this blend achieved an efficiency of 10.5 per cent, which is slightly more than that of the equivalent fluorine-free polymer. "It is a small but significant improvement," notes Osaka.

The researchers are confident that there is room for further improvement as they anticipate being able to increase the solubility of the materials by tweaking the chemical composition of the fluorinated polymers. This should improve the fabrication process of the solar cells, further boosting the power conversion efficiency and voltage. "The next stage may be developing further high-performance polymers showing efficiencies of 12 per cent, or hopefully even 15 per cent," says Osaka. ■

Reference

1. Kawashima, K., Fukuhara, T., Suda, Y., Suzuki, Y., Koganezawa, T., Yoshida, H., Ohkita, H., Osaka, I. & Takimiya, K. Implication of fluorine atom on electronic properties, ordering structures, and photovoltaic performance in naphthobisthiadiazole-based semiconducting polymers. *Journal of the American Chemical Society* **138,** 10265–10275 (2016).

MEDICINE

New drug tackles cancer at the stem

A drug that targets the production of cancer stem cells could stop therapy-resistant cancers from spreading in the body of bowel cancer patients

A drug that inhibits the development of cancer stem cells has been discovered by researchers from the RIKEN Center for Life Science Technologies, Carna Biosciences, Inc. and Japan's National Cancer Center (NCC)[1]. The inhibitor may provide a new therapy option for patients with bowel cancer that does not respond to established therapies.

There is increasing recognition that cancer is driven by special cells—cancer stem cells—that resist treatment and go on to cause recurrences, which are resistant to the original therapy. Researchers are hence trying to develop ways to target cancer stem cells.

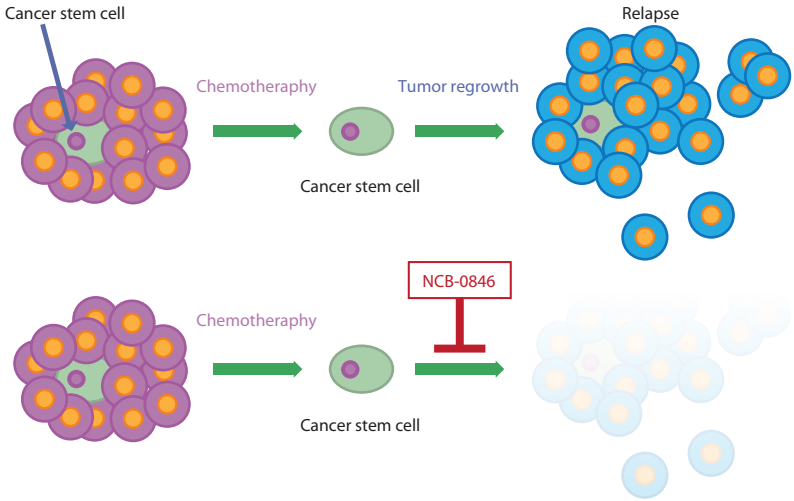
Bowel cancer is a significant cause of cancer death, accounting for 700,000 deaths annually worldwide. More than 90 per cent of bowel cancers carry mutations in Wnt signaling component genes, such as the *adenomatous polyposis coli* (*APC*) tumor suppressor gene. These mutations activate Wnt signaling, which leads to the generation of cancer stem cells.

Thus, therapeutics that block Wnt signaling are likely to eradicate cancer stem cells and cure the disease (see image). However, despite a wealth of data and research investment, no Wnt-inhibiting drug has been developed clinically.

Now, the team of researchers has developed a new compound, NCB-0846, which blocks Wnt signaling.

Previously, NCC researchers had identified Traf2- and Nck-interacting kinase (TNIK) as an essential regulatory component of the T-cell factor-4–β-catenin complex. TNIK regulates Wnt signaling in the most downstream part of the pathway, and its pharmacological inhibition has been anticipated to block the signal, even in bowel cancer cells with a mutation of the *APC* gene.

The team discovered NCB-0846, which can inhibit the kinase activity of TNIK, by screening a kinase-focused compound library and performing lead optimizations. X-ray co-crystal structure analysis revealed that NCB-0846 binds



Cancer stem cells are intrinsically resistant to conventional chemotherapy and radiotherapy. Cells that survive these treatments often grow back and eventually cause cancer relapse (top panel). Therapeutics that block the aberrant Wnt signaling are likely to eradicate the cancer stem cells and cure the disease (bottom panel).

to TNIK in an inactive conformation, which is likely to be essential for Wnt inhibition.

NCB-0846, which can be taken orally, was found to suppress the growth of patient-derived bowel cancer xenografts. It suppressed various cancer stem cell activities of bowel cancer cells and their expression of cancer stem cell markers.

"We hope that this new compound may give hope to patients with drug-refractory bowel cancer, as there is of yet no Wnt-inhibiting drug in clinical practice," says RIKEN team member, Mikako Shirouzu.

"We're very encouraged by our promising preclinical data for NCB-0846, especially considering the difficulty in targeting this pathway to date, and shortly we hope to conduct a clinical trial at the NCC hospitals," adds Tesshi Yamada of the NCC.

NCB-0846 is currently undergoing preclinical development.

Reference

1. Masuda, M., Uno, Y., Ohbayashi, N., Ohata, H., Mimata, A., Kukimoto-Niino, M., Moriyama, H., Kashimoto, S., Inoue, T., Goto, N. *et al.* TNIK inhibition abrogates colorectal cancer stemness. *Nature Communications* **7,** 12586 (2016).

PHYSICS

The height of accuracy

Two ultraprecise clocks, located several kilometers apart, have been used to measure differences in height to an accuracy of a few centimeters

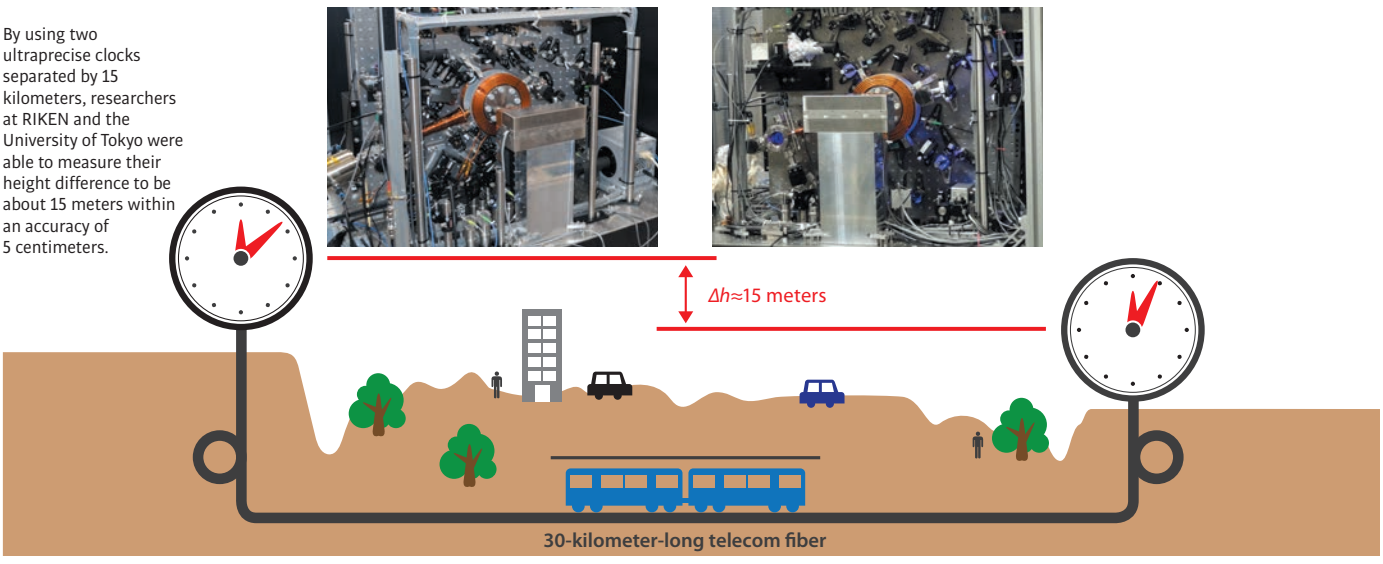
Researchers at RIKEN have created two clocks so accurate that they can be used to measure the difference between their elevations, even when separated from each other by 15 kilometers[1]. The clocks could eventually be used to monitor seismic activity.

Using clocks to measure height differences, rather than just time, may seem bizarre, but it is a natural consequence of Einstein's general theory of relativity, which predicts that the passing of time depends on the gravitational field. For example, a clock on the summit of Mount Everest will run ever so slightly faster than one at sea level, causing it to gain roughly 1 second every 30,000 years. So by measuring the differences in the times of two sufficiently accurate clocks, it is possible to estimate the difference in their heights.

By exploiting this effect, Hidetoshi Katori of the RIKEN Quantum Metrology Laboratory and co-workers measured the height difference between the two ultraprecise clocks to be about 15 meters, within an accuracy of 5 centimeters. This measured height difference was consistent with independent measurements made using conventional techniques for measuring elevation.

The clocks, known as optical lattice clocks, have been developed by the team over the last decade. They were linked to each other by an underground optical fiber (see image). "This is the first time that such ultraprecise clocks have been connected between distant laboratories," comments Katori.

This demonstration has two important implications. First, since the accuracy of the clocks is about 100 times higher than that of the SI definition of the second, they necessitate



By using two ultraprecise clocks separated by 15 kilometers, researchers at RIKEN and the University of Tokyo were able to measure their height difference to be about 15 meters within an accuracy of 5 centimeters.

a new, more accurate definition of the second. Katori envisages that this could happen in about a decade.

Another implication is that such clocks could be used to realize what Katori calls an "internet of clocks"—a network of ultraprecise clocks. "Such a clock network would function as a geopotential meter that can be used to monitor the motion of massive objects underground," explains Katori. This would allow scientists to monitor changes in seismic activity. If the network can be extended to a global scale, it could aid cosmologists' search for the elusive dark matter predicted to exist in the Universe.

The team has even loftier goals for the future. "By further improving the optical lattice clocks, we hope to demonstrate millimeter-level chronometric leveling in 2 to 3 years," says Katori. "In the future, we dream of replacing conventional height benchmarks by networked clocks." ■

Reference

1. Takano, T., Takamoto, M., Ushijima, I., Ohmae, N., Akatsuka, T., Yamaguchi, A., Kuroishi, Y., Munekane, H., Miyahara, B. & Katori, H. Geopotential measurements with synchronously linked optical lattice clocks. *Nature Photonics* **10,** 662–666 (2016).

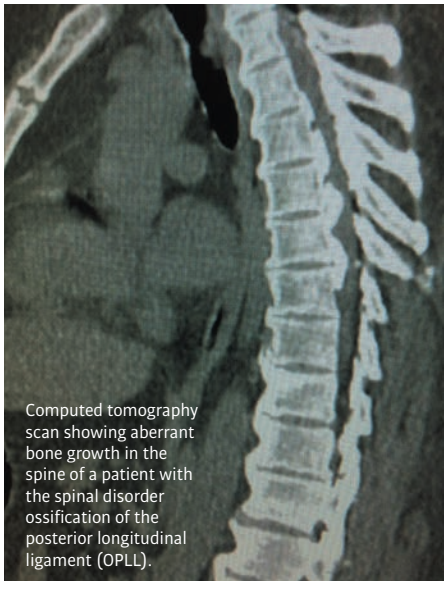
BIOLOGY

Sending bone growth down the spine

Gene causes unwanted bone growth in a spinal disorder common among Asian people

RIKEN researchers have shown how a genetic mutation increases the risk of a spinal disorder, which is prevalent throughout Asia.

Ossification of the posterior longitudinal ligament (OPLL) of the spine is a degenerative disease that afflicts more than 2 per cent of people in Japan. It results when bone starts developing in the soft tissue of the spinal cord, leading to nerve compression, pain and numbness—debilitating symptoms that can affect daily life.



Computed tomography scan showing aberrant bone growth in the spine of a patient with the spinal disorder ossification of the posterior longitudinal ligament (OPLL).

Over the years, scientists have found several gene variants that occur more often in people with OPLL, but it was not known definitively if or how any of these genes contributed to the patients' aberrant bone growth. So a team led by Shiro Ikegawa at the RIKEN Center for Integrative Medical Sciences tested the role of one potential susceptibility gene, known as *RSPO2*.

The team previously conducted a comprehensive gene sleuthing study, which found six sites in the genome that seemed to confer disease risk. One of these genomic segments included the gene *RSPO2*, which carries the instructions for making a protein called R-spondin 2. The protein was known to play a critical role in skeletal development, but the researchers wanted to drill deeper into how *RSPO2* works at a molecular level. They used mouse and human bone precursor cells to examine gene expression in a lab dish.

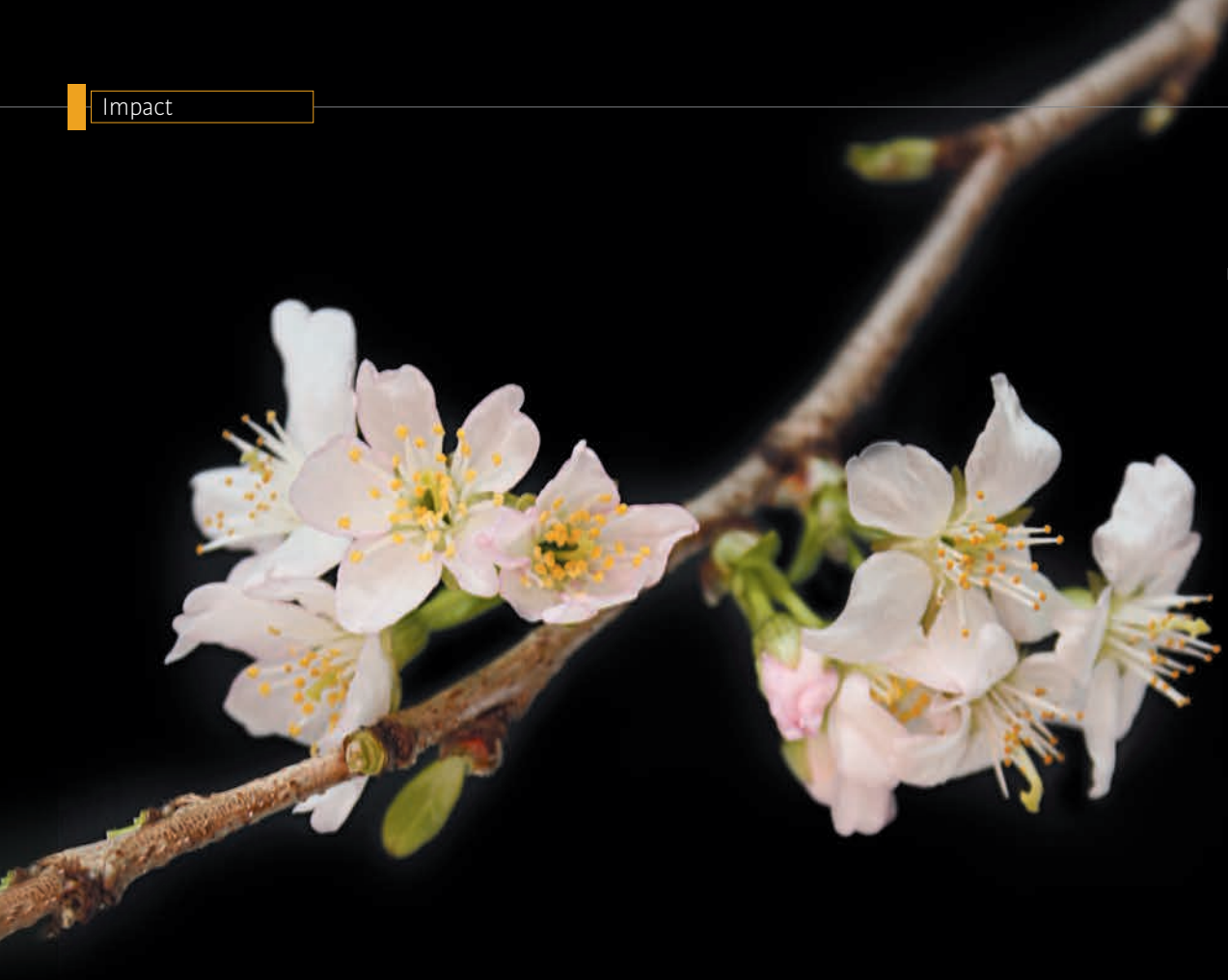
They showed that *RSPO2* normally acts to put the brakes on bone formation. Its presence inhibits the development of the early cartilage cells, which are later replaced by bone cells. However, the gene variant linked to OPLL decreases gene activity by changing the promoter region—the 'on switch'—of *RSPO2*[1]. Specifically, the disease-associated variant alters the binding of a transcription factor, which would otherwise allow *RSPO2* expression and thereby halt bone growth. Without this binding, stem cells in the spinal cord get misdirected into bone.

"Our findings provide new insights into the etiology and pathogenesis of OPLL, as well as a new target for treatment of the disease," says Masahiro Nakajima, who led the study. If drug companies can find a way to block the pathway unleashed by low *RSPO2* expression, Nakajima explains, they could help improve the lives of people with the disease.

The team plans to investigate the role of other potential OPLL genes in search of additional drug leads. "We believe it is possible to find other causal variants and susceptibility genes using the same approach," Nakajima says. ■

Reference

1. Nakajima, M., Kou, I., Ohashi, H., Genetic Study Group of the Investigation Committee on the Ossification of Spinal Ligaments & Ikegawa, S. Identification and functional characterization of *RSPO2* as a susceptibility gene for ossification of the posterior longitudinal ligament of the spine. *American Journal of Human Genetics* **99,** 202–207 (2016).



Nishina Otome is a heavy-ion bred cherry blossom that flowers all year round.

Delightful mutants

Tomoko Abe has bombarded thousands of plants with heavy ions to create unusual varieties

"These tearless, non-pungent onions will not only wipe tears away from the kitchen and the food processing facilities, but also will add a new dimension to the enjoyment of onion recipes around the world." This was the proud prediction of a team of Japanese researchers, including Tomoko Abe at the RIKEN Nishina Center for Accelerator-Based Science, in April 2016, in a paper describing the use of heavy-ion beams to breed sweet-smelling onions. Japanese curry company House Foods Corporation has since begun selling the onions as 'Smile Balls'.

But Abe has more than onions to smile about. Two decades ago, she became the first researcher to use a heavy-ion accelerator to generate mutant organisms. In that time, her group has invented a bright-pink petunia, a deep-red dahlia and a seedless verbena with many flowers that can be admired for a long time. More recently, they have begun producing new varieties of extremely nutritious, healthy and potent plants, fungi and single-celled organisms, from salt-resistant rice to hypoallergenic peanuts and sweet wormwood that produces increased amounts of a compound that kills malaria parasites.

Heavy ions

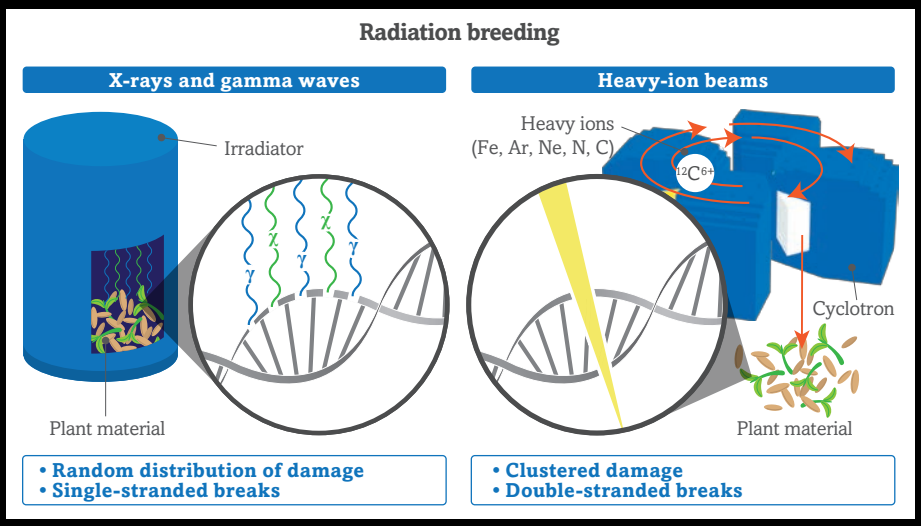
Mutations are the catalysts of genetic diversity. They occur spontaneously in nature, contributing to evolutionary processes that have shaped millions of species. Over millennia, plants and animals have developed many unique qualities that humans have exploited. Early explorers would travel exhaustively in search of exotic spices, legumes and herbal elixirs. Eventually, humans learned how to cultivate desirable traits, whether by selection and interbreeding, genetic modification or even controlled mutation.

Mutation breeding dates to the early 1920s, when American geneticists discovered x-ray radiation could increase the rate at which genomic errors appeared in fruit flies, barley and maize. Other techniques were later developed, such as gamma rays and chemical triggers of mutagenesis, to achieve as much genomic variation as possible without killing the cultivars. However, these techniques took days to months to make stable mutations because they introduced breaks only to one strand of the two-stranded DNA molecule, which could be easily repaired by the cellular machinery.

Abe found a way to irreversibly break both strands of DNA, creating mutations within seconds. In 1991, soon after joining RIKEN, she went to visit the RIKEN Accelerator Research Facility (RARF) during the annual public open day. The RARF housed the largest cyclotron in the world, capable of accelerating heavy ions to half the speed of light to produce a swathe of irradiation, eight centimeters in diameter, with a uniform distribution of intensity and

sufficient energy to penetrate tissue. It had been a tool for physicists for years, but Abe wanted to use it to breed plants.

The particle accelerator turned out to be an excellent mutant generator, and Abe spent the next few years determining which ions produced the optimal number of errors for which species: lighter carbon ions were ideal for cracking shorter genomes of *Arabidopsis thaliana*, for example, and heavier neon ions for longer rice genomes. In one hit of radiation, Abe could jumble the codes of hundreds to thousands of seeds, or other plant material. The plants were then grown on plots of land across Japan through a network of almost 200 companies, universities, prefectural and national government bodies, and monitored for any marvelous attributes or curious features. When mutants were found, they were cloned.



X-rays and gamma rays cause fewer permanent mutations than heavy-ion radiation in the DNA of plant material.

Flower power

An estimated 3,200 radiation-bred varieties of plants have been formally recognized worldwide. Half of these were produced using gamma rays, one-sixth with x-rays, one-tenth doused in chemicals, and fewer than a hundred under heavy-ion radiation. Abe's group alone has produced 30 varieties that are either in the market or close to being launched.

The first plants were sold commercially in 2001 at a central wholesale market in Hiroshima. They matured into large dahlia flowers with deep-red petals. "The warm color was ideal for the winter season," says Abe. And with the entire country heating up over the FIFA World Cup they were hosting with South Korea the following year, it seemed appropriate to name the dahlia 'World'.

Several irradiated yeast strains are used to produce high-quality sake in Saitama.

Abe's team have collaborated with farmers to create many attractive varieties. In 2007, they produced the first cherry blossom trees with pure, pale-yellow petals. 'Nishina Zao' was named after the founding father of modern physics in Japan—Yoshio Nishina—and the Zao mountain ranges in central Japan, where the original sakura trees grow. "Our customers now wish for blue cherry blossoms," says Abe, who has seen a mutant with bluish-tinted blossoms under fluorescent lighting. "It isn't quite blue yet."

Timing is a critical factor for spring sakura gazing in Japan, but in 2009, RIKEN released a variety that could blossom all year round. 'Nishina Otome' was a hit, especially as a centerpiece in traditional flower arrangements during the winter New Year holidays.

Overall, the income generated by companies selling plants bred at RIKEN using heavy-ion beam irradiation has amounted to about 200 million Japanese yen annually.

High-value crops

In more recent years, Abe's focus has shifted from floral beauties to high-value crops. Her team worked with the Saitama Industrial Technology Center to create yeast that is used by 20 breweries across Saitama prefecture to ferment flavored rice wine. "They have a distinct sake smell, and taste expensive," she says. The team has also modified fast-growing algae to contain more fats and oils. Mass-production of the lipid-rich water plants could offer a commercially viable and sustainable source of biofuels.

Soon after the devastating tsunami washed over Japan's eastern coast in 2011, Abe collaborated with a local government office and Tohoku University to test salt-resistant rice varieties on the seawater-contaminated paddy fields. One irradiated strain of a local grain called Manamusume (the farmer's dear daughter) had higher yields than a staple strain. Since 2014, RIKEN has been collaborating with two other large accelerator facilities in Japan—TIARA and W-MAST—through the Cross-Ministerial Strategic Innovation Promotion project funded by the government to develop higher-yielding varieties of rice.

Using advanced genome sequencing technology, the consortium has also begun to pinpoint the exact location of the coding errors they introduce. "Mutants have become increasingly important in modern genetic studies," says Abe. "The discovery of genes using the combination of mutants and genome sequencing technology may lead to a new field in biology, 'mutagenomics'." ■

© 2016 RIKEN

Fundraising for the RIKEN Centennial Project

2017 will mark the centennial of RIKEN's founding. To continue to serve as a fundamental research institute trusted by society, RIKEN plans to take this opportunity to deepen its ties with those who have supported the institute in the past and to develop new ties in Japan and overseas. RIKEN will be collecting contributions to commemorate the centennial anniversary and to carry out the RIKEN Centennial Project.

Use of donations

- Centennial Project laboratories
- Future leader fund
- Improvement of research facilities
- Centennial celebration
- Collecting, preserving, and displaying historical materials and records

http://www.riken.jp/en/about/support
http://100th.riken.jp/en/
since_1917@riken.jp

RIKEN Vitamin

In 1924, RIKEN researchers Katsumi Takahashi and Umetaro Suzuki succeeded in isolating and extracting vitamin A from cod liver oil, for the first time in the world. The vitamin supplement was later mass produced and sold as the popular product RIKEN Vitamin. The RIKEN cyclotron technology was used in the industrial-scale enrichment of vitamin A by distilling cod liver oil and crystallizing vitamin A at low temperature.

RIKEN

centers and facilities across Japan and around the world

Sendai

Center for Advanced Photonics

Tsukuba

BioResource Center

Wako

Center for Emergent Matter Science
Center for Advanced Photonics
Center for Sustainable Resource Science
Brain Science Institute
Nishina Center for Accelerator-Based Science
Radioactive Isotope Beam Factory
Center for Advanced Intelligence Project
Interdisciplinary Theoretical and Mathematical Sciences Program
Advanced Center for Computing and Communication
Cluster for Industry Partnerships
Cluster for Science and Technology Hub
Chief Scientist Laboratories
Associate Chief Scientist Laboratories
Distinguished Senior Scientist Laboratories
Initiative Research Units
Special Research Units
Research Groups
Global Research Cluster

Yokohama

Center for Sustainable Resource Science
Center for Integrative Medical Sciences
Center for Life Science Technologies

Nagoya

Osaka

Quantitative Biology Center

Kobe

Center for Developmental Biology
Center for Life Science Technologies
Advanced Institute for Computational Science
K computer

Harima

SPring-8 Center
SPring-8 Synchrotron Radiation Facility
SACLA X-ray Free Electron Laser Facility

USA

RIKEN-MIT Center for Neural Circuit Genetics
RIKEN BNL Research Center

Europe

RIKEN-Max Planck Joint Research Center (Germany)
RIKEN-KFU Joint Research Labs (Russia)
RIKEN Facility Office at RAL (UK)

Asia

RIKEN Beijing Representative Office
RIKEN Singapore Representative Office
RIKEN-Tsinghua Research Groups (China)
RIKEN-XJTU Joint Research Center (China)
RIKEN-SJTU Joint Research Center (China)
RIKEN-SIOM Joint Research Laboratory (China)
RIKEN-NCTU Joint Research Laboratory (Taiwan)
USM-RIKEN International Center for Aging Science (Malaysia)
RIKEN-NCBS Joint Research Center (India)
RIKEN-JNCASR-IISc Joint Research Center (India)
RIKEN-KRIBB Joint Research Center (South Korea)

RIKEN

RIKEN
RESEARCH

www.riken.jp/en/research/rikenresearch

itaintmagic.riken.jp

www.facebook.com/RIKEN.english

www.riken.jp/en

@riken_en

www.twitter.com/riken_en

LinkedIn

www.linkedin.com/company/riken

RIKEN 2016-022