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FALL 2016

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RIKEN RESEARCH

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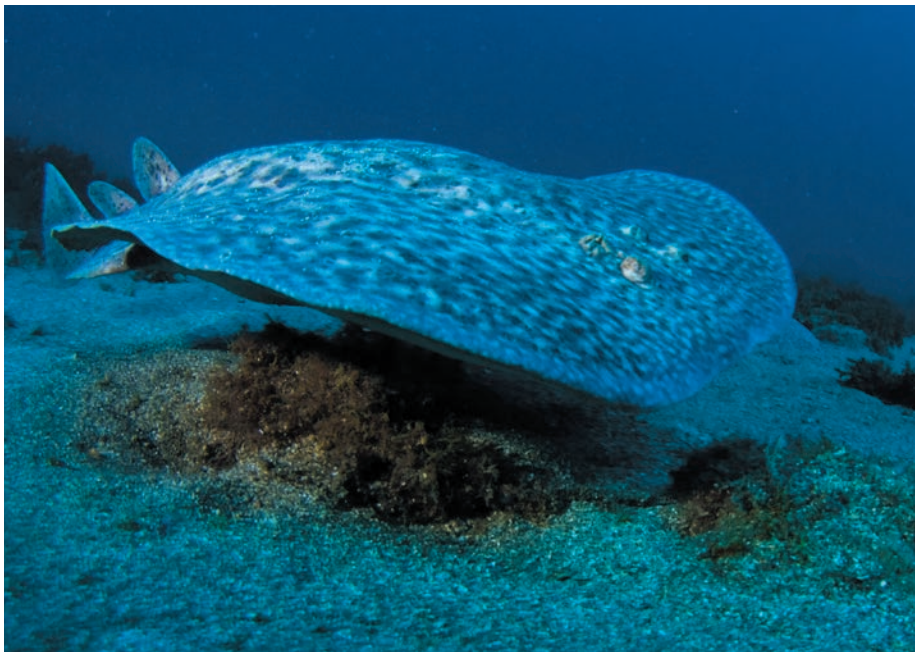

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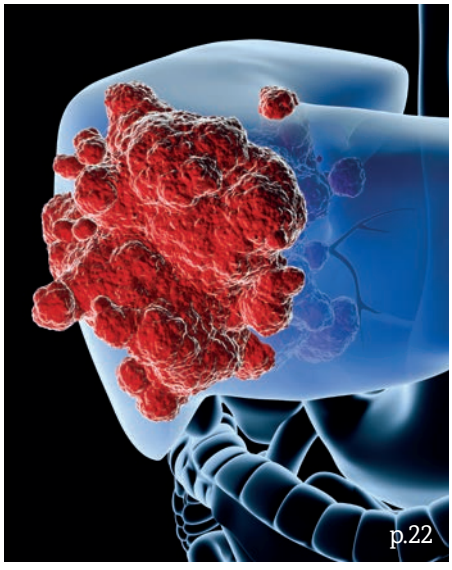

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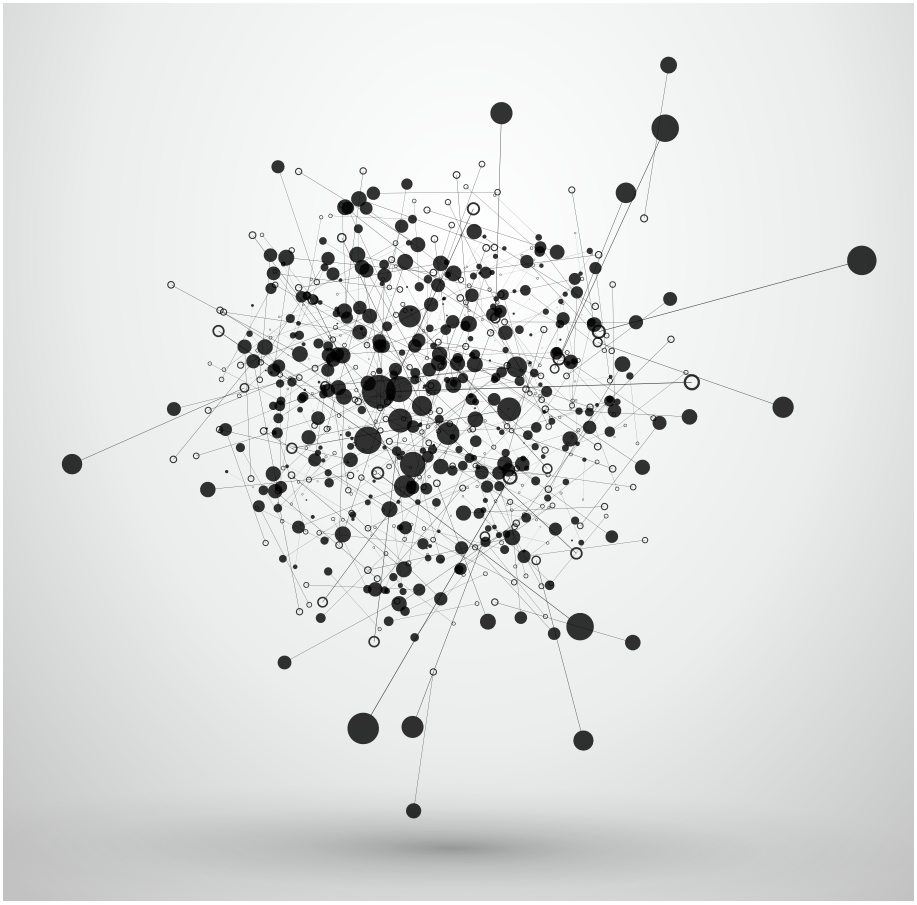

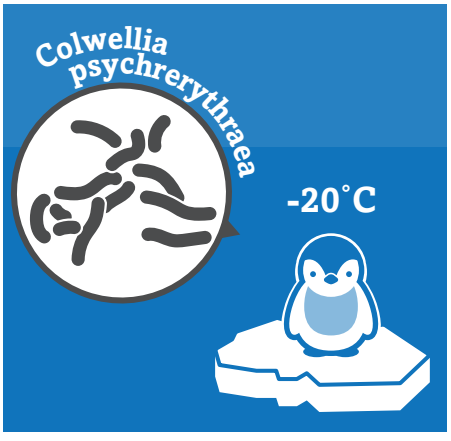

Asia's portal to a global genome database

Cover story: Organisms thrive even in the coldest regions of the Earth. **Page 34**

On 16 June 2016, the RIKEN Advanced Center for Computing and Communication and the University of California, Santa Cruz, (UCSC) launched an official replica of the enormous online register of genomic data, the UCSC Genome Browser Database. The first in Asia and the second in the world, the mirror site is designed to speed up access to the database for users in the region.

First created in 2000 to publish the entire human genome, the UCSC Genome Browser Database has become a powerful tool for analysis, hosting the entire assembly of nucleotide sequences for close to a 100 species. The database allows users to display a variety of data sets, such as those produced by the 1000 Genomes Project, ENCODE (Encyclopedia of DNA Elements), the International Cancer Genome Consortium, the International Human Epigenome Consortium (IHEC) and the RIKEN-led FANTOM5 (Functional Annotation of the Mammalian Genome) project.

Thanks to advances in next-generation sequencing technology, there has been a dramatic increase in published genomic data, which researchers in the life sciences have been studying for insights into the utility and function of genetic information. The Asian mirror site will facilitate further research in the field. Read about more such exciting developments at RIKEN in the "News" and "Briefs" sections.

The "Feature highlight" in this issue of *RIKEN Research* highlights research by Masanori Murayama at the RIKEN Brain Science Institute, who discovered a brain circuit that governs how sensory memories are stored during sleep. And the "Impact" article introduces readers to a host of organisms living in the most extreme environments, some even producing an enzyme used in the laundry detergent, Kao Attack, which is sold globally. We hope to bring you many more examples of the symbiotic relationship between science and society.

Building the mind of the post-K machine

Balazs Gerofi

Research Scientist

System Software Development Team
Flagship 2020 Project
RIKEN Advanced Institute for Computational Science

RIKEN is leading the design and development of the next-generation supercomputer —the successor of the K computer.

Please describe your role at RIKEN.
RIKEN is leading the design and development of the next-generation supercomputer—the successor of the K computer, which will be launched around 2020. Part of this effort is developing the system software, and I am working on its operating system and input–output subsystem.

System software is considered an enabler for conducting science in its traditional sense. Research in many fields—from climate modeling to drug discovery, astrophysics and alternative energy—has shifted entirely to computer simulations and many of these domains require large supercomputers to expand the state of the art.

How did you become interested in your field?
I have been fascinated with computers since my early childhood. My father also worked as a computer engineer and taught me the fundamentals of programming while I was in elementary school. I went to university to study mathematics and program design, and became a researcher to pursue my dreams of exploring the unknown.

What excites you most about your field?
Operating system code is at the heart of computers; a small change in the operating system 'kernel' can have a huge impact on everything else that runs on the system. With supercomputers, this becomes even more exciting because there are so many components connected to each other.

What has been the most interesting discovery in your field in recent years?
'Discovery' might not be the appropriate word for system software research because of the field's engineering nature. Of course, there have been breakthrough ideas in computer science. One very influential concept was the recognition of a single computer's resemblance to distributed systems. The growing number and diversity of components in computers led to specialized operating systems running side-by-side, an approach RIKEN is also pursuing for the post-K machine.

How has being at RIKEN helped your research?
Japan has been at the forefront of supercomputing for a long time. RIKEN is particularly appealing because it hosts the K computer and leads the development of the next-generation Japanese supercomputer. Access to cutting-edge hardware and a strong emphasis on international collaboration at RIKEN have been highly beneficial to my research. Recently, I had the opportunity to visit Intel Corporation in the United States for a couple of months, which gave me insights into the direction in which the computer-hardware field is headed.

What has been your most memorable experience at RIKEN?
One of my most memorable experiences was the new-year rice cake pounding ceremony (*mochitsuki*). I learned first-hand how rice cakes are made, and got to hammer down some *mochi*, which was quite good exercise and a lot of fun.

What do you wish you had known before arriving in Japan?
I am originally from Hungary. One of the most crucial things in moving to Japan is language ability, and I wish that I had put more effort into that in the beginning. By the same token, I think RIKEN should also promote more frequent interactions between Japanese and foreign researchers within the institution. ■

Clearing the brain

Meng-Tsen Ke

Foreign Postdoctoral Researcher

Laboratory for Sensory Circuit Formation
RIKEN Center for Developmental Biology

Please describe your current research.
I work on the application and assessment of optical clearing methods for imaging large brain tissues. Optical clearing enhances our ability to see deep into the brain by rendering tissue transparent. Combining these techniques with genetic labeling and advanced microscopy allows for the three-dimensional (3D) reconstruction of intact tissues such as neuronal circuits or solid tumor cell clusters.

How did you become interested in this field of research?
I started my PhD by dissecting neuronal circuits in the mouse olfactory system responsible for the sense of smell. But the lack of a suitable tissue clearing method made dissections difficult and time consuming. To reconstruct the mouse brain, I had to first slice samples into thin sections, image each section separately, and then carefully stitch them back together to get a total sum of the volume. The stitching process required a lot of corrections and modifications. To improve and speed up the flow of my experiments, I started to develop an optical clearing technique. This is just another example of a technology being invented out of necessity.

What excites you the most about your research?
The boom in optical clearing methods has been impressive. Currently, optical clearing techniques enable the 3D imaging of intact tissue in fixed samples at cellular to synaptic resolutions. But new concepts and ideas are continually pushing the field forward. In the near future, *in vivo* optical clearing for in-depth imaging will enable us to investigate cellular activity or subcellular dynamics at deeper brain regions under physiological conditions.

What has been the most interesting discovery in your field in the last few years?
Recent developments in super-resolution microscopy have enabled us to inspect fine structural details in neuronal circuits at nanoscale spatial resolutions. This technology fills the gap between picometer-scale electron microscopy and micrometer-scale light microscopy for developing comprehensive maps of synaptic connections. As the availability of commercial super-resolution microscopy widens and optical clearing techniques improve, we should easily be able to obtain high-resolution 3D images of fluorescence-labeled samples, while saving time and effort on image alignment.

How and when did you join RIKEN?
I joined RIKEN in 2011 as a PhD student through the institute's Joint Graduate School Program. After completing my PhD at Kyoto University, I continued my research at the same laboratory through RIKEN's Foreign Postdoctoral Researcher Program.

> *RIKEN is supportive and constantly encourages young scientists to pursue interesting, but risky, research topics.*

What is the best thing about working at RIKEN?
RIKEN is supportive and constantly encourages young scientists to pursue interesting, but risky, research topics. I am also impressed by the funding system and open atmosphere that is conducive to active discussions and interactions among researchers. RIKEN has given me access to the latest facilities and devices in the field.

Is there anything you wish you had known before coming to Japan?
As a Taiwanese, my transition to Japan was relatively smooth. Taiwan and Japan share cultural similarity compared with Western countries, and the common *kanji* characters have been useful in familiarizing me with the new language. ■

Careers at RIKEN

For further information, visit our Careers page:
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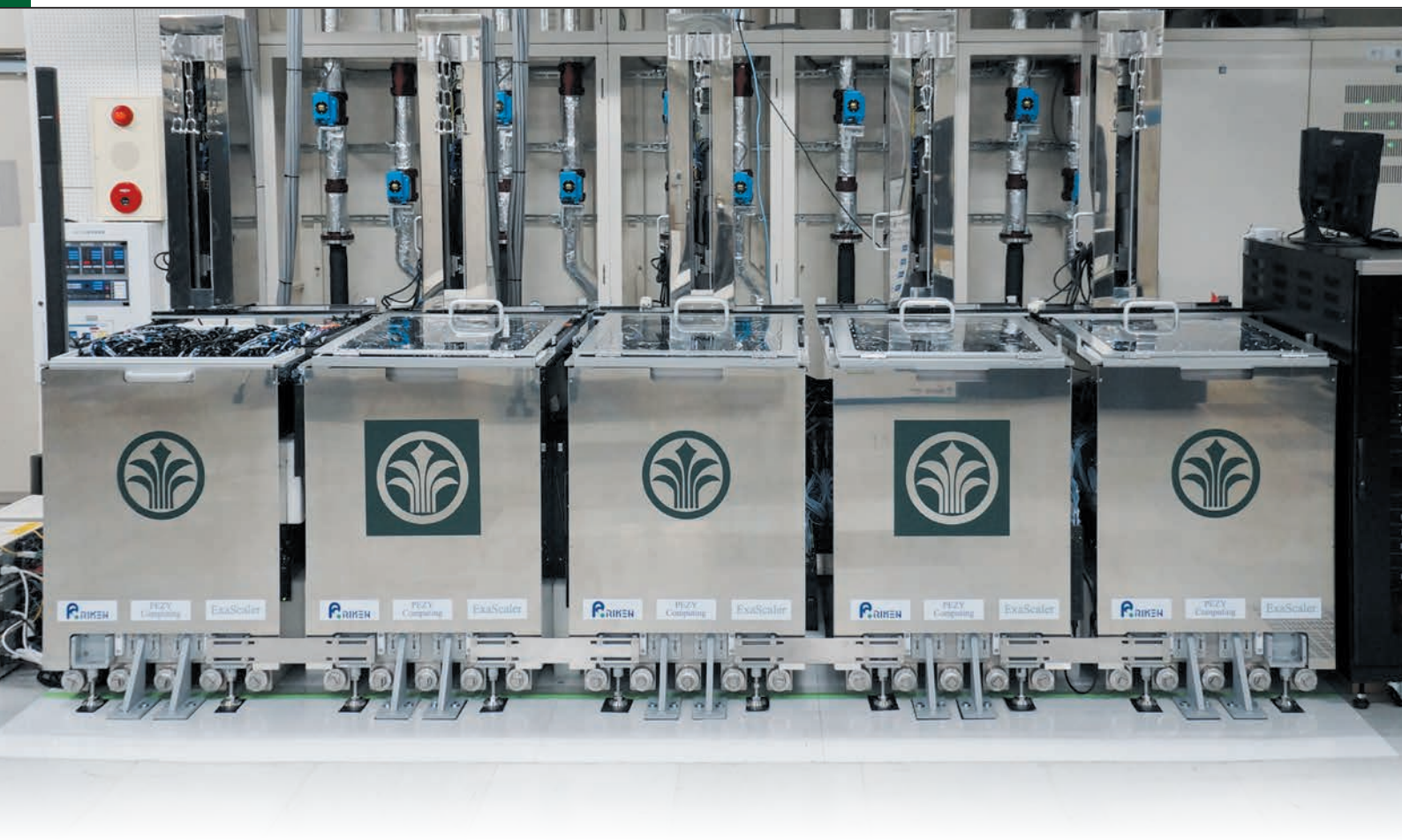
Briefs



Two supercomputers top the green list

RIKEN hosts the two most energy efficient supercomputers in the world and is the first institution to hold the top two spots simultaneously, according to The Green500 ranking published in June 2016.

Both supercomputers, Shoubu (see image) and Satsuki, use cutting-edge liquid immersion technology to achieve very efficient cooling of computing equipment, and were developed through collaborations between RIKEN and the companies PEZY Computing and ExaScaler Inc.

First place went to Shoubu, a system installed at the RIKEN Advanced Center for Computing and Communication (ACCC) in Wako, Japan. Shoubu is the first large-scale (or petascale) supercomputer to top the green list, and the first to hold the number-one spot for three consecutive rankings, over a period of 18 months. The ACCC is currently calling for applications from a wide range of fields to use Shoubu and accumulate knowledge on the computer. One example of a research achievement using Shoubu is the real-time modeling of the cerebellum of a cat.

Second place went to Satsuki, a super-compact system installed in the office of Toshikazu Ebisuzaki, chief scientist at the RIKEN Computational Astrophysics Laboratory, also in Wako. Occupying a mere three square meters of floor space, Satsuki is the first system of its size to make it on to the Top500 list of the world's fastest computers. "When I first heard the idea, I had trouble believing that it would be possible to install a supercomputer in my own office," says Ebisuzaki. "But thanks to the engineering efforts of our collaborators, we managed to do it."

Ebisuzaki plans to use Satsuki for a host of solutions, including many-body gravity simulations to study the orbital evolution of planet embryos and space debris.

www.riken.jp/en/pr/topics/2016/20160622_1

Spotlight on space x-ray camera

NASA honored the Japanese team that mounted an x-ray camera aboard the International Space Station (ISS) with an Innovation in Earth and Space Science award at the fifth annual ISS Research and Development Conference in San Diego on 14 July 2016. The Monitor of All-sky X-ray Image (MAXI) was designed by RIKEN, the Japan Aerospace Exploration Agency (JAXA) and several collaborators to survey the sky for celestial objects that emit x-rays, which will improve our understanding of the evolving Universe.

"It is a great honor to receive the prestigious award," says Tatehiro Mihara of the RIKEN–MAXI team. "We are very grateful to everyone at the MAXI team, including RIKEN and Japanese institutes for their continuous support for basic research and especially to JAXA for their long-term commitment to the development, launch and operation of MAXI."

Since its operation in August 2009, MAXI has scanned the sky every 92 minutes and has issued more than 350 alerts to an international community of astronomers as quickly as 12 seconds after sighting a sudden increase in x-ray flux. In as a little as four hours, MAXI's x-ray observation data is made publically accessible online. The data has been used in more than 150 peer-reviewed papers and has greatly contributed to high-energy astronomy research, including the first detection of the initial fireball stage of an exploding star as well as the discovery of six black holes.

maxi.riken.jp/news/en

From left: Julie Robinson, chief scientist for the International Space Station, Josh Cassada, NASA astronaut, Tatehiro Mihara, senior research scientist in the RIKEN–MAXI team, James Kirkpatrick, executive director of the American Astronautical Society, and Gregory Johnson, president and executive director of the Center for the Advancement of Science in Space.

An elemental honor

The International Union of Pure and Applied Chemistry (IUPAC) has received RIKEN's proposal to name element 113. Nihonium, with symbol Nh, named after the Japanese word for Japan, *Nihon*, will be formally introduced to the periodic table after a five-month public review, ending 8 November 2016.

In 2003, a research group led by Kosuke Morita at the RIKEN Nishina Center for Accelerator-Based Science began a quest to discover the superheavy element. They first synthesized element 113 in July 2004, then again in April 2005, and once more in August 2012. On the eve of the new year, 2016, the IUPAC formally recognized the RIKEN group for having discovered the element, earning them the right to propose a name for it.

In the centuries-long history of discovering chemical elements, only research groups in Western countries have had the privilege of naming new elements. RIKEN is the first institute in Asia to win the right to determine the name of a newly observed element. Morita and his group chose nihonium to recognize the strong support they have received from the Japanese public, especially in the form of continuous funding for basic scientific research. RIKEN hopes that the element's appearance in classrooms all over the world will inspire scientific minds of the future.

www.riken.jp/en/pr/topics/2016/20160608_1

NIH director visits RIKEN

On 13 June 2016, Francis Collins (see image, front row, left), director of the United States National Institutes of Health (NIH), and Roger Glass (back row, left), associate director for international research at NIH, visited the RIKEN Yokohama campus.

RIKEN Executive Director Shigeo Koyasu (front row, right) greeted the visitors and briefed them on the institute. Toshio Goto (back row, second from right), director of the RIKEN Program for Drug Discovery and Medical Technology Platforms, then gave a presentation. The delegation also toured some of the research facilities at the RIKEN Center for Life Science Technologies and the RIKEN Center for Integrative Medical Sciences, and exchanged views with scientists at RIKEN.

The visit concluded with a lively discussion about research trends in the life sciences in Japan and the United States, recent research grants offered by the NIH and opportunities to promote the circulation of young scientists.

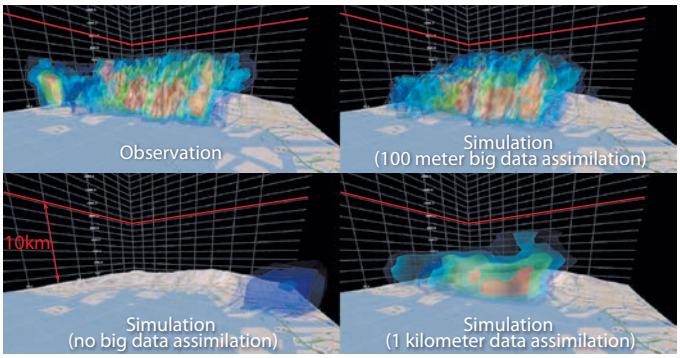
www.riken.jp/en/pr/topics/2016/20160614_1

News

Supercomputing torrential downpours

RIKEN's powerful K computer has been used by an international team of researchers led by Takemasa Miyoshi at the RIKEN Advanced Institute for Computational Science to accurately predict the occurrence of torrential rains. "Supercomputers are becoming more and more powerful, and are allowing us to incorporate ever more advanced data into simulations," says Miyoshi. "Our study shows that, in the future, it will be possible to use weather forecasting to predict severe local weather phenomena such as torrential rains, which can cause enormous damage and cost lives."

Supercomputer-based weather predictions typically use simulations with grids spaced at least a kilometer apart, incorporating new observational data every hour. However, due to the crude calculations, these simulations cannot accurately predict the threat of torrential rains, which can develop within minutes.



Thunderclouds observed in the morning of 11 September 2014 (top left) were best replicated by a K computer-powered weather system using 100-meter grid observational data (top right), compared with 1-kilometer grid observational data (bottom right) or conventional simulation models (bottom left). (Source of map data: Geospatial Information Authority of Japan)

Miyoshi's team used a technique known as big data assimilation, in which large-scale computer simulations are synchronized with observational data to produce high-resolution, three-dimensional maps of rain distribution, with 100-meter grid spacing, every 30 seconds. The system was able to accurately replicate a sudden storm that hit Kyoto on 13 July 2013.

www.riken.jp/en/pr/press/2016/20160809_1

Roasted plant waste quenches parched soils

Heat-treated biomass can improve the quality of poor soil in arid regions, researchers at the RIKEN Center for Sustainable Resource Science in Japan have shown. The biomass was able to increase the water and essential minerals retained by dry, nutrient-poor soil from Botswana. "Treating the poor soil with torrefied biomass improves a variety of factors that ultimately lead to greater plant growth," explains Jun Kikuchi, who led the study published in *Scientific Reports* in June 2016.

Torrefied biomass—sometimes called bio-coal—is a charcoal-rich substance produced by heating organic matter at relatively low temperatures, and has become a potential soil enhancer. Kikuchi and his team wanted to characterize the biological properties of soil treated with bio-coal. They applied torrefied residues of the biodiesel crop *Jatropha curcas* to dryland soil and compared the samples with untreated soil.

Tests showed that soil treated with 5 per cent biomass held 5 per cent more water than untreated soil, which is an important quality of fertile earth. The researchers also found that the essential minerals potassium, sodium and phosphorus were more available for uptake by plants. Not surprisingly, plants grown in the torrefied biomass were heavier and had thicker stems and longer roots.

www.riken.jp/en/pr/press/2016/20160617_3

Microorganisms from ancient tombs

In July 2016, the Japan Collection of Microorganisms (JCM) launched a new line of microorganisms isolated from two ancient burial mounds in Japan, which could help to elucidate the cause of biodeterioration of wall paintings in the tumuli. By making the collection of 730 filamentous fungi, bacteria and yeasts accessible to scientists, the JCM, which is operated by the RIKEN BioResource Center in Tsukuba and specializes in the collection, preservation and distribution of microorganisms, hopes to facilitate the conservation of valuable cultural artifacts.

Located in Japan's Nara prefecture, Takamatsuzuka and Kitora tombs are believed to have been built in the late 7th or early 8th century. Multicolored murals, painted over plastered stone, were discovered in Takamatsuzuka tumulus in 1972, and polychrome paintings were discovered using fiberscopy in Kitora tumulus in 1983. Initial attempts to conserve the murals on-site proved difficult, so they were temporarily relocated to a facility where they could be protected against further deterioration while being restored.

Researchers from the Agency for Cultural Affairs and the National Research Institute for Cultural Properties, Tokyo, isolated microorganisms from the original site to investigate their roles in the biodeterioration of the paintings. The project led to important findings on mural conservation and the discovery of new species, but neither organization could afford to continue to maintain the microorganisms. The collection was therefore acquired by the JCM to support further studies into conservation science.

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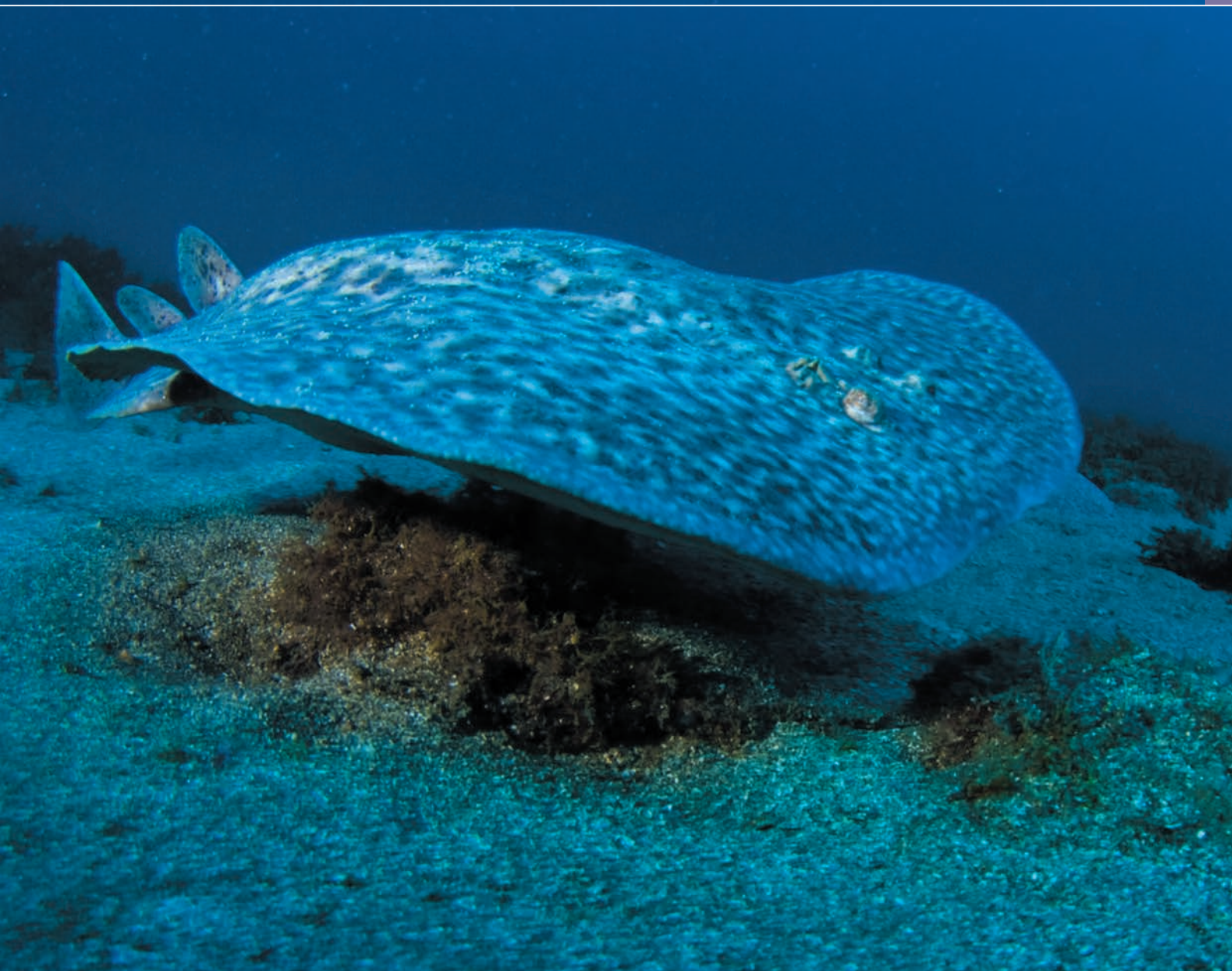
Microorganisms (dark spots in red circle) have been found growing on ancient murals in Takamatsuzuka tomb, which was built around 1,300 years ago in Japan.

Photo courtesy of the Agency for Cultural Affairs



Ray of hope for cleaner power

Researchers at RIKEN have generated and stored enough electricity to illuminate a light-emitting diode (LED) by physically stimulating the organs of an electric ray. The findings, published in *Scientific Reports* in May 2016, could lead to clean, super-efficient electric power generators inspired by nature.

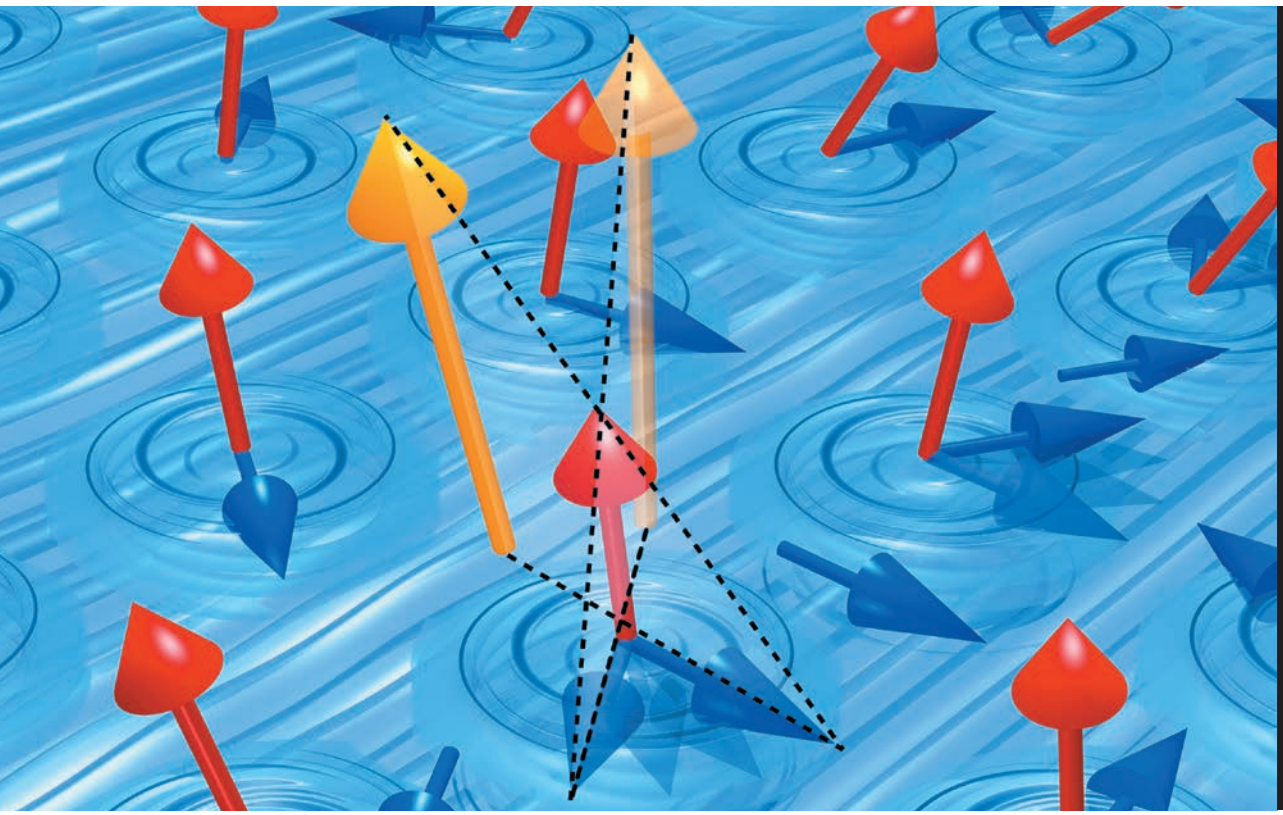
Global concern over the environmental impact of electric power generation has led to a push away from traditional thermal and nuclear power. Biofuel cells have recently been developed to meet the demand, but their performance remains inferior to conventional systems.

Scientists led by Yo Tanaka at the RIKEN Quantitative Biology Center in Osaka began work to develop a new type of electricity generator, based on the knowledge that electric rays known as torpedoes generate electric power with an efficiency of nearly 100 per cent. Torpedo fish have electric organs with densely aligned membrane proteins that convert the chemical energy of adenosine triphosphate (ATP) into ion transport energy, and a nervous system that controls the whole process.

The researchers began by looking at what happens in a live electric ray. Physically stimulating the torpedo generated a 10 millisecond pulse, with peak voltage of 19 volts and a current of 8 amperes. "We found that we were able to store enough electricity to light an LED light or drive a toy car," says Tanaka.

To generate more electricity, they removed the electric organ from the torpedo and chemically stimulated it by injecting a solution of the neurotransmitter acetylcholine though a syringe. This resulted in more than a minute of continuous current, with a peak voltage of 91 millivolts and 0.25 milliamperes of current.

www.riken.jp/en/pr/press/2016/20160531_3



Schematic diagram showing the mechanism of the spin current Doppler shift. The Dzyaloshinskii–Moriya interaction arises because of a Doppler effect due to an intrinsic spin current, which modifies the propagation of exchange interactions between spins (indicated by arrows).

PHYSICS

A changed exchange for magnetic materials

A method for controlling the magnetic texture of exotic materials offers a route to realizing the next generation of electronic devices that consume less energy

A model for understanding why unusual magnetic structures such as vortices arise in some materials has been developed by RIKEN researchers[1]. This model could help with the design of electronic devices offering lower energy consumptions.

Magnetism arises because of an electron property known as spin, which makes the electrons behave like tiny magnets. In a typical magnet, these spins align in a uniform direction due to an interaction between them called a symmetric exchange interaction.

On the other hand, an antisymmetric exchange interaction, known as the Dzyaloshinskii–Moriya interaction, causes adjacent spins to misalign slightly. This interaction leads to magnetic patterns that are much more complex than simple parallel ordering, such as magnetic vortices and helices.

Scientists are investigating these unusual magnetic textures as a route to spin-based information processing. However, a better understanding of how the Dzyaloshinskii–Moriya interaction arises, and methods for predicting its strength are needed to aid the development of better spintronic materials and devices that require much less power than conventional electronic devices.

"The Dzyaloshinskii–Moriya interaction has been known for more than half a century," says RIKEN scientist Toru Kikuchi. "But previous studies on its microscopic origin merely reported calculation results without presenting a physical picture of how it actually works."

Kikuchi and his co-workers from the RIKEN Center for Emergent Matter Science provide a more visual understanding of the interaction. They show that the Dzyaloshinskii–Moriya interaction arises because of a Doppler effect due to an intrinsic spin current: in certain kinds of magnets, a spin current is induced intrinsically without any external forces, and this intrinsic spin current modifies the propagation of exchange interactions between spins (see image). A similar effect can be observed in the way the wind modifies the propagation of sound waves.

The researchers show, moreover, that the strength of the Dzyaloshinskii–Moriya interaction is equal to the magnitude of the intrinsic spin current.

Kikuchi and his co-workers used this insight to develop first-principles calculations for predicting the strength of the Dzyaloshinskii–Moriya interaction. The results they obtained agreed strongly with previous experimental values for alloys of manganese iron germanium and iron cobalt germanium.

"Our picture indicates the possibility of controlling the Dzyaloshinskii–Moriya interaction using the spin current," says Kikuchi. "This may open up a new way to create novel devices." ■

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BIOLOGY | PRESS RELEASE

Baton in the brain orchestrates neurons

Experiments reveal how the mouse brain 'date stamps' neural signals responsible for producing mental maps

Two RIKEN researchers have pinpointed how the neurons that represent space in mice keep in time[1].

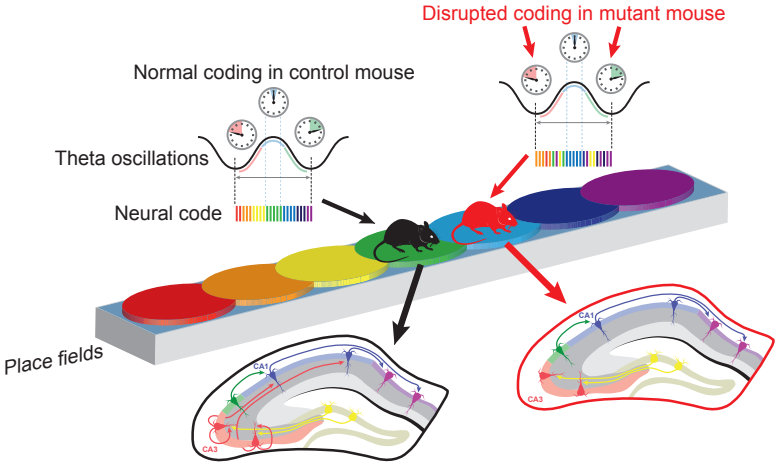
Just as orchestra members need a conductor to stay in tempo, neurons in the brain require well-timed waves of activity to organize memories across time. In the hippocampus—the brain's memory center—temporal ordering of the neural code is important for building a mental map of where you have been, where you are, and where you are going.

As a mouse navigates its environment, the central hippocampal area, CA1, relies on rhythmic waves of neural input from nearby brain regions to produce an updated map of space. When the researchers turned off the input from a nearby hippocampal area, CA3, the refreshed maps became jumbled. While mice could still perform simple navigation tasks and signals from single neurons appeared to represent space accurately, the population-level code, or 'orchestra', was out of time and flawed.

"The neural music didn't change, but by silencing CA3 input to CA1 in the hippocampus, we eliminated the conductor," says Thomas McHugh of the RIKEN Brain Science Institute.

McHugh and co-author Steven Middleton genetically engineered mice to express a nerve toxin that shut down the synaptic junctions between CA3 and other brain areas. Then, while mice ran up and down a track, the researchers recorded multiple individual neurons as well as the summed electric current from a larger group of neurons.

As the transgenic mice moved in the enclosure, individual neurons continued to update their activity at regular intervals. This cyclic organization of information, however,



In a normal mouse (black mouse), the hippocampal area CA3 ensures that memories are arranged in the right order. When communication from CA3 is blocked (red mouse), this arrangement of memories breaks down.

was missing across the population of neurons. "Without input from CA3, there was no global organization of the neural signals," says McHugh.

Without CA3 input, accurate prediction of the spatial location from the ensemble neural code was impaired. The mice still knew where it was, but small errors in representing space from individual neurons became compounded.

"If neurons don't activate in sequence, you can't organize memories across time," says McHugh. "Whether in mice or humans, you need temporal organization to get from here to there, to make decisions and reach goals." If shutdown of CA3 was possible in humans, McHugh suggests, memories would probably become useless and jumbled.

The researchers also observed a reduction in neural oscillations characteristic of CA3-to-CA1 communication. Disruptions to these oscillations have been identified in patients with diseases ranging from schizophrenia to Alzheimer's. Thus, a deeper understanding of how the rhythms of the brain organize information could shed light on the circuit mechanisms of these disorders. ■

Reference

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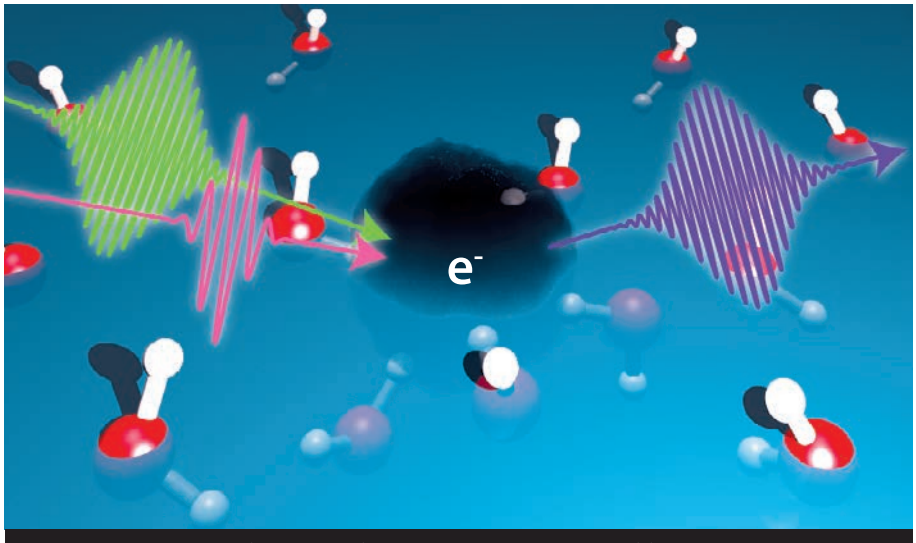
CHEMISTRY

The search for partially hydrated electrons

A powerful new spectroscopy technique can be used to probe the dynamic and unknown chemical processes that occur at the interface between air and water

From biology through to physics, boundaries and surfaces are the sites of some of the most significant interactions and transformations. RIKEN researchers have developed a spectroscopic technique to probe the processes taking place at a very important and fundamental boundary—the interface between air and water. Using this technique, they have detected the dramatic behavior of single electrons at the water surface[1].

Free electrons in bulk water attract several water molecules around them and are thus known as hydrated electrons. "Hydrated electrons are very reactive species and play important roles in chemical reactions," says Tahei Tahara of the RIKEN Molecular Spectroscopy Laboratory. But while hydrated electrons have been extensively studied in bulk water, their very existence at the water surface is still debated.



By combining two light beams (pink and green), it is possible to detect an electron (e^-) on the surface of water.

The spectroscopic technique developed by the researchers is called heterodyne-detected vibrational sum-frequency generation (HD-VSFG), and it detects chemical species on the surface of water. Like infrared spectroscopy, it uses light to identify the molecules present in a sample via the characteristic wavelengths of light they absorb.

In VSFG, two light beams combine to generate a third beam of light whose frequency is the sum of the frequencies of the two incoming light beams. This combined light beam carries the signals of the chemical species present (see image). "This light mixing process occurs only in regions where the 'upward' and 'downward' have different natures," explains Tahara. "At the air–water interface, the upward area is the air, while the downward area is the water. Consequently, VSFG signals are generated only at the interface."

The researchers shone ultraviolet laser light at the water surface, which causes electrons to split off some water molecules. Using HD-VSFG, they noticed a very short-lived signal in the spectral region that corresponds

to stretching of the oxygen–hydrogen bond. The researchers confirmed that this signal indicates the vibration of water molecules that hydrate newly formed electrons at the water surface.

Furthermore, the team showed that these electrons were only partially hydrated, rather than fully surrounded by water molecules as they are below the surface. The HD-VSFG results revealed that partially hydrated electrons are highly unstable, escaping into the bulk water to become fully hydrated within 100 picoseconds (10^{-10} second) of forming.

"Observing hydrated electrons is just the first step," Tahara says. "Because our new method can be utilized to study chemical reactions at liquid interfaces in general, we will be able to unveil dynamics and mechanisms of interfacial reactions that are almost unknown at the moment." ■

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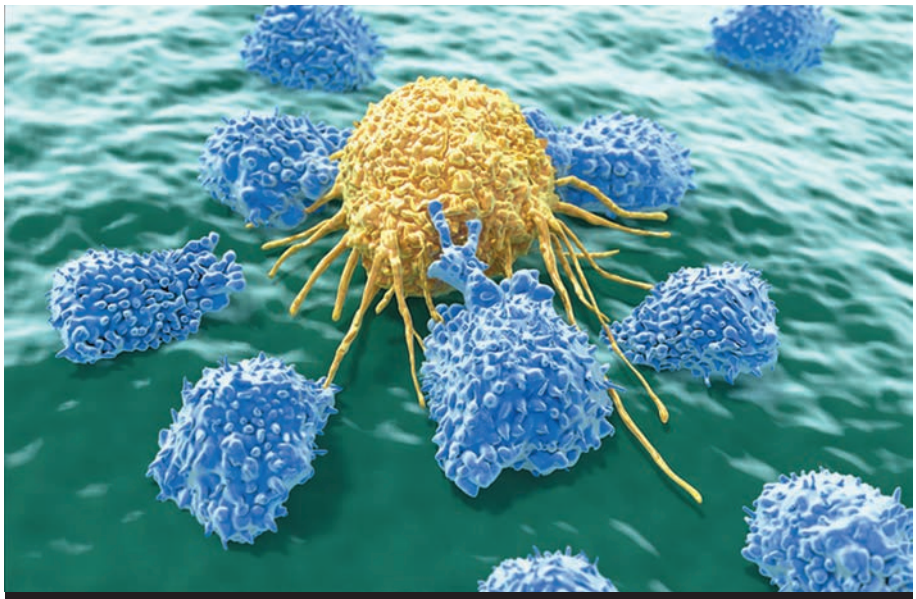
MEDICINE | PRESS RELEASE

Anticancer strategy mobilizes double-pronged attack

A vaccine made from modified cells that can activate both innate and adaptive immune responses has potential for fighting tumors, even years after it has been administered

Though various immunotherapy-based strategies are being used against cancer, they are often thwarted by the inability of the immune response to infiltrate the immunosuppressive tumor microenvironment and effectively fight cancer cells. Now, RIKEN scientists have developed a new vaccine containing modified cells that can stimulate both innate and adaptive immune responses, which allows the body to retain response memories and attack new tumor cells as they form. They found that the vaccine enabled killer $CD8^+$ T-cells—important players in the immune response against cancer—to enter the tumor microenvironment and target cancerous cells.



Natural killer T-cells (purple) attacking a cancer cell (yellow). A new anticancer vaccine has been developed that uses foreign cells modified by adding a natural killer T-cell ligand.

"Cancer cells have different sensitivities to the innate and adaptive responses, so it's important to target both in order to eradicate them," says Shin-ichiro Fujii of the RIKEN Center for Integrative Medical Sciences. "We have developed a special type of modified cell, called artificial adjuvant vector cells (aAVC), which we found can do this."

The aAVC cells are foreign cells that have been modified by adding a natural killer T-cell ligand, which permits them to stimulate natural killer T-cells, along with an antigen associated with a cancer. The group found that when these cells are activated, they promote the maturation of dendritic cells, which coordinate the innate and acquired responses. Dendritic cells are key because they can generate killer $CD8^+$ T-cells as well as activate the immune memory, which allows the body to recall and respond to a threat, even years later.

To find whether it worked in bodies, the researchers conducted experiments in mice with a virulent form of melanoma that also expresses a model antigen called ovalbumin (OVA)[1]. Tests in mice showed that aggressive tumors could be shrunk by vaccinating the animals with aAVC

cells programmed to display the OVA antigen. Following the treatment, tumors in the treated animals were smaller and necrotic in the interior—a sign that the tumors were being attacked by the killer CD8⁺ T-cells.

The researchers further found that in animals that had undergone the treatment, cancer cells injected even a year later were eliminated. "This indicates, that we have successfully created an immune memory that remembers the tumor and attacks it even later," says Fujii.

"Our therapy with aAVC is promising because typical immunotherapies have to be tailor-made with the patient's own cells," Fujii says. "We use foreign cells, so they can be made with a stable quality. Because we found that our treatment can lead to the maturation of dendritic cells, immunotherapy can move from local treatment to more systemic treatment based on immune memory."

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BIOLOGY

Super-sizing plants by tweaking just one gene

A simple genetic manipulation makes plants bigger and tougher, offering new research avenues and applications in the face of a warming planet

A single genetic change is all it takes to make plants produce more biomass and become more resilient to stress, a team of RIKEN researchers has found[1]. This advance, which is important in light of global climate change, will facilitate research into these processes and efforts to improve them.

The team had previously studied a family of four genes known as *pseudo-response regulator* (*PRR*) genes, which regulate a plant's biological clock by repressing the expression of other clock-related genes. They found that although mutations in individual *PRR* genes had little effect, plants with three of the four genes disrupted tended to flower later, tolerate drought better and produce more biomass.

However, establishing and maintaining three mutations in different species is time consuming and difficult, limiting broader applications of the method. To overcome this problem, a team led by Norihito Nakamichi and Hitoshi Sakakibara of the RIKEN Center for Sustainable Resource Science engineered a modified version of one of the genes, *PRR5*, to consistently activate its target genes.

PRR5-VP plants (upper row) are much more resilient to drought than normal plants (lower row).

The new construct, PRR5-VP, effectively eliminates the role of the *PRRs* by activating genes that they would normally repress. The resulting PRR5-VP plants exhibited the same late flowering, biomass increase and stress tolerance seen in the triple mutants.

In stress tests, a single day of freezing temperatures killed all the normal plants, but only half of the PRR5-VP plants. The PRR5-VP plants were also drought tolerant, surviving a 16-day imposed drought that killed nearly all of their normal kin (see image). Similarly, the engineered plants weathered salinity stress significantly better.

The PRR5-VP plants produced about twice as much biomass as their normal counterparts. While the increase was accompanied by slower growth, this drawback might be alleviated by precisely controlling when and where PRR5-VP is switched on.

"This was a proof-of-concept study, and I'm satisfied because the data were very beautiful and clearly demonstrate that this approach works," says Nakamichi.

Since PRR5-VP is a single genetic construct, it can be easily introduced to other species for further research or biotechnological applications. Follow-up work will investigate genes downstream of *PRR5*, which seems to act as a nexus of several pathways

—Nakamichi's team showed that *PRR5* increased biomass by delaying flowering but improved stress tolerance by regulating other processes.

The team will also investigate the role of *PRR5* in different species. "In other plants, *PRR5* might regulate physiological processes not found in the model species *Arabidopsis*," says Nakamichi. "This approach may reveal aspects of the evolution of the biological clock's genetic network." ■

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BIOLOGY | PRESS RELEASE

Foul or fragrant? Predicting odor preferences

A mathematical model based on fruit fly responses to various odors can be used to predict their responses to new odors

An all-RIKEN team can now predict how flies will respond to odors by developing a model for the activity of neurons in the olfactory processing center of the fruit fly brain[1].

For many animals, the sense of smell is central to various behaviors and critical for survival. But it had been unclear how the brain distinguishes between harmful and beneficial smells.

An odor activates a population of small neuronal structures called glomeruli in the first olfactory center of the brain. But the vast number of glomeruli (about 1,800 in mice and 5,500 in humans) impedes research.

To overcome this obstacle, Hokto Kazama of the RIKEN Brain Science Institute and coworkers exploited the simpler olfactory system of the fruit fly *Drosophila melanogaster*, which has only about 50 glomeruli. This allowed them to use two-photon calcium imaging to systematically record odor-evoked activity from almost all fly glomeruli in response to many different odorants.

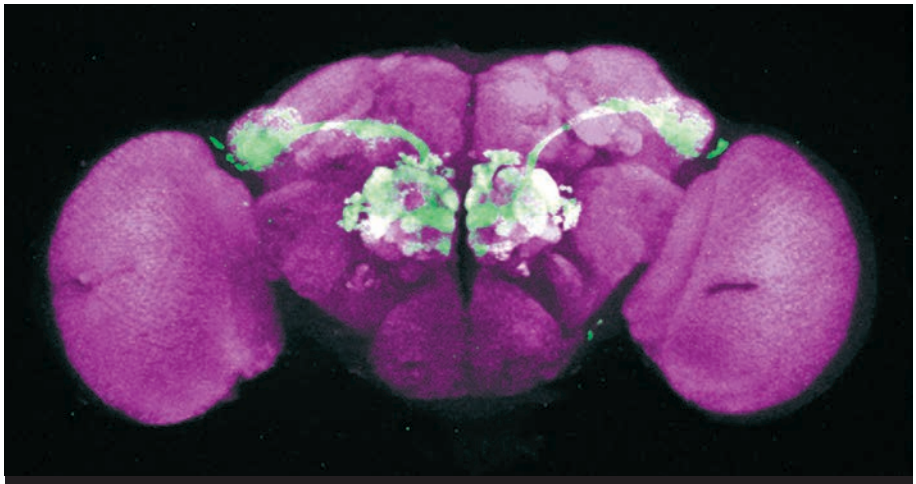
The researchers monitored fly behavior using a clever flight-simulator arena, in which the fly's head is fixed and surrounded by an olfactory and visual landscape that is rotated in real-time in response to wing movement. The flies' responses ranged from strong attraction to strong aversion, and they made judgments extremely quickly, sometimes within 200 milliseconds.

Based on these behavioral and physiological data, the researchers developed a mathematical model that explains how attraction and repulsion to odorants can be computed from the activity of olfactory glomeruli. In the model, each glomerulus contributes to attraction or aversion with a specific weighting. Summing the transformed and weighted activities of all glomeruli not only matched the real behavioral responses to the odors used to make the model, but also accurately predicted responses to new odorants. Contrary to conventional wisdom, the results imply that this computation requires most, if not all, glomeruli.

The model also predicted that the relative preference of odors would vary, and could even switch, depending on the nature of other odorants present. The team verified this by performing experiments in which the same odors were presented under different conditions.

"Not only does this demonstrate that even flies can adapt to their olfactory environment, it exemplifies the usefulness of our approach that combines physiological measurements with mathematical modeling of behavior and neural activity," adds Kazama.

Because the basic function and wiring of the olfactory system are well conserved from flies to humans, the study is expected to provide a deeper understanding of the principles and mechanisms of olfactory processing in the human brain. ■



By investigating the responses of glomeruli in fruit fly brains to various odors, researchers developed a model that can predict the responses of flies to new odors.

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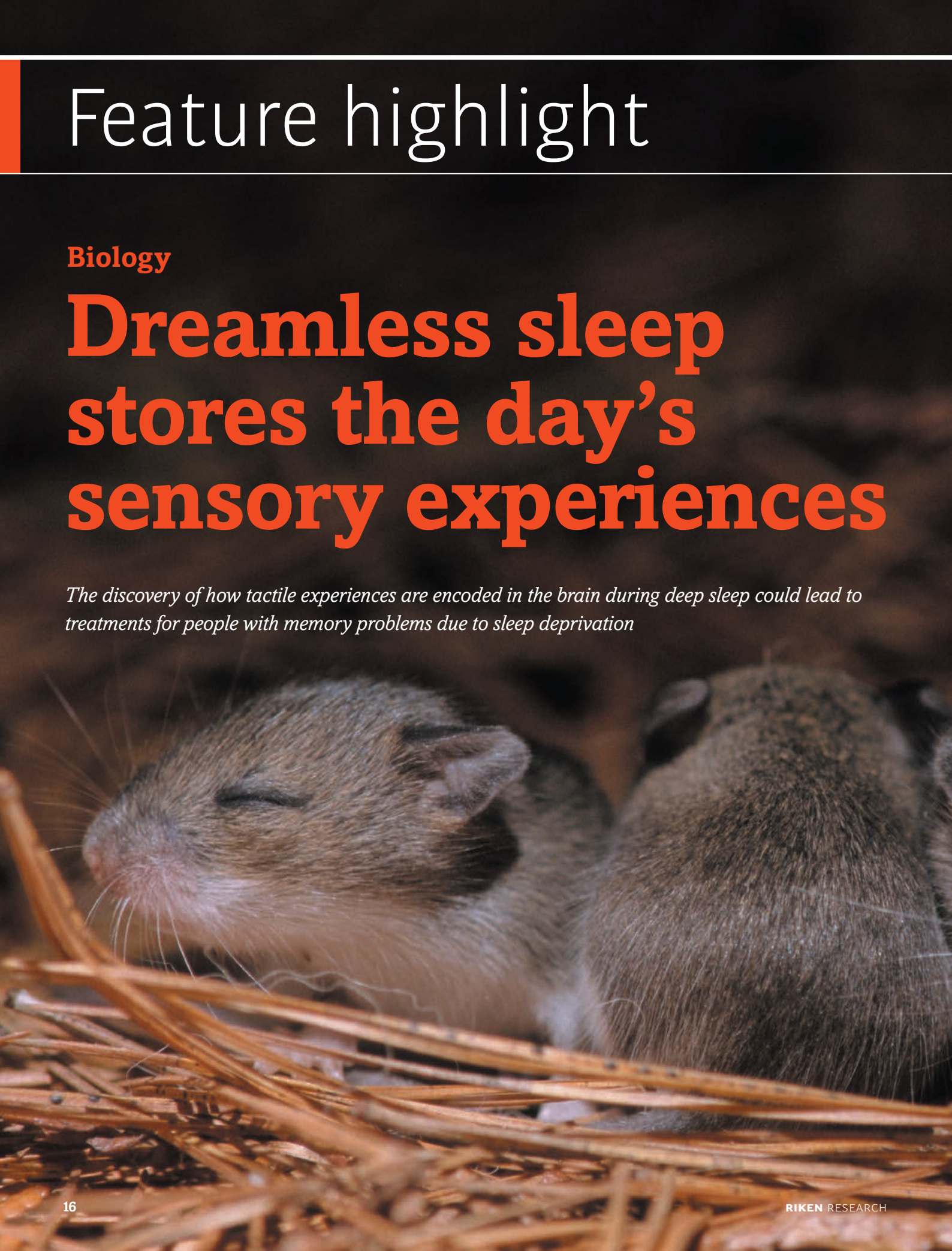
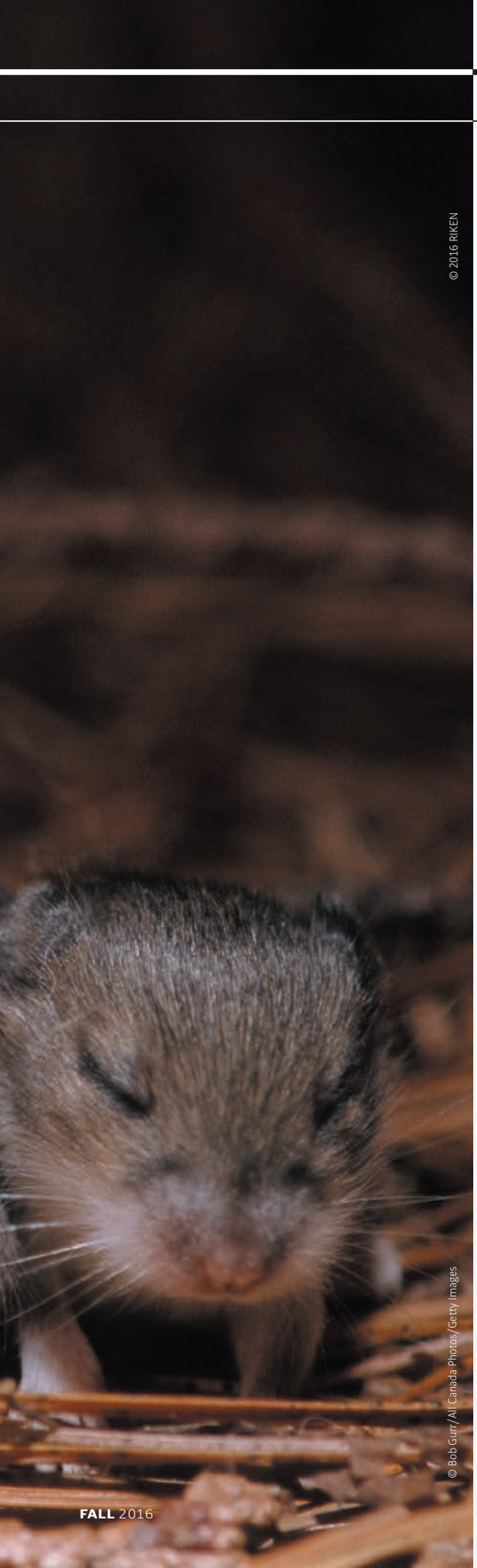
Feature highlight

Biology

Dreamless sleep stores the day's sensory experiences

The discovery of how tactile experiences are encoded in the brain during deep sleep could lead to treatments for people with memory problems due to sleep deprivation





A good night's rest is known to help people remember what they learned during the day. But just how sleep allows us to retain the memory of stimuli received through our five senses while awake has been a mystery.

Researchers at the RIKEN Brain Science Institute have mapped a neural circuit that helps the brain encode and store these kinds of sensory memories during sleep[1]. Their findings could be helpful for patients suffering from sleep deprivation and associated memory-loss problems.

"Our brain stimulation protocol might be able to reactivate sleep-deprived neurons to improve memory consolidation," says Masanori Murayama, head of the RIKEN Behavioral Neurophysiology Laboratory. "It would be fantastic if someone were to apply our protocol in the clinic and succeed in improving cognitive functions."

Brain boomerang

How the body responds to certain visual cues or the shapes of objects might not seem complicated. But the interpretation, categorization and prioritization of this kind of sensory information is actually highly complex: it requires learning, context and coordinated responses from the brain.

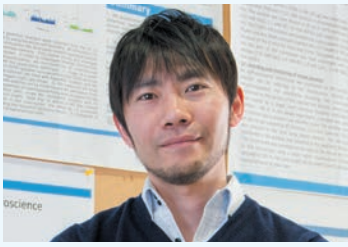
To better understand the neuroscience of this process, Murayama and his colleagues turned to mouse models of touch perception. In 2015, they discovered a two-stage brain circuit that enables mice to discriminate between different floor textures with their paws[2].

The scientists found that the skin must first send a signal to the brain's sensory cortex. This message-receiving part of the brain then relays the signal to the higher-order motor cortex—the part of the brain involved in planning, controlling and executing voluntary movements—before the neural signal travels back down to the sensory cortex. This brain boomerang is necessary for tactile processing, the researchers showed, and mice lose their accurate sense of touch without this 'top-down' control circuit from the higher-order motor processing center to the more primary sensory area of the brain.

Sleep on it

Building on those results, Murayama's team sought to determine how the brain turns new sensory experiences into long-term memories. They developed a simple task in which mice familiar with a cage that has an all-smooth floor were introduced to a new cage in which half the floor surface was bumpy (Fig. 1).

Mice do not have an innate preference for one texture over the other, but they do have a natural propensity to investigate new things in their environment. Consequently, the animals tended to spend more time exploring the part of the cage with the bumpy floor, indicating that they remembered the all-smooth cage and were less interested in it. But they



Masanori Murayama

Masanori Murayama was born in Miyagi prefecture, Japan, in 1977. In 2001, he graduated from the School of Life Sciences at Tokyo University of Pharmacy and Life Sciences, and obtained his PhD in 2006 from the same university. After four years of postdoctoral training at the Department of Physiology, University of Bern, Switzerland, he returned to Japan to become a team leader at the RIKEN Brain Science Institute. He has since received many research prizes from neuroscience societies and the government of Japan. Through his research, Murayama has discovered a unique neural circuit underlying somatosensory (touch) perception in mice during the awake state and perceptual memory consolidation during sleep. This circuit might also be related to other perceptual experiences, including chronic pain and itching.

Figure 1: Mice that remembered the smooth-floor experience on one half of a cage tended to spend more time exploring objects on the novel bumpy surface of the other half.

did this only after a good night's sleep, or the neurobiological equivalent.

Sleep deprivation impaired the animals' ability to remember the all-smooth cage. And so did an experimental manipulation in which Murayama's team used light-activated genetic technology to precisely inactivate the same top-down control circuit they had previously shown was responsible for touch perception.

The timing and direction of the inactivation were critical, however. Silencing the neural signaling bottom-up (from the sensory cortex to the motor cortex) had no effect on tactile memory. Neither did inactivating the top-down pathway (from the motor cortex to the sensory cortex) when the mice were awake or while they were asleep many hours after the initial learning period. Only when the researchers disabled the circuit during deep sleep immediately after the first exploration of the all-smooth cage did the mice subsequently forget the experience.

"Top-down information flow is selectively involved in memory consolidation during sleep," says RIKEN neuroscientist Daisuke Miyamoto, the first author of the new study.

Murayama was astonished by how quickly the brain's internal connections became hard-wired with the memories during the first bout of deep sleep —otherwise known as non-rapid eye movement, or non-REM, sleep. "We manipulated the circuit for only 30 minutes during non-REM sleep, which was long enough to control memory consolidation," he says. "A mouse usually sleeps 12 hours every day; so that's merely 4 per cent of their total sleep time!"

Hard-wiring perception

The researchers then tested whether they could artificially induce the firing of the top-down pathway to strengthen the memory of the initial tactile experience. As the mice entered deep sleep, Murayama and his colleagues stimulated the neurons in the top-down circuit in a synchronized fashion. This caused the mice to remember their experience of the smooth floor for twice as long, even in the sleep-restricted animals.

"Mimicking deep-sleep activity with stimulation in just two brain regions—the motor cortex and the sensory cortex—was enough to resolve memory deficits caused by sleep deprivation," Miyamoto says. "I was really surprised by that."

This finding suggests that it is not sleep per se that is needed for consolidating perceptual memory, but rather the coordinated activation of the top-down pathway. The researchers speculate that artificially stimulating the brain using magnets or electrodes could enhance memory processing in people. "If we improve the stimulation pattern for clinical applications, it might be possible to ameliorate memory deficits in patients with sleep disorders," Miyamoto says. "Furthermore, anti-synchronous cortical stimulation might be able to impair the consolidation of unpleasant perceptual experiences, such as those of victims of crime and violence," he adds.

Scientists at the RIKEN Behavioral Neurophysiology Laboratory have now begun to consider one particular sleep disorder by again looking at a mouse model. Like many people with chronic itches and rashes, these mice scratch their skin constantly, disrupting their sleep and impairing their memory. "If you can understand the itch circuit in the brain and suppress itch perception," Murayama says, "it will stop the itch–scratch cycle, and improve skin and cognitive function."

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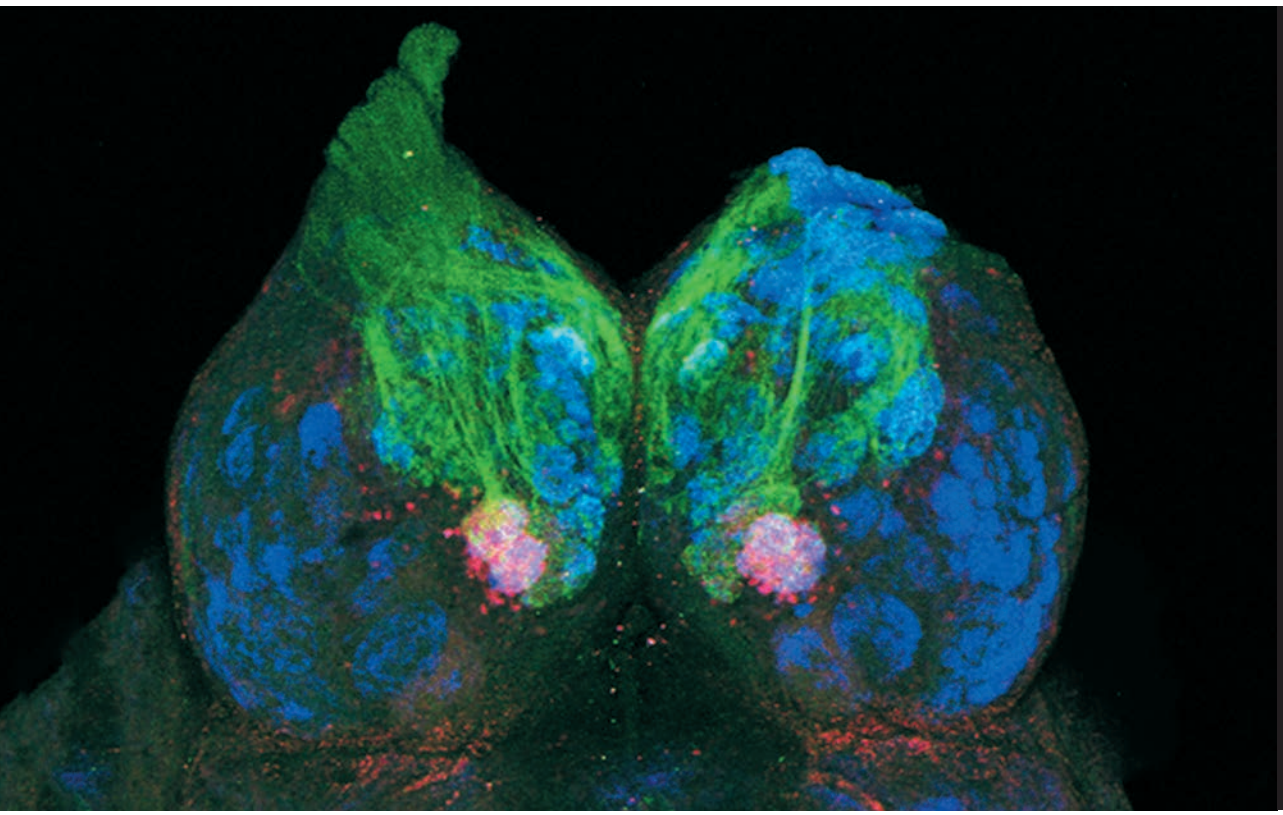

Neurons activated by the pheromone prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) send signals to specific regions (red) in the olfactory bulb of the brain.

BIOLOGY | PRESS RELEASE

Male zebrafish follow their noses

Female zebrafish release a chemical to attract mates, which males detect through the brain's smell pathway

A molecule involved in fish reproduction activates the brain via the nose, find RIKEN researchers[1]. Female zebrafish release the pheromone—a social and sexual signaling molecule used to attract the opposite sex—and males sense it through smell receptors in their noses.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a reproductive hormone present in female fish and mammals. In fish, it is also a pheromone that females secrete in their urine. Males normally swim toward even small concentrations of the molecule. However, the team, led by Yoshihiro Yoshihara at the RIKEN Brain Science Institute, found that male fish without a sense of smell were indifferent when $PGF_{2\alpha}$ was added to their tank.

$PGF_{2\alpha}$ synchronizes reproductive behaviors between female and male zebrafish, but this mechanism was not understood. After confirming that sensory tissue responsible for smelling was needed for males to sense $PGF_{2\alpha}$, the researchers found that only neurons known as ciliated olfactory sensory neurons are activated by it. They then searched within these neurons for the receptor that detects $PGF_{2\alpha}$.

Surprisingly, the key players were not prostaglandin receptors. Molecular labeling revealed that $PGF_{2\alpha}$ bound to only two olfactory receptors, which were evolutionarily quite different from prostaglandin receptors. The same or corresponding olfactory receptor genes are present in other

fish and mice, which suggests that other species may have a similar mechanism for reproductive communication.

The researchers found that through these olfactory receptors, $PGF_{2\alpha}$ activates a direct, dedicated neural pathway to the brain areas responsible for eliciting courtship behavior in male fish. The ciliated olfactory sensory neurons send signals to specific regions in the olfactory bulb of the brain (see image), which in turn relay them to distinct forebrain areas.

Hardwired pathways like this are common for innate behaviors, says Yoshihara, and it may have been an evolutionary accident that the $PGF_{2\alpha}$ molecule was well-matched to certain olfactory receptors, facilitating the use of the 'smell pathway' for reproductive purposes.

Finally, the researchers tested the response to $PGF_{2\alpha}$ in male fish lacking the genes for one of the olfactory receptors they had identified. These fish were not drawn to $PGF_{2\alpha}$ in their tank, spent less time chasing female fish, and were less successful at spawning. A smell receptor thus seems to be the gateway for $PGF_{2\alpha}$ into the male fish brain. Pheromone signaling works hand in hand with other senses such as vision to bring about the courtship dance that increases a fish's chances of mating. ■

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BIOLOGY

Corralling factors that manage migration

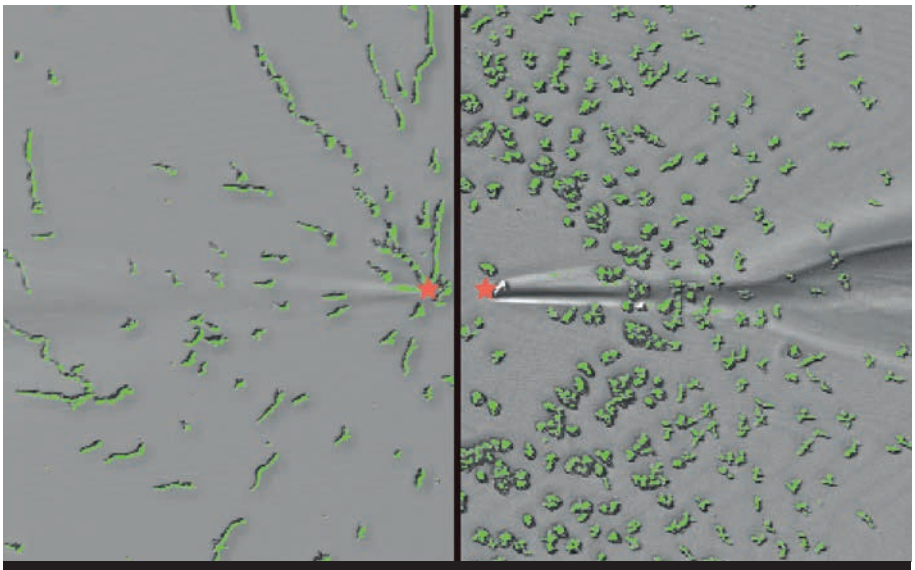
Cells use a sequestration mechanism to ensure that they can respond sensitively to migration cues

For many cells, growth and migration are directed by the concentration of attractive molecular signals in their environment. Using the slime mold *Dictyostelium* as a model, RIKEN scientists have uncovered a novel mechanism that governs the remarkable sensitivity of this response, which is known as chemotaxis.

One of the chemical lures that stimulate chemotaxis in *Dictyostelium* is a molecule called cyclic adenosine monophosphate (cAMP). Yoichiro Kamimura at the RIKEN Quantitative Biology Center notes that *Dictyostelium* has an extremely fine-tuned ability to respond to even minor local increases in cAMP levels. "Cells can detect a very small density difference—as small as a few per cent—over a range of concentrations that spans as much as 100,000-fold," he says. How cells achieve this feat, however, remains poorly understood.

cAMP acts on a class of protein known as G-protein-coupled receptors (GPCRs), which reside in the cellular membrane and initiate changes in cell behavior via activation of heterotrimeric G-protein complexes. Kamimura and colleagues identified a protein called Gip1 that interacts with this G-protein and appears to play a critical role in establishing the sensitivity of *Dictyostelium* chemotaxis. They showed that cells deficient in Gip1 could react appropriately to low concentrations of cAMP, but largely lost their ability to process signals from higher concentrations of this molecule[1] (see image).

Subsequent experiments revealed that, in the absence of chemotactic signals, Gip1 normally keeps G-protein localized in the cellular interior. The interaction between cAMP and GPCRs generates a cue for the G-protein to separate



Dictyostelium cells normally migrate (left) toward a high concentration of cAMP signal (red star), but *Dictyostelium* cells lacking the Gip1 protein (right) lose their capacity to respond to such cues.

from Gip1 and migrate to the cell membrane, where it contributes to the chemotactic response.

Based on these findings, the researchers conjecture that sequestration by Gip1 establishes a biased internal distribution of G-protein that ensures that cells can respond effectively to high concentrations of chemotactic signals. Kamimura notes this is the first time that such a molecular mechanism has been observed for controlling the dynamic range of signals to which cells can respond.

Many mammalian cells employ chemotaxis, and Kamimura points out that a similar process might control the migration of white blood cells toward disease-associated signals in the body. As a next step, the team will explore precisely how Gip1 controls G-protein localization.

Given that GPCR signaling is involved in numerous other cellular processes—including many diseases—these findings could have implications that extend well beyond chemotaxis. "This study could be a seed that grows into a new molecular tool for manipulating G-protein activity," says Kamimura. ■

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CHEMISTRY | PRESS RELEASE

Titanium flexes its mussels

Attaching biologically active molecules inspired by nature to titanium surfaces could lead to artificial joints and implants that are more biologically compatible

Titanium is used in many biomedical applications such as artificial joints and dental implants. While it is strong and does not harm tissues, the metal lacks some of the beneficial biological properties of natural tissues such as bones and teeth. RIKEN researchers, inspired by the ability of mussels to attach very tightly even to metallic surfaces due to special proteins found in their byssal threads, have attached a biologically active molecule to a titanium surface[1]. This advance paves the way for implants that are more biologically beneficial.

By attaching mussel proteins (left) to titanium surfaces (right), researchers have developed biocompatible implants.

The work stems from earlier discoveries that mussels can adhere to smooth surfaces very effectively thanks to the protein L-DOPA, which is known to be able to bind very strongly to smooth surfaces such as rocks, ceramics and metals. Interestingly, the same protein functions in humans as a precursor to dopamine and is used as a treatment for Parkinson's disease.

"We thought it would be interesting to try to use various techniques to attach a biologically active protein—in our case, we chose insulin-like growth factor-1 (IGF-1), a promoter of cell proliferation—to a titanium surface like those used in implants," explains Chen Zhang, a researcher at the RIKEN Nano Medical Engineering Laboratory.

Using a combination of recombinant DNA technology and treatment with the enzyme tyrosinase, the researchers were able to create a hybrid protein that contained active parts of both the growth factor and L-DOPA. Tests showed that the proteins were able to fold normally, and further experiments in cell cultures demonstrated that the IGF-1 was still functioning normally. Thanks to the incorporation of L-DOPA, the proteins bound

strongly to the titanium surface. Even better, they remained attached when the metal was washed with phosphate-buffered saline, a water-based solution. "This is similar to the powerful properties of mussel adhesive, which can remain fixed to metallic materials even underwater," says Zhang.

The team is enthusiastic about the potential of the discovery. "We are very excited by this finding because the modification process is a universal one, which could be used with other proteins. It could allow us to prepare new cell-growth-enhancing materials, with potential applications in cell-culture systems and regenerative medicine," says Yoshihiro Ito, the team leader. "And it is particularly interesting that this is an example of biomimetics, where nature can teach us new ways to do things. The mussel has given us insights that could be used to allow us to live healthier lives." ■

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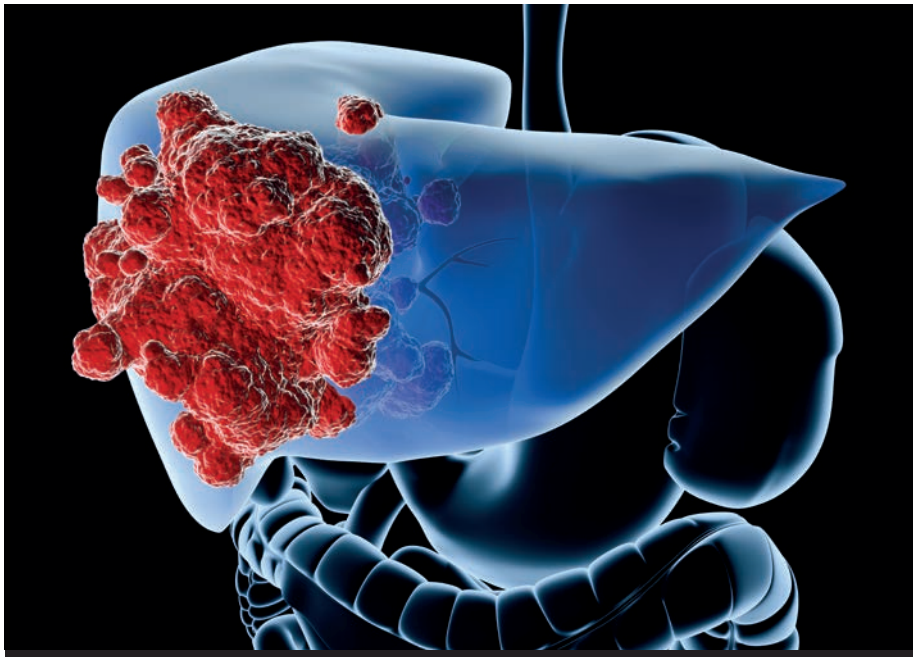
MEDICINE

The genomic landscape of liver cancer

A huge DNA sequencing effort has exposed the mutations behind liver cancer in Japanese patients

In one of the largest ever genomic studies of a single-organ cancer, scientists in Japan have discovered a multitude of mutations responsible for causing liver cancer[1]. The research, led by a team from the RIKEN Center for Integrative Medical Sciences, could help doctors develop personalized drug regimens tailored to each patient's genetic signature.

"Whole-genome sequencing revealed that about 40 per cent of liver cancers had a mutation of the genes related to therapeutic targets and that are hence expected to be actionable," says Hidewaki Nakagawa, senior author of the study.



Liver tumors (like the one shown in red here) are major killers. A large genomic study has shown that liver cancer can be classified into six different types.

The fifth most common cancer and the fourth leading cause of cancer-related death in Japan, liver cancer was responsible for an estimated 19,000 deaths in the country last year alone. Chronic infection with hepatitis B and C viruses can often lead to liver cancer, but so can alcohol abuse, metabolic diseases and certain environmental toxins, suggesting that many different genetic drivers can interact with lifestyle factors to bring about liver tumors.

In search of mutations that could explain the diversity of the disease, Nakagawa and his colleagues sequenced the entire genomes of liver cancers from 300 Japanese patients. That much sequencing yielded more than 300 terabytes of data, and the analysis required using the supercomputer SHIROKANE—the fastest in the life-science research sector in Japan.

The researchers discovered 25 genes that were repeatedly mutated in different patients' cancer samples, causing changes in tumor suppressors or other regulatory proteins. They also found a number of recurrently mutated sites in the genome that did not code for a protein, as well as structural rearrangements that popped up time and again with an effect on nearby gene expression.

Looking across the mutational landscape, Nakagawa and his team found that liver cancer among Japanese patients could be broken down into six types—and that these types were strongly linked to survival outcomes.

"Our study revealed that the prognosis of patients with mutations in some genes is worse than others," says Akihiro Fujimoto, who worked on the research at RIKEN before moving to Kyoto University Graduate School of Medicine. "Although validation with an independent cohort is required, this result can contribute to the prediction of patient prognosis in the future."

Knowing the different liver cancer types and the mutations that underlie them could also help scientists develop new, precision drug treatments. "Our analysis identified novel driver gene candidates, which can be targets for therapies," says Fujimoto.

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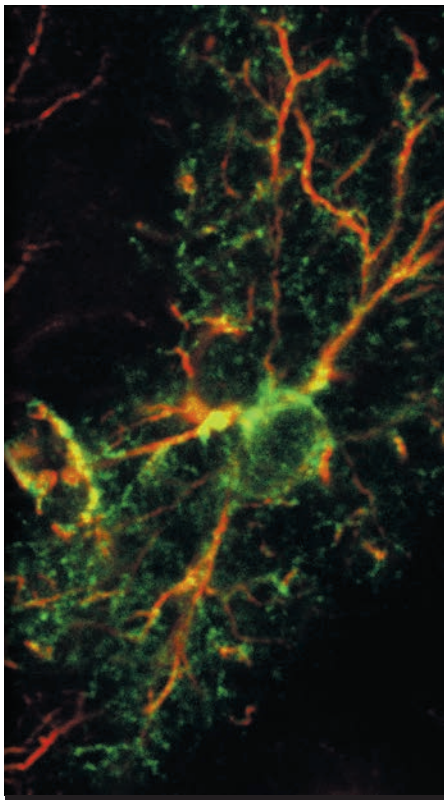
BIOLOGY | PRESS RELEASE

Under-appreciated brain cell active in neurological changes

Brain cells previously thought to play a mainly supporting role have been shown to be active in determining the strength of connections between neurons



Fluorescence micrograph showing expression of a fluorescent dye due to astrocyte activity (green).

Star-shaped glial cells in the brain known as astrocytes help control the strength of connections between neurons, find RIKEN researchers[1].

Whenever we learn something new or are affected by experiences, it is due to changes to synapses—the connections between neurons in our brains—from the formation of new synapses to the strengthening or weakening of existing synapses. Until recently, synaptic strength was thought to change only at synapses of active presynaptic neurons, but the truth turns out to be more complex.

"We have found an active mechanism that helps to increase variation in synaptic strength," explains Yukiko Goda at the RIKEN Brain Science Institute. "Surprisingly, it comes from astrocytes, which previously were thought to play mostly passive roles in the brain."

Astrocytes are often described as support cells for neurons. While recent studies have shown that they might have some global effects on neuronal transmission, Goda's team found that they can have very local effects at the individual synapse level.

To investigate the effects of astrocyte activity on synaptic strength, the team set up a culture of hippocampal neurons and astrocytes. They then examined the strengths of two neurons that were each connected to a third target neuron at separate synapses but were not connected to each other under different conditions (see image).

Expected changes in synaptic strength were found at a synapse when the presynaptic neuron to which it was connected was stimulated by electrical pulses. The researchers found that this was often accompanied by changes at the other, non-stimulated, synapse.

Testing showed that the changes at these 'heterosynapses' were not related to the postsynaptic neuron, but were blocked by a *N*-methyl-aspartate (NMDA) receptor antagonist. Further testing showed that blocking astrocyte activity also prevented changes at the heterosynapses. When neurons were not experimentally stimulated, blocking astrocyte NMDA receptors caused synaptic strengths of converging inputs on a given neuron to become more equal, whether in culture or intact hippocampal slices. "We found that astrocyte activity helps maintain normal variation of synaptic strengths, even in the absence of strong, plasticity-inducing stimulation," explains Goda.

These findings are not just academically important. "As synaptic dysfunction is thought to trigger or exacerbate many

neurological diseases, a deeper understanding of how synaptic communication is regulated will aid in discovering disease mechanisms and developing treatments," says Goda. "Our work shows that astrocytes could be a potential target of novel therapeutics."

"Our next goal is to determine the precise signaling mechanism by which astrocytes target presynapses," she adds. ■

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PHYSICS

Solar cells benefit from electronic 'catastrophes'

Spontaneous redistribution of charge at nanometer-thin interfaces unlocks a fundamentally different technique for harvesting solar energy

By precisely fabricating nanoscale junctions between two metal oxides, RIKEN researchers have found a way to transform an electrically neutral semiconductor into a polarized material capable of capturing large quantities of light-generated charges[1].

Individually, lanthanum aluminate ($LaAlO_3$) and strontium titanate ($SrTiO_3$) are insulators. But when these materials are put into intimate contact at a 'heterointerface', a highly conductive two-dimensional (2D) zone is formed. One reason these intriguing interfacial properties emerge is the so-called polar catastrophe: chemical ions at the abrupt, structurally stable $LaAlO_3$–$SrTiO_3$ junction cause charges to rearrange and produce polarized fields that screen the 2D layer from the rest of the material.

Recently, Masao Nakamura and colleagues from the RIKEN Center for Emergent Matter Science developed a 'correlated' solar cell that exploits the strong electron correlation in transition-metal oxides to generate multiple charges from a single photon. This technology is promising because it reduces much of the energy loss that normally occurs in solar cells due to heat.

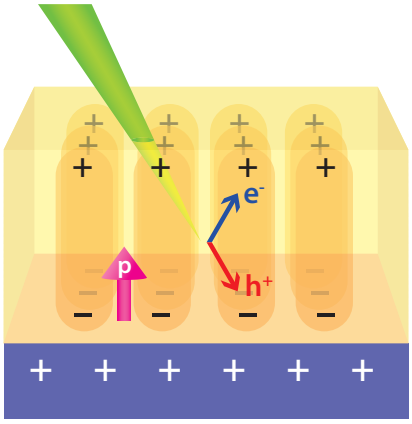
However, the method suffers from poor energy conversion. The team realized that part of the problem was relying on conventional diffusion processes to catch light-generated charges. "Unfortunately, correlated electron materials have low mobilities, which means their active layers are very thin," explains Nakamura. "We considered different ways to extract photocarriers than those employed in typical junctions, and that led us to study the photovoltaic effect in polar materials."

Some semiconductors have non-symmetric crystal structures that generate larger photovoltages than those predicted by theory. The asymmetric frameworks in these materials help 'shift' electrons toward a collection point independent of the charge's mobility, a feature the RIKEN team harnessed by creating a custom-made polar interface.

To ensure a sharp polar discontinuity, Nakamura and co-workers chose an iron-based lanthanum oxide ($LaFeO_3$) and deposited it in a 30-nanometer layer on a $SrTiO_3$ substrate. When illuminated by laser light, the new interface displayed an intriguing switching of photocurrent direction simply by contacting different sides of the junction (see image)—clear evidence that the atomic structure was influencing the behavior of the entire bulk material.

Using techniques such as piezoresponse force microscopy and electron holography to characterize the junction, the researchers discovered that although the $LaFeO_3$ layer was originally nonpolar, polar catastrophe forces spontaneously switched its charge distribution.

"Polar materials could act as high-efficiency solar cells, but they usually have large band gaps and hence absorb small amounts of visible light," says Nakamura. "Our work suggests that heterointerfaces can be used to tailor these substances." ■



A schematic depiction of a spontaneously polarized solar cell. When light (green beam) strikes a heterointerface with a net electrical polarization (pink arrow), photogenerated charge carriers (e^- and h^+) can be recovered no matter how slowly they move.

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Cell biology

Controlling the dynamics of life

Researchers at RIKEN are pursuing the promise of unprecedented control over health and disease with the emergence of a truly interdisciplinary field of study. Experimentalists in cell biology are expanding comprehensive and quantitative analysis techniques, while mathematicians, physicists and philosophers are continuing a legacy of applying abstract thought to describe the dynamics of living organisms.

Mariko Okada is head of the Laboratory for Integrated Cellular Systems at the RIKEN Center for Integrative Medical Sciences
Since joining RIKEN in 2000, Okada has been exploring a systems biology approach, combining experiments and computational methodologies to understand cellular regulatory mechanisms. Her current interests are in the mechanisms that induce specific cellular outcomes.

Biologists and physicists are often seen as being at opposite ends of science. While both attempt to explain and predict the world around us using the language of science, the dialect of physics and mathematics isn't easy to translate into the dialect of biology.

In the field of biology, these two dialects are converging in a way never seen before. The techniques and approaches used by these seemingly disparate branches of science are being exchanged to yield new and far-reaching benefits.

On one hand are the reductionists, who try to explain complex systems by breaking them down into simpler component parts. With access to a vast repertoire of microscopic experimentation and observation, these keen-eyed examiners try to implicate faulty genes and mutant proteins in diseases, or infer details about living organisms from cells isolated in Petri dishes.

On the other hand are the theorists, who try to establish fundamental principles in the biological universe. A single-nucleotide shift, they argue, cannot possibly explain the behavior of an entire organism. Instead, the many molecular components continuously interact to form an integrated, robust and dynamic network that expresses a wide range of biological states.

Both approaches are limited in their ability to describe biological processes. The former stops time and confines space to take a closer look, while the latter listens for faint harmonies in a cacophony of noise. An integrated approach is now taking hold of the field, with researchers at RIKEN at the forefront of taking control of health to sideline disease.

Parts of a puzzle

The origins of modern molecular biology are often traced back to a series of studies in the mid-twentieth century in which scientists first isolated DNA, determined its chemical components, and revealed its three-dimensional double-helix structure.

These breakthroughs were followed by the discovery of restriction enzymes that could cleave DNA at specific sites and detach short manageable fragments from the tangled string of the larger molecule. Technologies emerged, such as polymerase chain reaction (PCR), to clone millions of copies of these DNA fragments for closer analysis.

The 1970s saw the arrival of simple and quick methods for sequencing purified strands, culminating in the 2000s with the completion of the Human Genome Project to spell out all 3 billion letters in the multi-volume book that is the human genome. Further automation and refinement of sequencing techniques have dramatically sped up and reduced the cost of gene sequencing.

These advances have enabled the careful and controlled study of individual genes. By editing, cutting and pasting sections of the genetic code, often to generate mutations and model organisms, researchers have been able to link specific genes with specific cellular processes and, ultimately, diseases.

Until recently, well-respected molecular biologists could spend their entire lives studying the biological function of a single gene or molecule. But the same technologies that facilitated such diligent research, have also led to an explosion of information about the rest of the DNA, RNA and proteins swimming in the cellular soup. The genomes of thousands of microbe species have been sequenced, and thousands more of individual specimens within the same species. Added to these complete genomic registers have been the characterization of entire sets of RNA transcripts, epigenetic modifications, proteins and metabolites produced by an organism.

This phenomenal production of information transformed a nascent field of bioinformatics into a booming industry —the 'omics' industry—turning raw data into insights. Researchers, especially

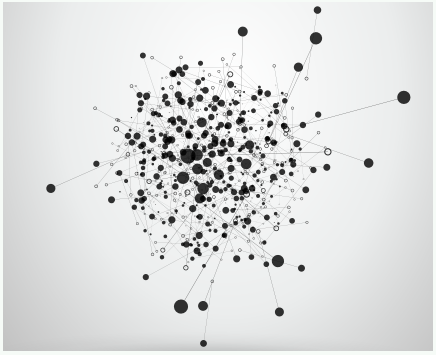

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Figure 1: Complex webs linking multiple genes, RNA transcripts, epigenetic modifications, proteins and metabolites in living organisms are being developed from massive databases.

in the United States, began developing computational techniques and statistical models to annotate and analyze the data using powerful supercomputers. The result is a complex web linking multiple genes to multiple metabolites and multiple products (Fig. 1).

But even after overcoming the difficulties of piecing together the billions of puzzle pieces of living organisms, many argue that this approach brings researchers no closer to understanding how the different parts connect over time, giving rise to the dynamics of life. A somewhat older, but less well-known approach, to molecular biology offers an alternative.

Summing up cells

Pen, paper and a clear head established the theoretical foundations for molecular

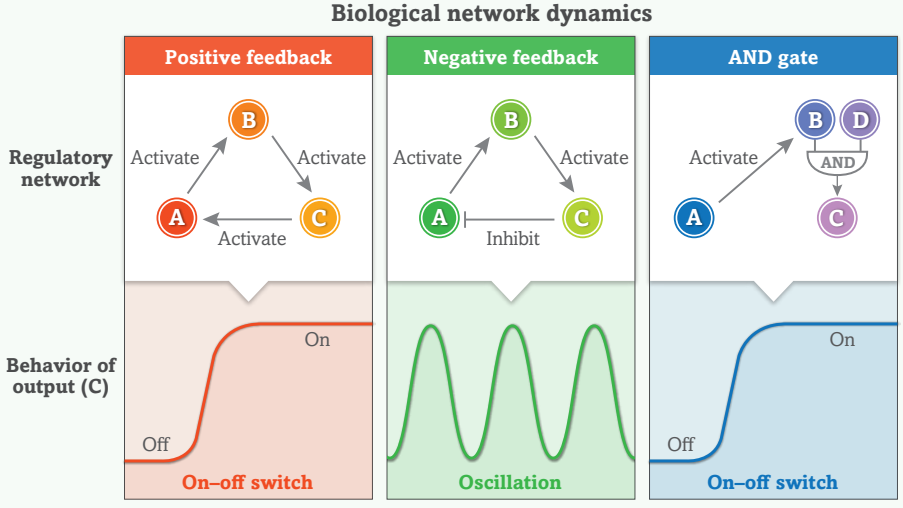

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Figure 2: Biological networks are controlled by self-regulating pathways of positive and negative feedback.

biology, with the application of differential equations, control theory and non-equilibrium thermodynamics to describe moving molecular networks.

Biologists and biophysicists investigated these concepts in the belief that universal rules also applied to living systems. Well-described rules gave researchers control over biological states, which could eventually be used to correct malfunctioning systems and cure human diseases.

Biological processes typically involve a series of chemical reactions, linked together like a chain of dominoes. The dominant view prior to the establishment of these theoretical frameworks was that a single step in the pathway controlled the rate of production and that speeding up or slowing down that one activity could increase or decrease the output. But several researchers in Europe used mathematical techniques to prove that control over the pathway was shared between many different steps.

These techniques have been applied to the study of a wide range of biological systems, from signaling pathways to gene expression. They reveal the robust and adaptive nature of biological networks, and explain why only a small number of diseases can be attributed to single genetic mutations. Proponents of this theoretical approach have even found that the same pattern of genetic expression can manifest as more than one physical trait, contradicting the reductionist view that organisms are no more than the sum of their parts.

With the gradual convergence of previously disparate disciplines, mathematicians and physicists have begun to explore new territories. In 2013, RIKEN launched the Interdisciplinary Theoretical Science Research Group (iTHES) to drive theoretical science in fields ranging from sub-nuclear physics to cosmology and biology. And, in April 2016, it established the Center for Advanced Integrated Intelligence Research to carry out fundamental studies into informatics. But in the field of molecular biology, the Laboratory for Integrated Cellular Systems at the RIKEN Center for Integrative Medical Sciences, has made remarkable strides in merging the two worlds of theory and experiment.

Chain reaction

An interdisciplinary approach to molecular biology involves finding coherent theories to explain the reams of gene and protein expression data acquired from high-throughput experiments. The framework gives meaning to the static networks produced by the 'omics' industry.

Biological networks are self-regulating. Steps further along the pathway can control earlier events in one of two ways: positive or negative feedback. Imagine a triangle of three genes: gene A activates gene B, gene B activates gene C, and gene C then activates gene A to create a positive feedback loop (Fig. 2). Researchers use mathematical models to explain what effect such regulatory mechanisms, or motifs, might have on cellular dynamics. The activate–activate–activate motif, for example, results in a steep increase in activity, which can set a threshold in the system. But if C instead inhibits A to create a negative feedback loop, the result is often a series of oscillations—an important mechanism governing cyclic behavior such as circadian rhythms and cell cycles.

In May 2014, researchers at the RIKEN Center for Integrative Medical Sciences identified an important role of a positive feedback mechanism in controlling the switch-like response of a transcription factor in a type of white blood cell known as B cells[1] (Fig. 3). These cells defend the body against foreign invaders by producing specialized antibodies that tag pathogens for destruction by other parts of the immune system. Studies that improve our understanding of the underlying mechanisms of B cell activation can help to explain how irregular activity could lead to immune deficiency or lymphoma.

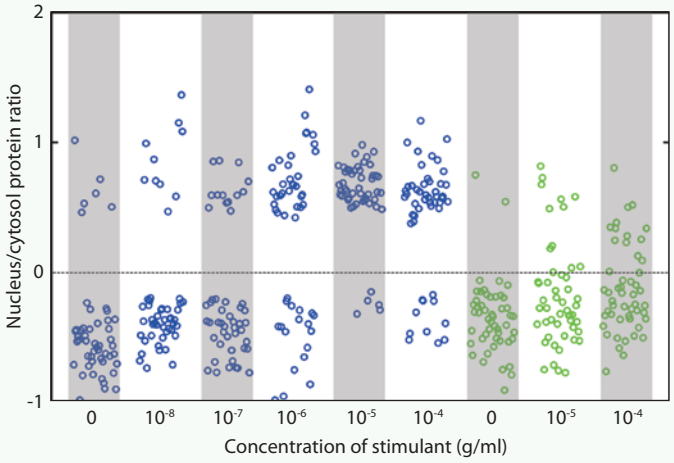

Figure 3: A transcription factor found in B cells is activated and moves from the cytosol to the nucleus in a switch-like manner (blue). Cells in which the mechanism stimulating this switch-like response was deactived exhibited a more graded response (green).

Earlier in May 2010, the RIKEN team, in collaboration with a systems biologist at University College Dublin, found a different regulatory motif to explain the switch-like behavior of a transcription factor in a breast cancer cell line[2]. The circuit includes an additional molecule, D, and a concept borrowed from control theory: the 'AND gate'. Both B and D have to activate C to produce the switch-like outcome. The finding also helps to explain how the same pattern of activity can result in different cellular outcomes, depending on the presence or absence of an AND gate.

Interdisciplinary approaches to science require patience and a willingness to approach problems from a different perspective, as the language of physics and mathematics is not easy to translate into the language of biology. Often, such interactions fail to extract simple rules about cellular behavior. But when they do, you can be certain of their far-reaching benefits over time.

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Research highlights

The finding that entropy is fundamentally related to the time symmetry of a system could have important implications for the entropy of black holes.

PHYSICS

Finding order in chaos

The entropy of thermodynamic systems is found to be related to a certain symmetry of time, which has implications for the entropy of black holes

Entropy is related to the disorder of a system: the more disordered a system is, the higher its thermodynamic entropy will be. Despite this connection to disorder, two researchers have shown how entropy is related to a certain time symmetry of a system[1].

Symmetries are fundamental to physics because they are deeply connected to the unchangeableness of certain physical properties. For example, an object moving in a system that remains unchanged when moved to different places will have a constant speed—physicists say that its momentum is conserved. Similarly, a system that does not change with time (that is, it is independent of time translations) will always keep the same energy—a well-known principle called the conservation of energy.

In 1915, Emmy Noether discovered that this connection between symmetry and the conservation of physical parameters has a fundamental mathematical root. But entropy could not previously be understood within this framework since the laws of thermodynamics state that the entropy of a closed system will remain the same or increase. This means that no apparent symmetry is associated with thermodynamic entropy; it is not symmetric in time, but is related to the fact that time only proceeds forward. "Entropy is a special quantity in physics," explains Yuki Yokokura of the RIKEN Interdisciplinary Theoretical Science Research Group (iTHES). "It connects the microscopic and macroscopic worlds and is vital for understanding the 'arrow of time.'"

Now, Yokokura, with Shin-ichi Sasa of Kyoto University, has shown mathematically that processes that conserve entropy can be

connected to a symmetry through Noether's theorem. Instead of being related to uniform time translations (which correspond to the conservation of energy), the conservation of entropy is related to a special non-uniform time translation.

The non-uniform time translation can be understood by considering particles in a cylinder with thermally insulated walls and a very slowly moving piston. Then, the entropy of the system will be conserved, but its energy will change due to the work done by the piston. The symmetry of the system under such a non-uniform time translation (corresponding to the conservation of entropy) means that the properties of the particles in the system remain the same with each time step by an amount determined by the system's thermodynamic properties in a time-dependent way.

This could shed light on very different processes. "For example, the symmetries related to black hole entropies are similar to the ones we considered in our study," says Yokokura. "This may help us understand the microscopic origin of black hole entropy." ■

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BIOLOGY | PRESS RELEASE

Untangling ambiguity in neural circuits

Scientists have discovered how rats process unclear associations between stimuli in their brains

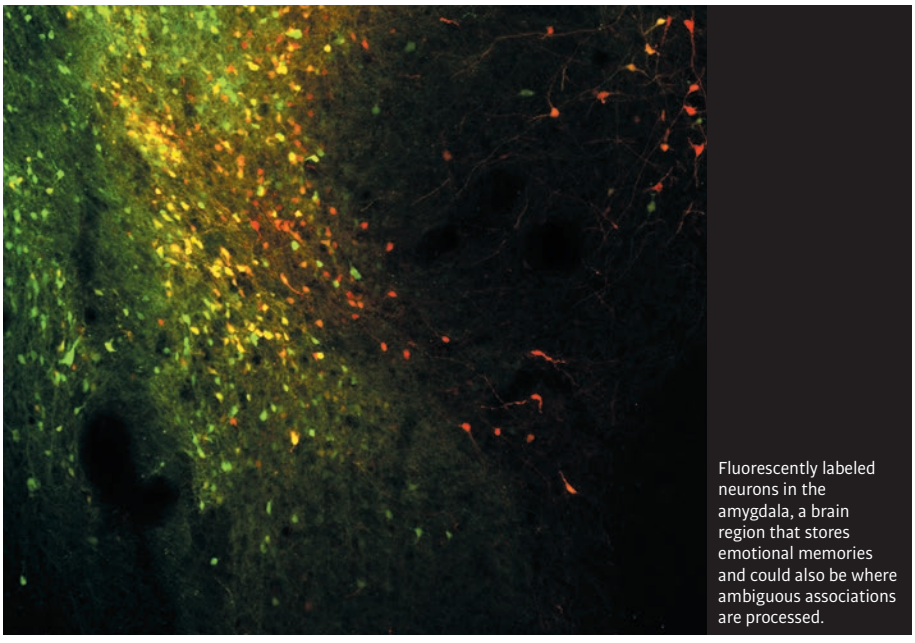
Humans and animals continually face ambiguous circumstances. We blame our food if we become sick after eating, for example, but if we fall ill having not eaten, the causal link becomes ambiguous. Now, RIKEN scientists have discovered where and how such ambiguous associations are processed in rat brains[1].

Learning how to predict dangerous relationships in the environment—such as between odors and unsafe food and between lightning and thunder—is essential for survival. While much is known about how experiences become linked with unpleasant outcomes when there are clear associations, it was unclear how these links are updated in the brain for ambiguous relationships.

Now, a team led by Joshua Johansen of the RIKEN Brain Science Institute has discovered that ambiguity in these types of associations can be observed in rats that had previously learned a tone–shock association and were then given shocks without the tone. They found traces of both the memory and its associated uncertainty stored in neural circuits of a central brain area called the amygdala.

On hearing a tone, rats that have learned the association usually freeze in anticipation of the shock. But they showed less anticipatory freezing when they learned that shocks can also be unaccompanied by a tone, because the causal link between tone and shock was not clear. In the brain, this uncertainty was reflected in weaker neural connections between the auditory system (where tones are processed) and the amygdala, a brain region critical for storing unpleasant memories. Strong connections that formed after learning the tone–shock association were reversible if the relationship later became ambiguous.

By manipulating brain activity during learning with optogenetics—a technique



Fluorescently labeled neurons in the amygdala, a brain region that stores emotional memories and could also be where ambiguous associations are processed.

to control brain cell activity with bursts of light—the researchers showed that specific neurons in the amygdala were responsible for enabling the uncertainty. When those neurons were silenced as animals experienced shocks without a preceding tone, surprisingly, rather than acting unsure and freezing less often, the animals always acted as if they expected to be shocked when they heard the tone. This indicated that the amygdala not only stores unpleasant memories, but also plays an active role in an animal's evaluation of the associated level of ambiguity.

"We believe we've discovered a new framework for the amygdala and how its neurons process ambiguous associations," says Johansen. "Our results are important for understanding how memories and associations are formed and modified."

The findings may also have implications for understanding conditions involving ambiguous associations, such as anxiety, as well as for designing more-flexible computer architectures. ■

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CHEMISTRY

Hunt for battery electrolytes makes good use of an old foe

Carbon dioxide used to synthesize possible electrolytes for rechargeable lithium-ion batteries

A simple method for making a family of potential battery electrolytes has been developed by RIKEN researchers, using ubiquitous carbon dioxide as one of the key chemical ingredients[1].

Electrolytes carry charged ions between the two electrodes of a battery and are usually dissolved in solvents such as carbonates. Because they are stable, environmentally benign and good at transporting lithium ions, lithium borate salts are being studied as next-generation electrolytes for rechargeable lithium-ion batteries. But chemists lack efficient methods for producing these salts, making it difficult to optimize them for use in batteries.

Coal-powered power stations, such as the one shown here, as well as many other industrial plants generate carbon dioxide, which researchers can potentially use as a raw material to make better electrolytes for lithium-ion batteries.

Zhaomin Hou of the RIKEN Center for Sustainable Resource Science and colleagues have now developed a simple method that uses carbon dioxide to produce a wide range of lithium boracarbonates, which combine some of the chemical properties of both lithium borate salts and carbonate solvents. "Their unique structure and composition could make these compounds useful as electrolytes in lithium-ion batteries," says Hou.

Researchers around the world are searching for ways to use carbon dioxide, which is one of the key drivers of human-driven climate change. In principle, the gas could be used as a low-cost chemical feedstock, but this application is limited because of carbon dioxide's low reactivity.

Hou's team discovered that a particular copper-containing catalyst spurred the reaction between carbon dioxide, a common reagent called bis(pinacolato)diboron, and a series of different organic molecules known as aldehydes. "We were surprised that the multicomponent coupling reaction took place in such a remarkably selective and efficient fashion," says Hou.

Supplying carbon dioxide at five times atmospheric pressure, they ran the reaction with a series of 18 aldehydes and produced lithium boracarbonates with yields between 53 and 91 per cent.

"Different starting aldehydes yield boracarbonate products with different substituents," explains Hou. "These substituents could influence the electrochemical and physical properties of the products, thus enabling systematic screening of lithium electrolyte candidates."

The researchers used x-ray crystallography to confirm the structure of one of these products. They also suggested a reaction mechanism that explains how the copper catalyst helps to activate the reagents so that they combine with carbon dioxide. "These findings will help us design new reactions with various substrates," says Hou.

The team now plans to investigate the electrochemical properties of lithium boracarbonate compounds and test them as electrolytes in rechargeable lithium-ion batteries. "We will also continue to investigate the efficient and selective synthesis of novel, useful chemicals using carbon dioxide as a feedstock," adds Hou. ■

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MATERIALS | PRESS RELEASE

Moving film harnesses the power of humidity

Humidity variations cause a new film to curl up and flatten out and even to 'walk' across a surface

A film that curls up and straightens out when exposed to barely measurable fluctuations in the ambient humidity has been developed by RIKEN scientists[1].

"Our study began from a serendipitous finding," explains Daigo Miyajima of the RIKEN Center for Emergent Matter Science. "When we placed a compound called guanidinium carbonate into a high-temperature oven, we found that it formed not only a powdery substance—as is usual in similar processes—but also a yellowish film that stuck to the substrate surface."

The researchers discovered they could remove the film from the substrate by soaking it in warm water and that it was extremely light despite its toughness. To their surprise, under ambient conditions, the film would suddenly bend and straighten out again, without any obvious external stimulus.

The group found that the key was tiny, indiscernible changes in the ambient humidity. They discovered, for instance, that bringing a water drop close to the film would cause it to straighten, but that this did not happen when there was no air motion in the box that the film was in.

The researchers weighed the film when it was stretched out and curled up and found that the curled configuration was very slightly heavier. They concluded that it was desorbing water on one surface and that the bonds between the water molecules and the polymer created mechanical stress, which caused the film to curl up. The change happened extremely rapidly—taking just 50 milliseconds when the film was exposed to ultraviolet light.

The motion was also powerful. When the film was placed on a flat surface and made to curl up, it could jump to a height

Photographs showing a newly discovered film curling up in response to increasing humidity.

of 1 centimeter, which is 10,000 times the film thickness. It was also durable: when they irradiated the film repeatedly with ultraviolet light, it bent and straightened more than 10,000 times without noticeable deterioration.

In a final experiment, the researchers covered one half of the film with thin gold to stop it absorbing and desorbing water and then subjected it to repeated curling and straightening. The film walked over a surface, dragging itself as half of the film bent and relaxed.

"In the same way that a mechanical watch takes advantage of the natural movements of the wrist to gain energy, this film takes tiny fluctuations in the ambient humidity and transforms them into mechanical energy," says Takuzo Aida, who led the team. "This type of material will be useful for creating a sustainable society." ■

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BIOLOGY

Keeping viral DNA at bay

Scientists find that an epigenetic interplay keeps virus-derived DNA repressed in embryos, but not in the surrounding placenta

The human genome is riddled with virus-derived sequences considered to be remnants of viral infections during our evolutionary past. A new study by RIKEN researchers explains how these so-called endogenous retroviruses (ERVs) are repressed in the developing embryo, but also finds that they spring into action in the cells of the placenta[1].

"In my field, people mostly think that ERVs are harmful and therefore should be silenced in all cells," says Jafar Sharif of the RIKEN Center for Integrative Medical Sciences, who led the study with colleagues at RIKEN and collaborators in Japan, Canada and the US. "But our work shows that ERV expression takes place even under perfectly normal conditions, such as in the placenta during development."

Sharif and his colleagues first wanted to clarify how these viral sequences are silenced in the embryonic lineage.

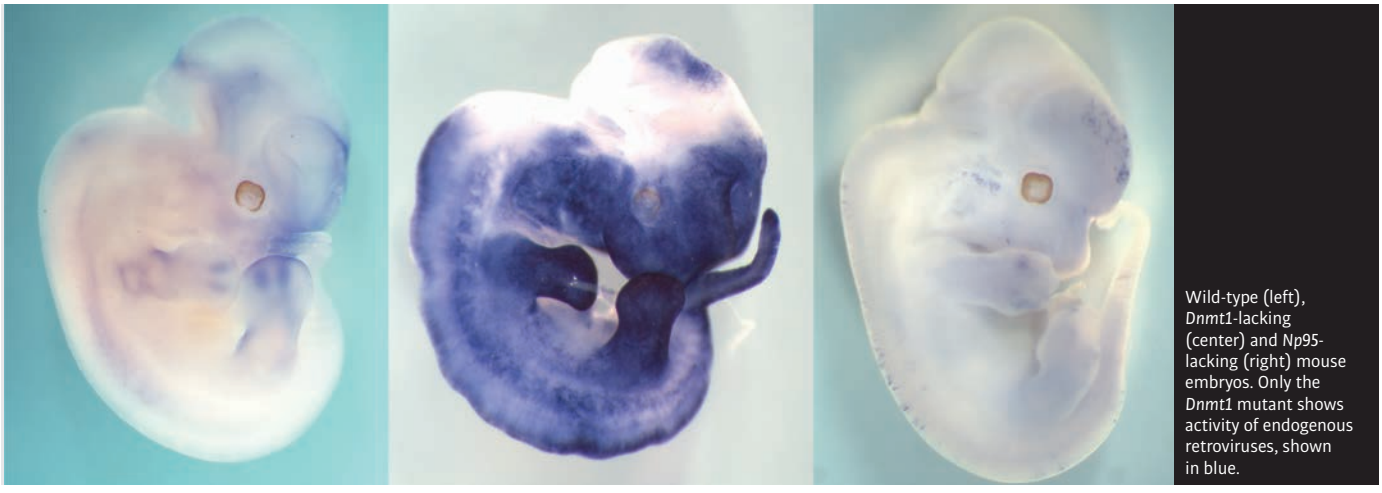
An enzyme called DNMT1 was known to help to inactivate these rogue genetic elements by ensuring that methyl tags are placed in the right locations on the DNA backbone to maintain proper gene expression and regulation.

When functioning DNMT1 is absent, the viral elements kick into action, which was thought to be due to a lack of DNA methylation. But that turned out to be only part of the story. The RIKEN team has shown that the partially methylated DNA wrought by depletion of DNMT1 actually attaches itself to a second protein called NP95, which triggers the regulatory cascade responsible for releasing the brakes on viral repression.

The researchers discovered that prolonged binding with NP95 disrupted the way DNA was packaged into chromatin fibers and allowed ERVs to be expressed. They confirmed the crucial role of Np95 by removing the protein in mouse embryos, either alone or in combination with Dnmt1 removal. In both cases, the viruses remained dormant (see image).

"I was very surprised," says Sharif of this unexpected finding. "In fact, at first I thought my experiments were not going well when I found that deleting the *Np95* gene together with the *Dnmt1* gene inexplicably extinguished the activation of ERVs."

But more surprising was that the Np95 protein and ERVs are highly expressed in placental cells.



Wild-type (left), *Dnmt1*-lacking (center) and *Np95*-lacking (right) mouse embryos. Only the *Dnmt1* mutant shows activity of endogenous retroviruses, shown in blue.

This discrepancy between ERV activity in embryonic and extra-embryonic tissues remains a mystery.

"There is still no physiological explanation for why these ERVs are expressed in the placenta," notes Sharif, who intends to find out why. "I like to think that ERV expression must have some biological meaning, or else it would not be tolerated in the placenta," Sharif says. ■

Reference

1. Sharif, J., Endo, T. A., Nakayama, M., Karimi, M. M., Shimada, M., Katsuyama, K., Goyal, P., Brind'Amour, J., Sun, M.-A., Sun, Z. *et al.* Activation of endogenous retroviruses in *Dnmt1*$^{-/-}$ ESCs involves disruption of SETDB1-mediated repression by NP95 binding to hemimethylated DNA. *Cell Stem Cell* **19,** 81–94 (2016).

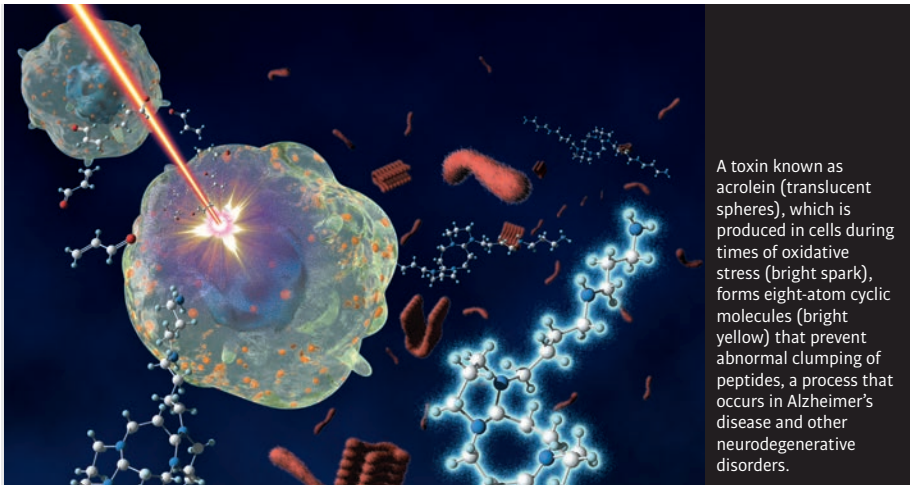
BIOLOGY | PRESS RELEASE

Deadly toxin has useful biological role

Previously considered a dangerous substance that promotes disease, a toxin released by cells may help in the fight against Alzheimer's

A toxin produced in cells during times of oxidative stress could help prevent the abnormal clumping of peptides associated with Alzheimer's disease and other neural diseases, find RIKEN scientists[1]. The key to this beneficial function is a chemical process known as 4+4 cycloaddition, where two molecules with backbones of four-atom chains combine to form an eight-atom, ring-like structure.

The RIKEN group found that in some circumstances, the toxin acrolein combines with a class of molecules called polyamines to make substances that prevent the fibrillization of Aβ40 peptides. "What is remarkable is that the reaction involves acrolein and a class of substances known as polyamines, which are all associated with oxidative stress," says Ayumi Tsutsui of the Biofunctional Synthetic Chemistry Laboratory. "Polyamines are known to play very important biological roles, but the mechanisms are still poorly understood."



A toxin known as acrolein (translucent spheres), which is produced in cells during times of oxidative stress (bright spark), forms eight-atom cyclic molecules (bright yellow) that prevent abnormal clumping of peptides, a process that occurs in Alzheimer's disease and other neurodegenerative disorders.

Acrolein levels are correlated with the progression of diseases such as cancer and stroke. "It made sense to see acrolein simply as a dangerous substance that triggers disease, and many researchers saw it that way," explains Tamotsu Zako of the RIKEN Bioengineering Laboratory. "But in previous work, we discovered that acrolein could bind with polyamines to form eight-atom cyclic molecules, and we wondered what biological role these rings might play."

The team was surprised to find that these substances seemed to prevent peptides known as amyloid-beta from aggregating—a process linked to the progression of Alzheimer's disease. The group tested the hypothesis by incubating Aβ40 peptides in mixtures of acrolein, a polyamine known as spermine, and a cyclic compound formed by acrolein and polyamines. Neither of the first two molecules alone affected the clumping, known as fibrillization, but the cyclic compounds turned out to be powerful inhibitors.

The researchers also found that when acrolein and polyamines were added together into a living cell, they combined naturally through 4+4 cycloaddition to create the diazacyclooctane molecule. "This is important for several reasons," says Katsunori Tanaka, who led the team. "First, it gives us insights into the mechanism through which polyamines—which we know to be tremendously important biologically—exert their action. And secondly, because acrolein and polyamines combine naturally in cells to form these powerful anti-fibrillization substances, it may open the way for us to influence the progression of terrible neurological disorders such as Alzheimer's."

The group hopes to extend their experiments to Aβ42, which is more prone to fibrillization than Aβ40 and is also believed to play a key role in Alzheimer's disease. ■

Reference

1. Tsutsui, A., Zako, T., Bu, T., Yamaguchi, Y., Maeda, M. & Tanaka, K. 1,5-diazacyclooctanes, as exclusive oxidative polyamine metabolites, inhibit amyloid-β(1-40) fibrillization. *Advanced Science* advance online publication, 1 June 2016 (doi: 10.1002/advs.201600082).

Superbugs with superpowers

Organisms discovered in extreme alkaline environments produce enzymes suitable for preserving smells, capturing tastes and cleaning clothes

Koki Horikoshi was an inveterate collector. As a young boy, he would hoard old radios, spending hours pulling them apart and rewiring them to tune in to the latest hits. But his most curious collection, the one for which the world best remembers him, has expanded our understanding of the Earth's biosphere.

During his lifetime, Horikoshi, who died in March 2016, catalogued the largest number of microorganisms that thrive in alkaline environments above pH 9—conditions that are toxic to humans. It turns out that these superbugs produce some of the most effective enzymes for cleaning laundry and preserving wasabi's pungence.

A basic diet

Born in Saitama in 1932, Horikoshi first encountered alkaliphiles through a chance experiment as a graduate student in the laboratory of chemist and microbiologist Kin-ichiro Sakaguchi, at the University of Tokyo. Sakaguchi was convinced that sake's flavor was the product of fungal self-digestion, and so every day Horikoshi tasted the fluids secreted by cultures of fungal strains.

Koki Horikoshi (left) and Toshiaki Kudo (right).

One day in November 1956, Horikoshi was about to undertake another round of tasting, only to find that the fungus in one of the cultivation flasks had disappeared overnight. In its place was the bacterium *Bacillus circulans*. Horikoshi proved that it had digested the fungus —the first bacteria found with fungus digestion abilities. *B. circulans* smelled of ammonia and grew weakly on a conventional neutral diet, but adding a pinch of basic baking soda increased the broth's pH, causing the cells to proliferate and produce the mold-killing enzymes.

The discovery, published in two *Nature* papers in 1958 and 1959, whetted Horikoshi's appetite for alkaliphiles. But the main course was yet to come.

Extreme living

In 1963, Horikoshi received his PhD and joined RIKEN. By 1968, his research had plateaued, so he flew to Europe for inspiration. In Florence, Italy, he was struck by the architecture, which was so different from the Japanese temples and tea houses constructed during the same period. He wondered whether a radically different environment could influence culture. Maybe even for culturing bacteria.

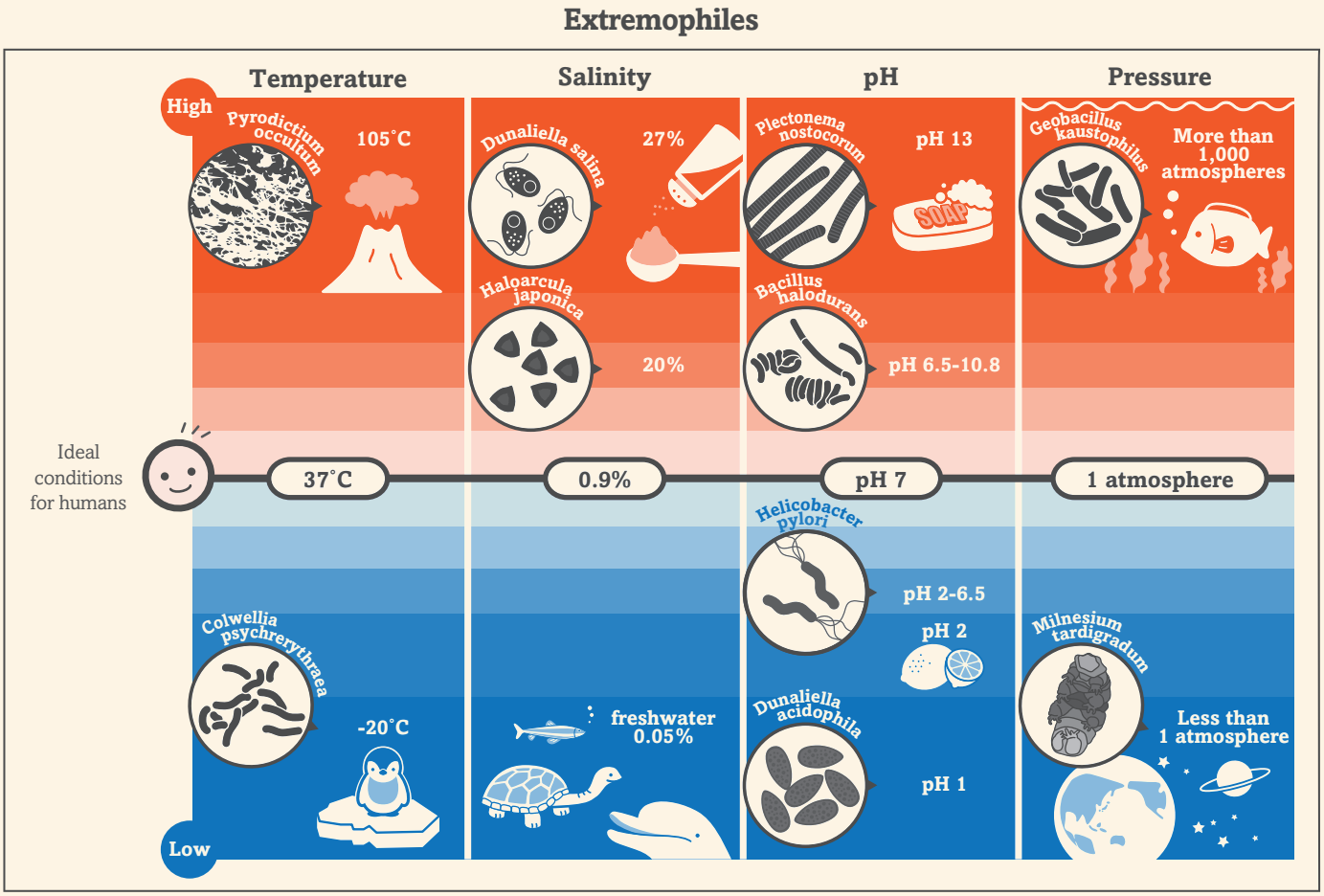
Back at RIKEN, Horikoshi collected soil samples from sites across campus and incubated them on an alkaline brew. The next day, he discovered a host of alkaliphilic bacteria.

> *Before Horikoshi's epiphany, biologists believed that only the narrow pH range that is tolerable by humans could sustain life.*

Our skin can deal with a good lathering and a quick rinse of alkaline soaps and shampoos, but prolonged contact is toxic. Before Horikoshi's epiphany, biologists believed that only the narrow pH range that is tolerable by humans could sustain life.

In the decades since, Horikoshi, who was promoted to chief scientist at RIKEN in 1974, has led groundbreaking studies into the diversity, physiology, molecular biology and genetics of alkaliphilic bacteria, yeast and fungi.

In 1984, Horikoshi led a major government initiative called the Superbugs Project to hunt for living organisms in the world's most extreme environments. His team pursued extremophiles not just at the upper and lower boundaries of pH, but also at the farthest reaches of temperature, pressure, salinity and toxicity. They found crimson, triangular bacteria in the salt fields of the Noto peninsula and rod-shaped bacteria tolerant



Living organisms inhabit the farthest reaches of temperature, pressure, salinity and pH.

to toxic toluene in the forests of Kumamato in southern Japan. In 1996, a submersible scooped alkaliphiles out of the Mariana Trench, more than 10 kilometers underwater. And in 1997, Horikoshi became editor-in-chief of the new academic journal *Extremophiles*.

Trap and clean

Alkaliphiles and extremophiles are not merely subjects of curiosity; they produce enzymes that have widespread commercial application. Horikoshi discovered two enzymes in particular that have made their way into millions of homes.

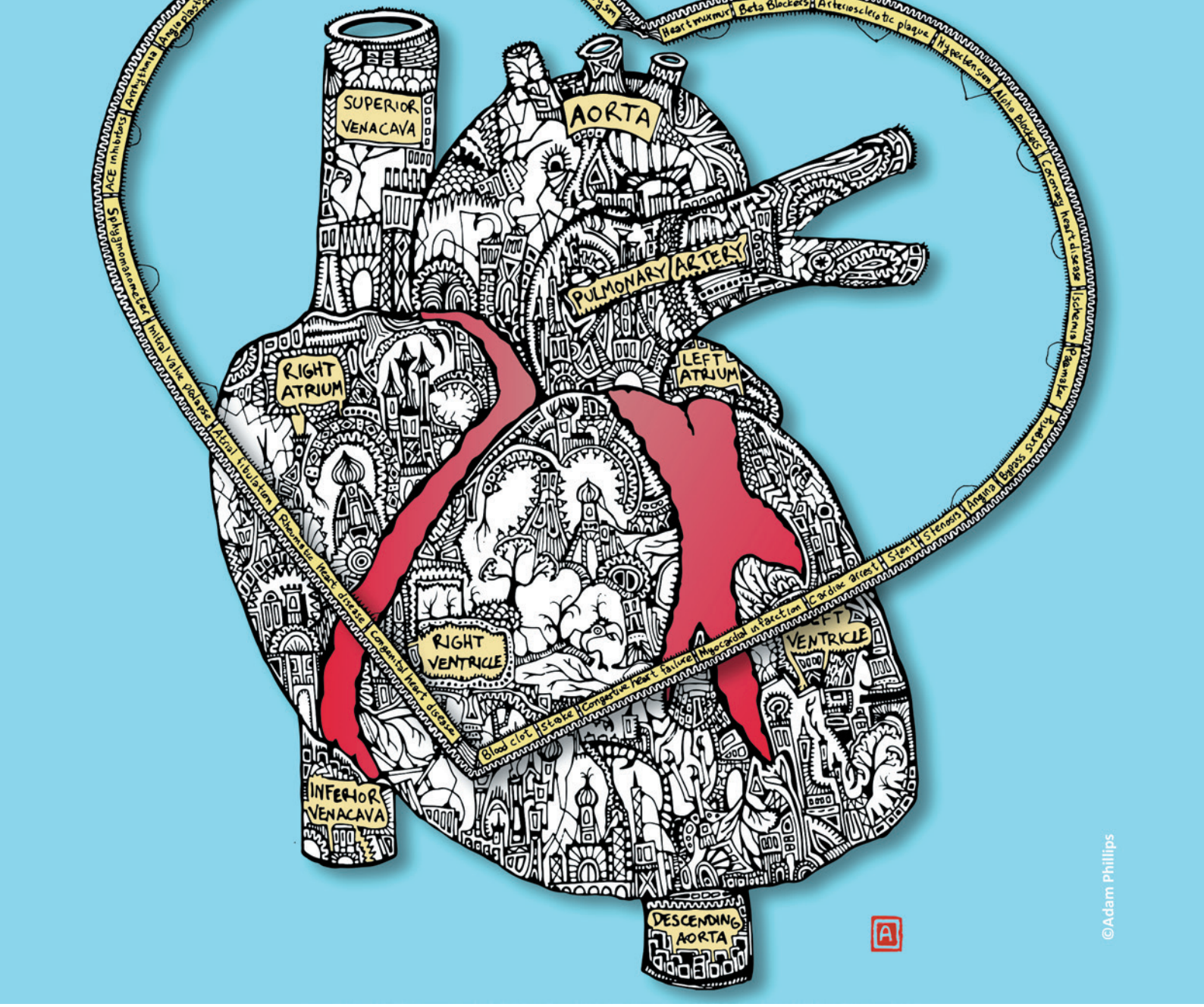
The laundry detergent Attack contains an enzyme produced by alkaliphilic bacteria.

The first was a donut-shaped sugar molecule called cyclodextrin, which can make unstable compounds stable and turn liquids into solids by trapping compounds in its central hole. But its application had been limited due to the toxic substances required to obtain high concentrations of the product, cyclodextrin.

Horikoshi's team discovered an alkaliphilic bacteria that could efficiently produce cyclodextrin from starch. The bacteria slashed the cost of producing 1 kilogram of cyclodextrin from US$530 to US$15. In 1976, a Japanese food manufacturer began mass-producing the enzyme, which is now a staple of many foods, pharmaceuticals and chemicals. It is used to prevent wasabi's pungent flavor from evaporating, to ensure well-mixed water and fat in butter and margarine, and to transform liquid sake into powder.

In collaboration with the chemical and cosmetics company Kao Corporation, Horikoshi's team also discovered that the enzyme cellulase could effectively remove dirt stuck to cotton fabrics. They took an alkaliphilic *Bacillus* bacterial strain that yielded large amounts of cellulase, and used genetic engineering techniques to increase production to an industrial scale. Kao introduced the enzyme to a line of laundry detergents called Attack. Six months after its launch in Japan in 1987, Attack had garnered half the detergent market. Continually improved Attack products are sold today in nearly a dozen countries.

Horikoshi died at 83 in March 2016, but his legacy lives on. "Horikoshi-sensei was a great and wonderful scientist who has inspired many young researchers," says Toshiaki Kudo, a microbiologist who joined Horikoshi's RIKEN lab in 1975. Kudo has extracted bacteria from biologically extreme environments such as the termite gut and puffer fish intestines. "We hope to continue his dream of pushing open new research fields." ■



Call for RESEARCH-INSPIRED ART

All too often, science and art are viewed as separate worlds. At RIKEN, we feel that scientific research can be artistic and that art based on scientific investigation can be beautiful and provocative. To celebrate the scientific method and artistic expression, we're calling for research-inspired art. If you are a scientist with artistic flair, please submit your (digital) work to it-aint-magic@riken.jp. We are looking for photos, drawings, music, or anything three-dimensional that you can take a picture of and send to us.

Here are the rules: base your work on any scientific paper published in 2015–2016. Include your name and scientific field (or your major if you are a student), a reference and a short description of the work. Once approved, your work will be uploaded to our blog, *It Ain't Magic*. If you've based your work on research conducted at RIKEN, especially a recent press release or *RIKEN Research* highlight, your submission could even be published in an upcoming issue of *RIKEN Research*.

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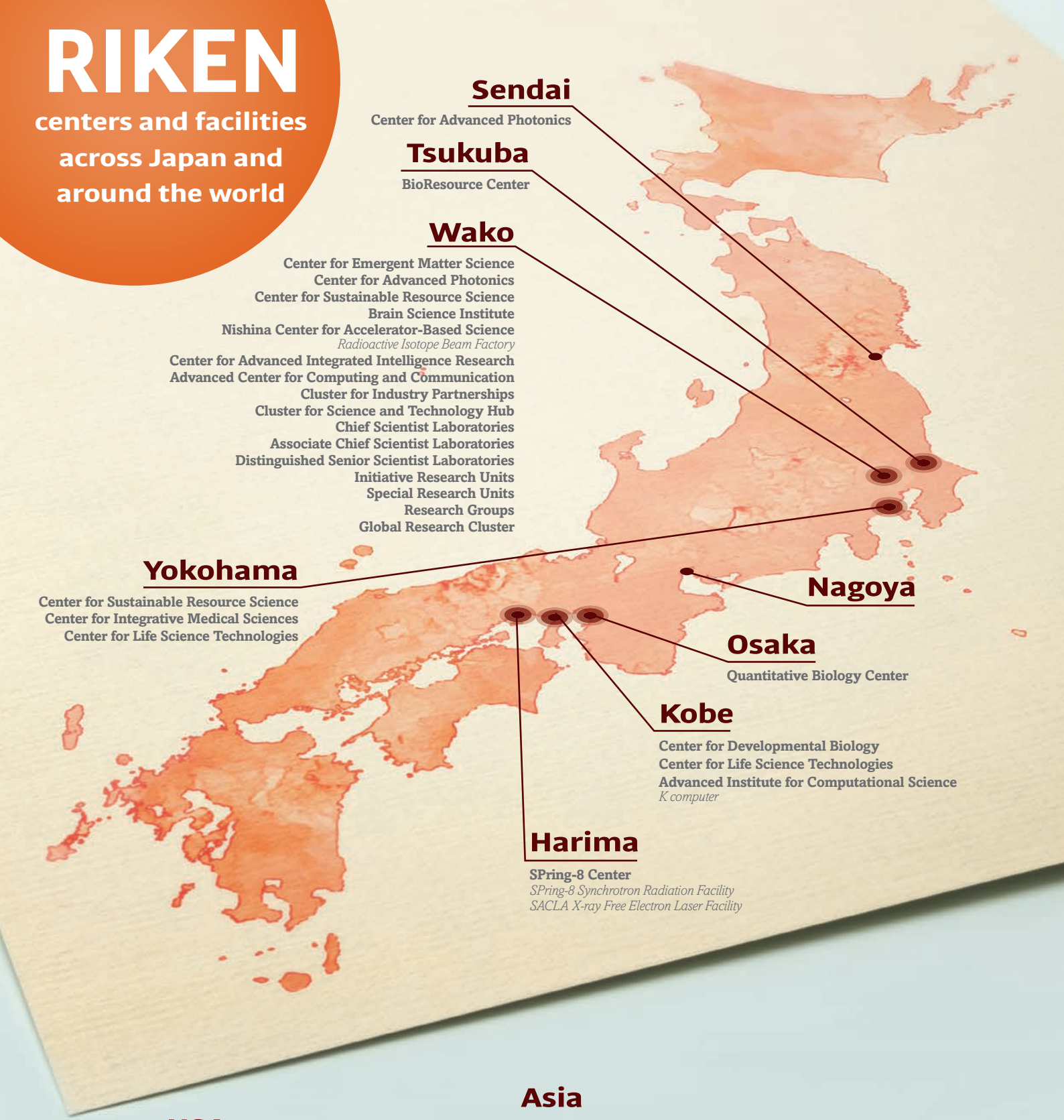

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