RIKEN RESEARCH

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Edging ahead

SPring-8 Storage Ring accelerator building in Harima

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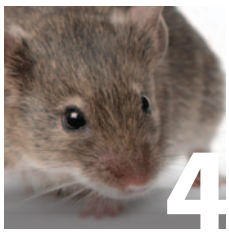

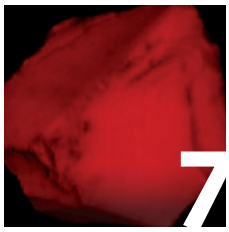

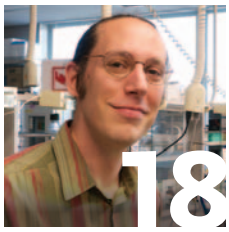

RIKEN RESEARCH

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Medicine

Experimental compound offers fragile X treatment

Studies in mice indicate that a daily dose of a kinase inhibitor could reverse the underlying morphological defects responsible for fragile X syndrome

Figure 1: Mice with fragile X syndrome (FXS) but expressing neither the PAK nor FMRP proteins display greatly improved symptoms.

Fragile X syndrome (FXS) is the most common inherited form of autism and intellectual disability, characterized by hyperactivity, repetitive behaviors and seizures. Although there are a number of medications that can help individuals to cope with the behavioral and mental health issues associated with the syndrome, as yet there are no proven therapies that directly impact the root cause of the disease. However, this could now be about to change with the RIKEN-led discovery of a compound that not only treats the symptoms in adult mice but also reverses the morphological defects associated with the syndrome[1].

The international research team, led by Susumu Tonegawa of the RIKEN–MIT Center for Neural Circuit Genetics (CNCG) at the Massachusetts Institute of Technology (MIT) in the United States, recently demonstrated the remarkable effectiveness of their promising compound—called FRAX486—in model FXS mice. "The effect was dramatic," says Tonegawa, a Nobel laureate and also director of the RIKEN Brain Science Institute. "With only one dose, the mouse was cured."

The development of FRAX486 began six years ago when Tonegawa and his colleagues were studying mice engineered to have low activity of an enzyme known as p21-activated kinase (PAK). Unexpectedly, they noticed that the animals had relatively few dendritic spines in their brains, and the spines that were there tended to be short and stubby. Dendritic spines are small neuronal protrusions that assist with neuron-to-neuron communication in the synapse. The researchers recognized that the absence of these spines in PAK-mutant mice was the exact opposite of what is seen in people with FXS and in mouse models of the disease. In FXS mice, which lack a working copy of the gene that encodes the fragile X mental retardation protein (FMRP), researchers typically see a profusion of long and thin dendritic spines in the brain.

The observation that mice lacking PAK and FMRP display mirror-opposite phenotypes led Tonegawa's team to test an intriguing hypothesis: perhaps PAK and FMRP antagonize each other to regulate spine morphology. Inhibiting PAK activity might therefore correct some of the defects associated with FXS. To test this idea, the researchers engineered a mouse to express neither PAK nor FMRP. To their surprise and delight, these mice had normal numbers of dendritic spines, unlike those only missing FMRP. Mice lacking both proteins were also less anxious and displayed fewer repetitive behaviors than FXS model animals. "It looked like the mouse was cured," says Tonegawa. "We never thought it would come out that nicely," he adds.

A promising venture

In 2007, soon after the team's first promising results were published, Tonegawa was contacted by Jay Lichter of Avalon Ventures—a US venture capital firm. Lichter was interested in working

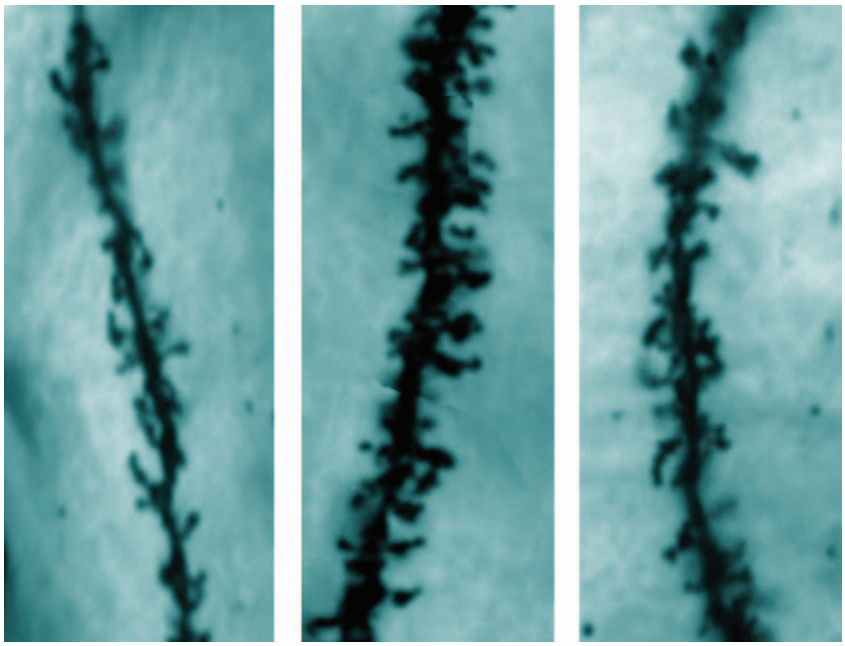

Figure 2: The brains of FXS mice treated with FRAX486 display a similar density of dendritic spines (right) to that of healthy mice (left), and a lower density than the brains of FXS mice given a sham treatment (center).

with Tonegawa to develop a drug that could chemically inhibit PAK. They hoped that such an agent could yield the same therapeutic benefit as the genetic manipulation reported by Tonegawa's research team but be applicable as a treatment in humans. Later that year, Tonegawa and Lichter founded the biotechnology company Afraxis to further develop the PAK inhibitor concept.

Scientists at Afraxis embarked on a search for a potent inhibitor of PAK by screening a library of 12,000 small molecules known to block kinase enzymes. Their search yielded one promising hit: FRAX486, a compound that selectively inhibits its target and also readily crosses the blood–brain barrier, staying active in the brains of mice for many hours. The researchers found that a daily dose of FRAX486 achieved a steady state of active drug levels in the brain.

Next, Tonegawa's research group undertook further characterization of the FRAX486 compound. This required new tests to be developed to quantify the effectiveness of the drug. Correspondingly, Bridget Dolan, a member of Tonegawa's laboratory at the CNCG, created an assay that involves the measurement of sound-induced seizures in FXS mice. She found that most mice injected with higher doses of FRAX486 were immune to seizures 8 hours after treatment, whereas all untreated FXS mice suffered seizures, with around half subsequently dying from respiratory arrest.

Face the FRAX

Dolan also showed that treating FXS mice with the drug ameliorated the autism-like symptoms of the disorder, such as hyperactivity and circling behavior. Meanwhile, collaborators at the National Institute of Mental Health and Neuro Sciences in India demonstrated that FRAX486 rescued morphological abnormalities in FXS mice. Just 8 hours after a single dose of the drug, the density of dendritic spines in FXS mice returned to levels similar to those found in healthy mice and well below the density in sham-treated FXS animals (Fig. 2). Physiological tests performed by a research team at Seoul National University in South Korea also revealed that FRAX486 treatment had no impact on body weight.

Importantly, all the beneficial effects were seen in adult mice that had already developed many of the symptoms of FXS. "FRAX486 is not only preventative, but also curative after the mouse gets sick," notes Tonegawa.

In early 2013, Genentech—a US-based subsidiary of the Swiss drug company Roche—acquired the patent portfolio behind FRAX486 and is now running further tests on the drug and related compounds with the hope of advancing a lead candidate into human trials in the near future.

1. Dolan, B. M., Duron, S. G., Campbell, D. A., Vollrath, B., Shankaranarayana Rao, B. S., Ko, H.-Y., Lin, G. G., Govindarajan, A., Choi, S.-Y. & Tonegawa S. Rescue of fragile X syndrome phenotypes in *Fmr1* KO mice by the small-molecule PAK inhibitor FRAX486. *Proceedings of the National Academy of Sciences USA* **110**, 5671–5676 (2013).

ABOUT THE RESEARCHER

Susumu Tonegawa received his PhD from the University of California, San Diego in the United States. He then undertook postdoctoral work at the neighboring Salk Institute for Biological Studies before moving to the Basel Institute for Immunology in Switzerland, where he performed his landmark immunology experiments. In 1987, Tonegawa was awarded the Nobel Prize in Physiology or Medicine for "his discovery of the genetic principle for generation of antibody diversity." Using advanced genetic manipulation techniques, Tonegawa is now unraveling the molecular, cellular and neural circuit mechanisms that underlie learning and memory. He is currently Picower Professor of Biology and Neuroscience at the Massachusetts Institute of Technology (MIT) and director of both the RIKEN-MIT Center for Neural Circuit Genetics and the RIKEN Brain Science Institute.

Tiny genes orchestrate plant shape

A subset of short genes hidden inside plant genomes may be important in setting plant growth patterns

Although thousands of entire genomes have been sequenced, our understanding of their detailed workings remains far from complete. Researchers continue to find new genes, determine their function, and map how they interact to build organisms. Working on the well-studied model plant *Arabidopsis thaliana*, Kousuke Hanada and colleagues from the RIKEN Plant Science Center* have revealed that a subset of tiny genes scattered through the genome may control the patterning of development[1].

To discover new genes, researchers must first mine huge volumes of genomic data to locate sections that have the basic structure of a gene, called 'open reading frames' (ORFs). Hanada's team focused on short ORFs (sORFs), which encode sequences of just 30–100 amino acids—compared with the average gene length of 600 amino acids in *A. thaliana*. Such sequences are often overlooked during annotation of the genome.

The research team identified nearly 8,000 sORFs through computer analysis of the *A. thaliana* genome. They then used expression analysis to exclude pseudogenes—genetic remnants that are no longer switched on or 'expressed'. The team achieved this by using a specially designed fluorescence microarray to generate an 'expression atlas' of highly expressed—and therefore likely to be functional—genes. On the microarray chip, genes fluoresce with an intensity proportional to their expression level. Through this approach, the researchers showed that 27% of the originally identified sORFs were highly expressed.

Figure 1: A normal *Arabidopsis thaliana* plant (left), and one overexpressing an sORF (right).

Hanada and colleagues then compared these likely functional sequences with those in 16 other plant genomes to identify which of their sORFs correspond to genes present in multiple species and are therefore highly conserved. This comparative genomics approach makes it possible to identify genes that have been evolutionarily conserved due to the essential functions they provide. The search revealed that more than half of the sORFs were similar to genes from other species.

Taking the 473 highly expressed and highly conserved sORFs, the researchers produced mutant *A. thaliana* plants in which these genes were overexpressed. A surprisingly high proportion of the mutants, close to 10%, had unusual traits, such as altered size, pale leaves, bent stems, or flowers with five instead of four petals. In contrast, overexpression of the previously known functional genes for *A. thaliana* only produced visible alterations in 1.4% of mutants.

The research demonstrates that sORFs potentially have pronounced effects on plant growth and form, and highlights the importance of these tiny elements in the genome. "Future work will focus on determining how the genes function when expressed normally," says Hanada.

1. Hanada, K., Higuchi-Takeuchi, M., Okamoto, M., Yoshizumi, T., Shimizu, M., Nakaminami, K., Nishi, R., Ohashi, C., Iida, K., Tanaka, M. *et al.* Small open reading frames associated with morphogenesis are hidden in plant genomes. *Proceedings of the National Academy of Sciences USA* **110**, 2395–2400 (2013).

* Reorganized into a new center from April 2013

Capturing light in an efficient dye trap

A fortuitous discovery leads to a new class of organic dyes that overcome a major limitation of this promising light energy conversion technology

Chemical compounds that can efficiently capture and convert light energy are in high demand as key components of inexpensive solar cells and advanced optical sensors. Carbon-based organic dyes are particularly strong candidates for such applications due to their low cost compared with the conventionally used silicon, but the lackluster performance of organic dyes has so far hindered their uptake by industry. Shuichi Enomoto, Shinichiro Kamino and colleagues at the RIKEN Center for Life Science Technologies have now discovered an organic dye that overcomes one of the major hurdles of this promising technology[1].

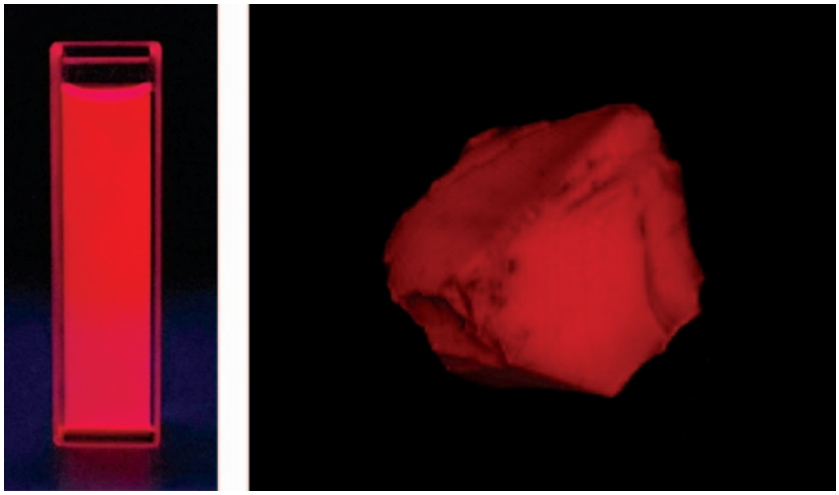

Figure 1: The ABPX dye produces fluorescence in both solution (left) and aggregate state (right).

A wide range of organic dyes are known to exist and their performance is limited by a tendency for the molecules to form aggregates that dissipate absorbed light energy. When an organic dye molecule absorbs a photon of light, it receives a kick of energy that can be put to use either by channeling the energy into an electric current in a solar cell, or re-emitting the light to indicate the presence of a target molecule, as part of an optical sensor. The aggregation of neighboring dye molecules dissipates some of the captured energy before it can be used—a process known as aggregation-induced quenching.

Enomoto's team discovered a compound known as an aminobenzopyrano-xanthene (ABPX) dye that is not affected by aggregation. "We fortuitously discovered the ABPX dye in the process of synthesizing rhodamine B, another organic fluorescence dye," says Kamino. Investigating the compound's structure and photophysical properties in solution revealed that aggregate formation did not impede its energy-emitting performance (Fig. 1).

The researchers found that the key to the ABPX dye's improved performance despite aggregation is its extra bulk. The compound's carbon skeleton is almost twice as large as that of rhodamine B and incorporates side groups that prevent neighboring molecules in aqueous solution from stacking together too closely, something that causes molecules to rapidly dissipate their captured energy in other dyes.

An additional unique property of the dye is that it is readily converted into a singly charged 'monocation' or doubly charged 'dication' in response to its environment. Each ion emits light at a different wavelength, which the researchers believe can be exploited to make optical sensors. "We have developed optical sensors for detecting target metal ions by taking advantage of this property," says Kamino.

The team are already working on new iterations of the dye that retain the useful aggregation properties while demonstrating improved light capture properties. "We are currently working on energy-conversion materials for organic solar cells," Kamino says.

1. Kamino, S., Muranaka, A., Murakami, M., Tatsumi, A., Nagaoka, N., Shirasaki, Y., Watanabe, K., Yoshida, K., Horigome, J., Komeda, S. *et al.* A red-emissive aminobenzopyrano-xanthene dye: elucidation of fluorescence emission mechanisms in solution and in the aggregate state. *Physical Chemistry Chemical Physics* **15**, 2131–2140 (2013).

X-rays in the fast lane

An amplification mechanism for x-rays promises high-energy pulses at pulse lengths short enough to observe the movement of electrons around atoms

X-ray free-electron lasers (XFELs) produce higher-power laser pulses over a broader range of energies compared with most other x-ray sources. Although the pulse durations currently available are enormously useful for the study of materials, even shorter pulses are needed to observe features such as electrons at subatomic scales. Takashi Tanaka from the RIKEN SPring-8 Center has now proposed a theoretical pulse-amplification scheme that allows for the production of ultrashort x-ray pulses at extremely high energies[1].

Methods previously proposed for shortening XFEL laser pulses typically fail to deliver sufficiently high energies for practical use. "In order to observe the motion of electrons inside atoms and molecules during chemical processes, shorter and brighter x-ray pulses are needed," says Tanaka.

The researcher's proposed solution involves shortening the electron pulse used to generate the x-rays by filtering out all but the middle part of the pulse, rejecting the weakest leading and trailing pulse components. The filtered electron pulse is then sent on a curved path—helped by a matching optical laser that stabilizes the electron movements—to divide the electron pulse into a series of small 'bunches'.

These electron bunches are then converted into weak x-ray laser pulses, which are sent through a delay line that shifts the leading 'target' pulse so as to coincide with the last of the corresponding electron bunches (Fig. 1). In a further amplification process, the target pulse is strengthened using a laser, leaving the rest of the pulses unaltered.

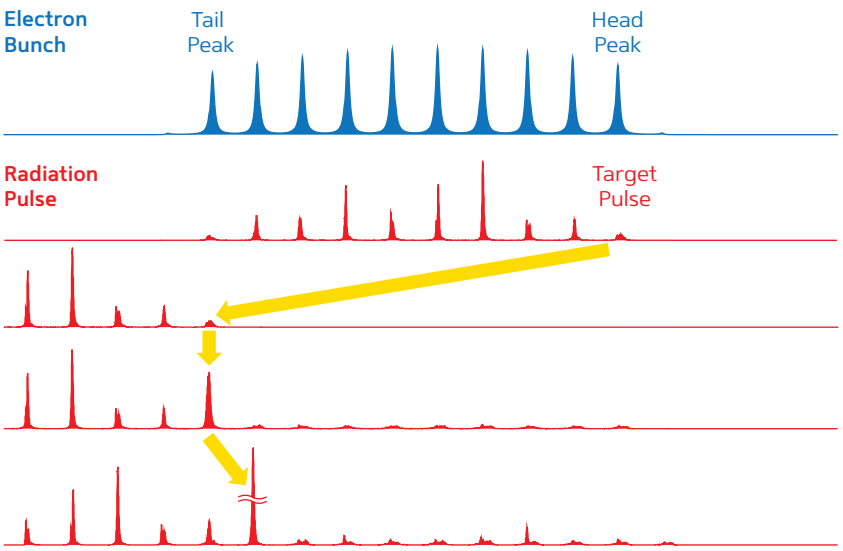

Figure 1: The proposed x-ray pulse generation scheme. A sequence of electron pulses (blue, top row) generates a matching sequence of x-ray pulses (second row). The pulses are then delayed such that the leading target pulse matches up with the last pulse of the electron bunches (third row). Repeating the process for the other electron pulses (fourth row) leads to a very high energy, ultrashort x-ray pulse.

Repetition of this last step a number of times amplifies the target pulse by many orders of magnitude. The predicted peak power is on the order of terawatts—more than the combined power output of all the nuclear power plants in the world. In addition, the predicted pulse duration is impressively short at 53 attoseconds, a duration which corresponds to traveling a distance of less than 16 nanometers at the speed of light.

Currently, the combination of exceptionally high power and short pulse length would be unmatched and could open many possibilities for the study of materials under extreme conditions. Tanaka expects the next step to be the realization of his proposal using the facilities at RIKEN's SPring-8 synchrotron complex in Harima. "We'd like to implement this scheme in the near future, but before that we need to develop relevant components such as the magnets needed to precisely control the timing between the electron and x-ray pulse," says Tanaka.

1. Tanaka, T. Proposal for a pulse-compression scheme in x-ray free-electron lasers to generate a multiterawatt, attosecond x-ray pulse. *Physical Review Letters* **110**, 084801 (2013).

The changing phase of quantum materials

A theoretical model that predicts how the properties of topological insulators vary under external influence could aid the search for an ideal material for quantum computers

Matter is categorized as either conductive, semiconductive or resistive to the flow of electrons based on its bulk properties. However, physicists have now predicted a new state of matter in which the bulk of the material is insulating—resisting electron flow—but where electrons are free to move along its edges. The possibility of such a material, known as a 'topological insulator', has caused a great deal of excitement among physicists because its surface conducting states are unusually stable, making them a promising resource for use in quantum computers. Bohm-Jung Yang and Naoto Nagaosa from the RIKEN Center for Emergent Matter Science and their coworkers have now devised a general theory for how an insulator changes into a topological insulator[1], which should aid in the practical search for such materials.

A full understanding of a material requires knowledge of how its properties vary as the external environment changes. Increasing the pressure of a gas, for example, can change it to a liquid, and higher pressures cause its atoms to bond together to form a solid crystal. Such changes of state at temperatures near absolute zero are known as quantum phase transitions. The theoretical model developed by Nagaosa's team describes quantum phase transitions involving topological insulator states.

"We want to understand how two insulating phases with distinct topological properties can be transformed from one to the other when external perturbations are applied," explains Nagaosa. "Our theory shows the importance of atomic symmetry in understanding this topological phase transition."

A topological quantum phase transition was recently experimentally observed in bismuth thallium sulfide selenide, a compound with an 'inversion symmetric' atomic arrangement—a structure that looks the same when reflected with respect to a point. The model put forward by Nagaosa and his colleagues goes beyond such materials by understanding phase transitions in 'noncentrosymmetric' materials, which do not exhibit this simplifying property.

The researchers' sophisticated model predicts that three-dimensional noncentrosymmetric materials can either change directly from a conventional insulator to a topological insulating state, or pass through an intermediate semi-metal state (Fig. 1). Their model provides detailed estimates for the temperature dependence of many of the properties of this semi-metallic phase, and the conditions required for a phase transition, known as quantum critical points. "The unique physical properties of the semi-metallic state that we have identified will provide a useful guideline for experimental proof of a topological phase transition in three-dimensional noncentrosymmetric systems," says Nagaosa.

1. Yang, B.-J., Bahramy, M. S., Arita, R., Isobe, H., Moon, E.-G. & Nagaosa, N. Theory of topological quantum phase transitions in 3D noncentrosymmetric systems. *Physical Review Letters* **110**, 086402 (2013).

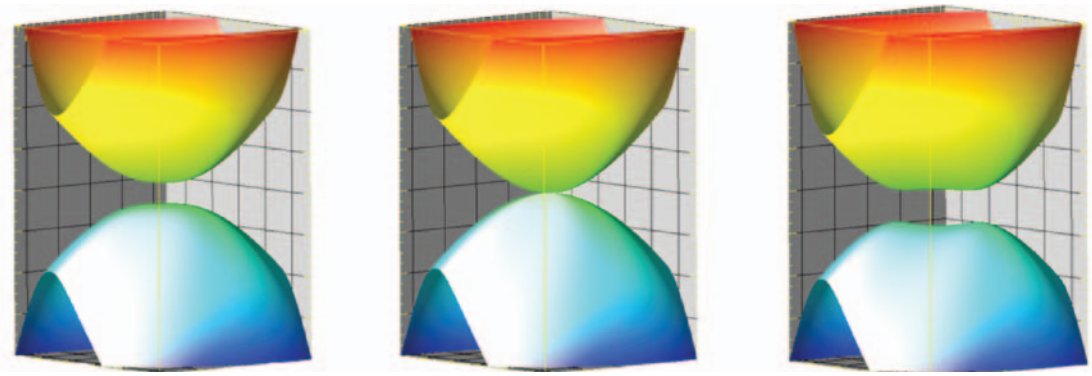

Figure 1: General energy band diagrams for topological insulators showing the change from insulator (left) to semi-metal (center) and topological insulator (right) with increasing pressure.

Bringing life into focus

Technical improvements provide major enhancements to the resolution and exploratory power of confocal microscopy in three-dimensional imaging of living tissues

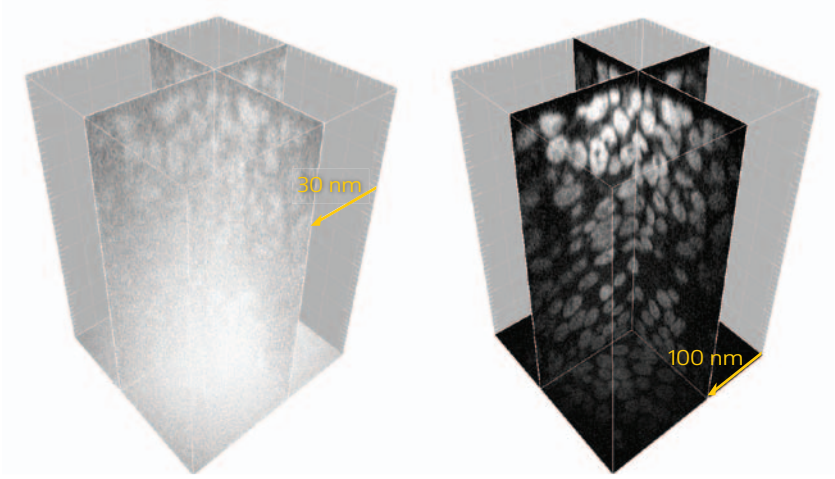

Figure 1: Imaging using conventional spinning-disk confocal microscopy (left) and the new configuration (right) developed by Yuko Mimori-Kiyosue and colleagues at RIKEN.

Spinning-disk confocal microscopy is an optical imaging technique that can be used to generate detailed three-dimensional fluorescence images of living cells and their contents. Although a powerful tool for observing dynamic processes in living organisms, it has proved difficult to use for all but the thinnest biological specimens. Motivated by a need to see more deeply into living cells, Yuko Mimori-Kiyosue at the RIKEN Center for Developmental Biology and colleagues have now made major technical improvements to the technique that deliver greatly improved resolution and clarity[1].

Mimori-Kiyosue's team conducts research on cellular structures called microtubules. Visualizing their dynamic behavior and arrangement in live mice proved frustrating. "We didn't have a microscope that enabled imaging of fast-moving, submicrometer-sized structures in cells within thick specimens," she says.

In confocal microscopy, a laser scans a fluorescently labeled sample and the emitted fluorescence is observed through a pinhole that blocks most of the out-of-focus light. Although the technique is able to generate sharp three-dimensional reconstructions, the scanning process is too slow for use on living cells. The spinning-disk method accelerates the process through the rotation of a disk containing many pinholes, which splits the laser into multiple beams. Unfortunately, this approach is limited by 'pinhole cross-talk'—the unwanted intrusion of background fluorescence through adjacent pinholes. Pinhole cross-talk becomes particularly severe in thick tissue samples due to high background levels.

The researchers overcame this problem with a strategy known as 'two-photon illumination', which generates fluorescence only within the focal plane of the image. While background signal is eliminated, the strategy does require the use of more powerful lasers. In response, they developed new spinning-disk units with special microlenses that efficiently condensed laser light, with pinholes spaced further apart to further minimize cross-talk.

When combined with a more sensitive high-resolution digital camera, these modifications yielded remarkably improved image quality (Fig. 1), allowing the research team to clearly image the dynamic behavior of microtubules in fruit fly and worm embryos as well as mouse oocytes at depths of up to 100 micrometers. The researchers now intend to use the technique to investigate genetically modified mice expressing fluorescently tagged microtubule-associated proteins.

The maximum tissue depth and sample area that can be imaged by this technique are currently limited by the power of the lasers used, but such limitations are anticipated to be short-lived. "Following the development of the next generation of high-powered lasers, we expect this system to have a large impact in the field of life sciences," Mimori-Kiyosue says.

1. Shimozawa, T., Yamagata, K., Kondo, T., Hayashi, S., Shitamukai, A., Konno, D., Matsuzaki, F., Takayama, J., Onami, S., Nakayama, H. *et al.* Improving spinning disk confocal microscopy by preventing pinhole cross-talk for intravital imaging. *Proceedings of the National Academy of Sciences USA* **110**, 3399–3404 (2013).

It's all in the symmetry

The low-temperature properties of a material can now be predicted from its symmetry thanks to a solution to an elusive problem in theoretical physics

A sphere looks the same no matter how it is rotated. Squash it on one side, however, and this symmetry is broken. A similar change from a high-symmetry state to a low-symmetry state defines many phase transitions in solids such as magnetic ordering, superconductivity and crystallization. By solving a long-standing problem in theoretical physics, Yoshimasa Hidaka of the Quantum Hadron Physics Laboratory at the RIKEN Nishina Center for Accelerator-Based Science has now developed a general theory that allows the low-temperature properties of such systems to be predicted from their symmetries[1].

Physicists use the term 'spontaneous symmetry breaking' to describe a system of particles that chooses a state with a lower symmetry than the physical equations that describe it. The simplest example is a ferromagnetic material, in which the magnetic spins of electrons lower their energy by lining up with their neighbors. At low enough temperatures, all of the spins point in one direction. Theoretically, the magnet has the same energy whether the spins all point northwest or southeast; in other words, the theory is rotationally symmetric, while the ordered spins are not.

Very little energy is required to disrupt the ordered states that result from spontaneous symmetry breaking. In the magnet, for example, these disturbances correspond to ripples—called spin waves—in which the spins precess around their ordered positions (Fig. 1). Knowing the number of ways these disturbances can occur allows theorists to calculate low-temperature properties such as a material's heat capacity. In principle, this number depends on how symmetry is broken in a system. "If a general counting rule is known, low-energy or low-temperature behaviors can be predicted," explains Hidaka.

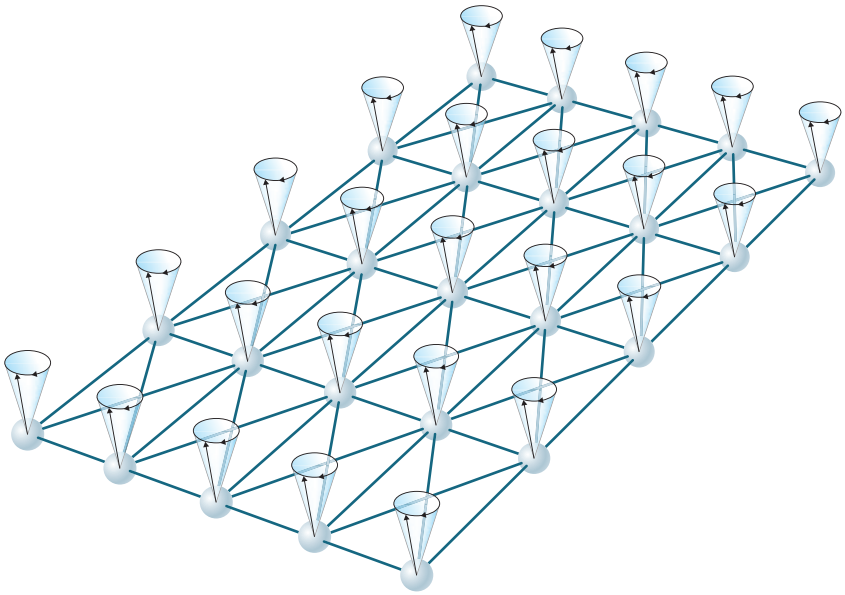

Figure 1: Spin waves cause the magnetic spins in a ferromagnet to precess around their ordered positions.

In the 1960s, physicists derived a counting rule for relativistic systems—those in which particles travel at close to the speed of light. This rule, called the Nambu–Goldstone theorem, says that the number of allowed disturbances equals the number of symmetries broken in a phase transition.

Hidaka was interested in finding a more general version of this rule that would apply to non-relativistic systems like solids or liquids—something that theorists have been trying to do for 50 years. He succeeded by adapting a theory used to describe the statistical motion of particles.

"The generalization of the Nambu–Goldstone theorem has been a long-standing problem. This general counting rule is universal," he says. Hidaka believes the new theorem could also be helpful in the study of neutron stars, which have been difficult to model theoretically.

1. Hidaka, Y. Counting rule for Nambu-Goldstone modes in nonrelativistic systems. *Physical Review Letters* **110**, 091601 (2013).

The solution to natural cell imaging

Scientists at RIKEN use x-ray diffraction to image whole, hydrated cells in their natural state for the first time

Most cells exist in a hydrated state and often live suspended in solution. In order to be imaged, cells must generally be frozen or dried, and then stained with substances such as heavy metals. Unfortunately, these processes can also alter the structure and chemical composition of the cells, resulting in inaccurate observations. Imaging the internal structures of whole, intact cells in their natural state has therefore been a particular challenge for scientists.

Changyong Song and colleagues from the RIKEN SPring-8 Center in collaboration with scientists from across Japan, Korea and the UK have now developed an x-ray diffraction microscopy method that allows whole, fully hydrated cells suspended in solution to be imaged for the first time[1].

X-ray diffraction microscopy works by analyzing the wave distortion created when an x-ray hits an object—rather like the patterns generated when waves pass an object in water. Coherent diffraction imaging (CDI) utilizes these diffraction patterns to build up detailed images of objects at the nanometer scale.

Song and his team overcame the reservations of others in the field to successfully image hydrated objects suspended in water by CDI. "The CDI technique is very sensitive to the sample and its surroundings," says Song. "Some researchers believed that the solution would produce significant noise, and that samples would hover around insecurely in solution. We sought to challenge this belief."

The team developed a hydrated specimen holder comprising two very thin membranes between which whole, hydrated cells could be sandwiched in solution (Fig. 1). "A uniformly thin layer of water between the membranes does not produce noticeable diffraction signals," explains Song. "The cells are held tightly in solution, and images are therefore exclusively from the fully hydrated cell samples."

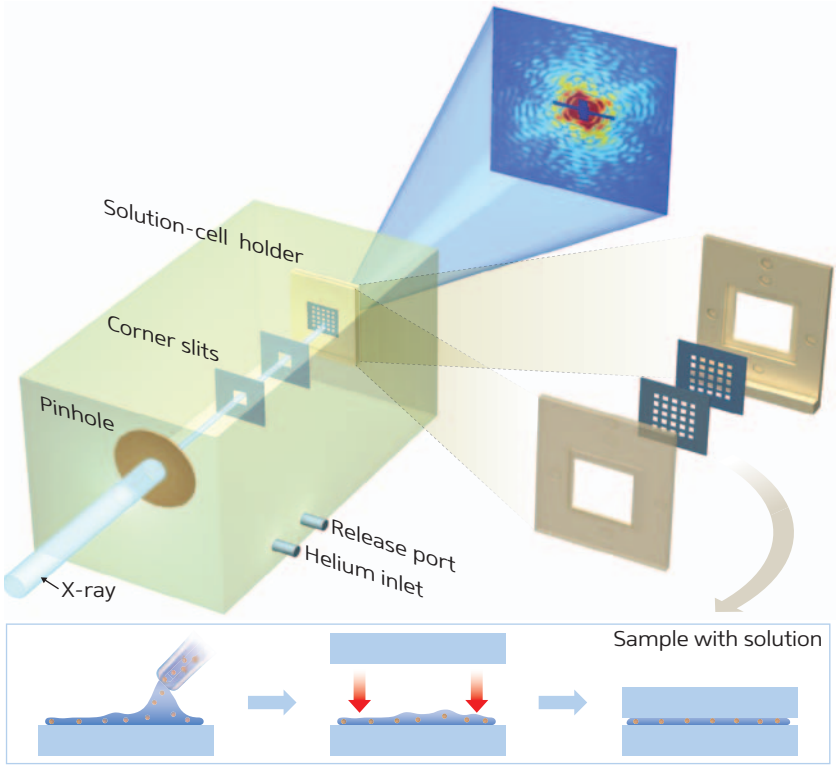

Figure 1: By using membranes to hold a living cell in a uniformly thin layer of water, the 'wet-CDI' technique allows detailed nanoscale imaging of whole, fully hydrated cells in their natural state.

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This 'wet-CDI' technique allowed the team to visualize cell morphologies and structures smaller than 25 nanometers in size, with no degradation in image quality. Wet-CDI could also easily be adapted for three-dimensional imaging.

"We expect wet-CDI to open a route to dynamic cellular imaging to chase developmental progress in cells at nanoscale resolutions," says Song. "It can also be immediately adapted to newly established x-ray free-electron lasers such as SACLA at the RIKEN SPring-8 Center. This will allow us to take nearly radiation-free snapshots of living cells or organelles."

Wet-CDI is a dramatic step forward in the quest to image whole cells in their natural state, and could have applications in such diverse fields as biology, medicine, materials science and petroleum research.

1. Nam, D., Park, J., Gallagher-Jones, M., Kim, S., Kim, S., Kohmura, Y., Naitow, H., Kunishima, N., Yoshida, T., Ishikawa, T. & Song, C. Imaging fully hydrated whole cells by coherent x-ray diffraction microscopy. *Physical Review Letters* **110**, 098103 (2013).

Dealing with drought

Characterizing components in a plant stress-mitigation pathway provides hope for the development of drought-tolerant crops

Figure 1: Difference in plant growth after 13 days of drought stress and 3 days of rewatering for 3 week-old wild-type (left) and *ahp2,3,5* triple-mutant (right) *Arabidopsis* plants.

Today's crops are subjected to all kinds of stresses. Drought and high salinity are particularly damaging to plant growth and development, limiting crop productivity across the world. Developing crop varieties that can adapt to such stresses has therefore never been more important. A team led by Lam-Son Tran at the RIKEN Center for Sustainable Resource Science has now characterized a key set of genes that transmit signals relating to drought and salinity stresses in the model plant *Arabidopsis thaliana*[1].

Plants deal with stress through a process involving sensing and response. The cytokinin-related phosphorelay is a multi-step pathway that mediates abiotic stress mitigation. It comprises histidine kinases, which receive cytokinins, histidine phosphotransfer proteins (known as AHPs in *Arabidopsis*), and response regulators that control downstream genes. Histidine kinases have previously been shown to regulate stress signalling pathways, but evidence regarding the identity and function of AHPs has been lacking.

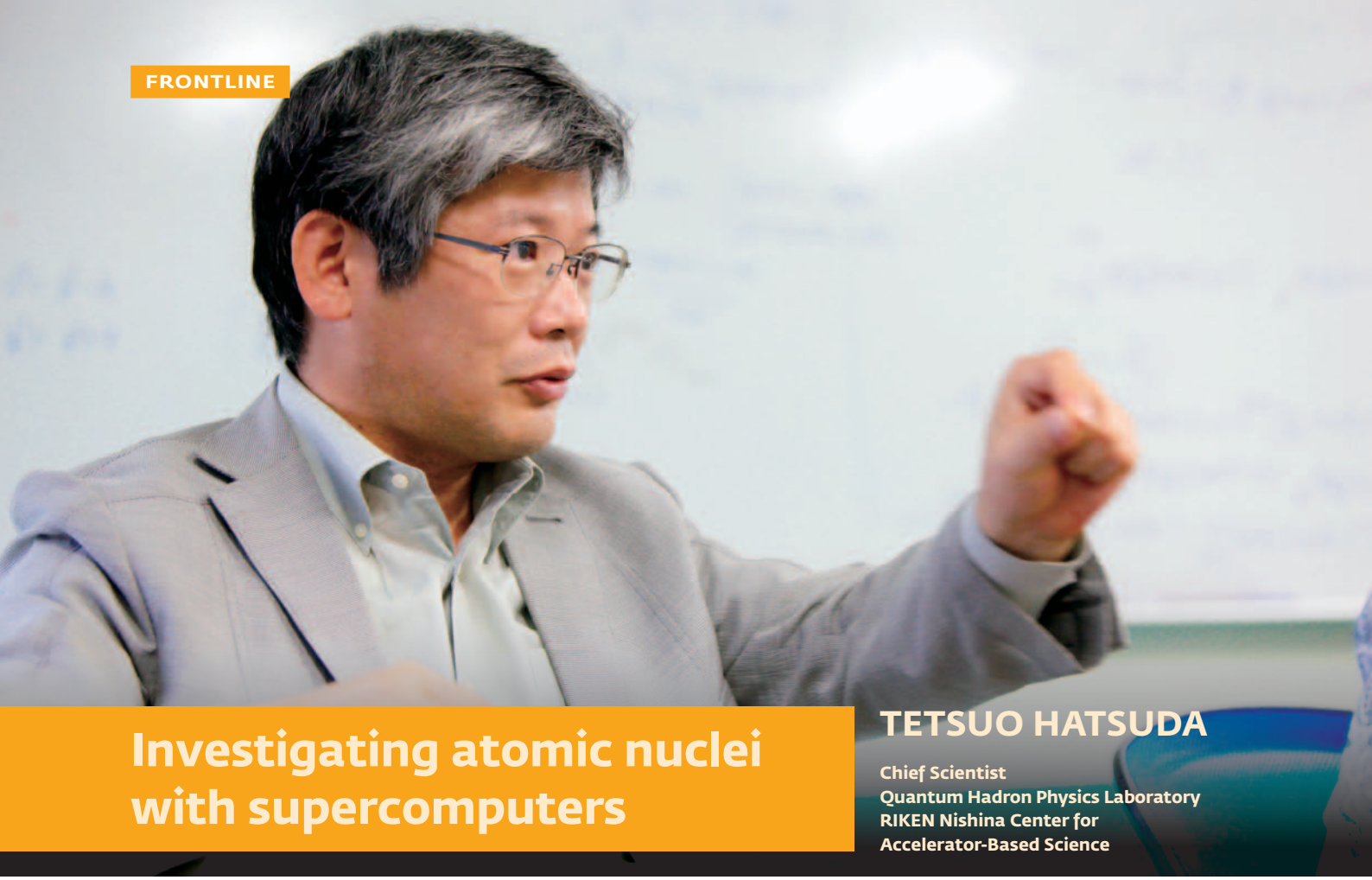
Tran's team identified three AHPs—AHP2, AHP3 and AHP5—that provide negative control of drought stress responses. Grown under a water deficit (Fig. 1), *ahp2,3,5* triple-mutant plants were found to be highly drought tolerant. Double mutants showed a lower tolerance, but still higher than that of wild-type plants, indicating that the genes function most effectively in combination. "In other biological processes, however, each gene may have a specific function of its own," says Tran.

The researchers found that expression of the *AHP* genes decreased in response to drought or salinity. This downregulation is likely achieved by stress-induced reductions in cytokinin levels, upstream of the phosphorelay. However, expression of individual *AHP* genes decreased by only 20–30% under lowered cytokinin, further emphasizing their combined role.

To identify stress-related genes controlled by the phosphorelay, the researchers conducted a genome-wide transcription analysis, comparing wild-type and *ahp2,3,5* mutant *Arabidopsis* plants. Under drought stress, 4,977 genes were significantly upregulated in mutant plants compared to the wildtype, and another 3,431 were downregulated. Several of the upregulated genes were found to be involved in the synthesis of cuticular waxes and cell-wall fatty acids. This corroborated the team's observation that the increased protection from water loss found in mutant plants was achieved by lowering electrolyte leakage through the cell membrane.

The team's findings have huge potential importance for producing transgenic crops with enhanced drought and salinity tolerance through rapid deactivation of the cytokinin-related phosphorelay. "The tricky part will be to silence the histidine phosphotransfer genes only upon drought or salinity stress, to avoid any negative effects on plant growth," says Tran.

1. Nishiyama, R., Watanabe, Y., Leyva-Gonzalez, M. A., Ha, C. V., Fujita, Y., Tanaka, M., Seki, M., Yamaguchi-Shinozaki, K., Shinozaki, K., Herrera-Estrella, L., & Tran, L.-S. P. *Arabidopsis* AHP2, AHP3, and AHP5 histidine phosphotransfer proteins function as redundant negative regulators of drought stress response. *Proceedings of the National Academy of Sciences USA* **110**, 4840–4845 (2013).



Investigating atomic nuclei with supercomputers

TETSUO HATSUDA

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RIKEN Nishina Center for
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The RIKEN Nishina Center for Accelerator-Based Science (RNC) was named after Yoshio Nishina, the father of atomic physics in Japan and RIKEN's fourth director. At the RNC's Quantum Hadron Physics Laboratory, Tetsuo Hatsuda and other researchers are using supercomputers to further the work of Nobel Prize-winning theorists Hideki Yukawa and Sin-Itiro Tomonaga, who worked in Nishina's laboratory at RIKEN while pioneering research into electrons and atomic nuclei.

Calculating the magnetic forces of the electron

The basis of some of the questions being explored using RIKEN's supercomputers can be traced to the late 1940s when Tomonaga, along with Richard Feynman, Julian Schwinger and others, established the discipline of quantum electrodynamics (QED), which is the quantum field theory of photons and charged particles, and forms a fundamental thread running through the Standard Model of modern physics. Many students at the University of Tokyo at the time—including Toichiro Kinoshita, now professor emeritus at Cornell University in the United States and visiting researcher at the Quantum Hadron Physics Laboratory—participated in a seminar by Tomonaga, then a professor at Tokyo Bunrika University (presently the University of Tsukuba).

"In those days, I was studying physics under the tutelage of Kunihiko Kodaira (a recipient of the Fields Medal for mathematics in 1954), who was the only researcher of quantum field theory at the University of Tokyo," says Kinoshita, in retrospect. "However, since Kodaira specialized in mathematics, University of Tokyo students who wanted to learn about quantum field theory rushed to Tomonaga's laboratory instead. Yoichiro Nambu (the 2008 Nobel Laureate in Physics) was among them."

In 1952, Kinoshita and Nambu joined the Institute for Advanced Study in the United States. They subsequently engaged in many years of research at the University of Chicago and Cornell University, respectively. In 1966, Kinoshita spent a year working at CERN (the European Organization for Nuclear Research) in Switzerland where he was astonished by their experimental data on muons.

Muons, like electrons, are fundamental particles and possess both charge and magnetic properties. The *g*-factor is a measure of the magnetic forces of these particles (known as the magnetic moment), and in quantum mechanics has the value of the integer 2, as calculated by the Dirac equation. However, in 1947, US physicist Polykarp Kusch (recipient of the 1955 Nobel Prize in Physics) showed experimentally that the value of the *g*-factor is not precisely 2, but instead deviates by about 0.1%. In 1948, Schwinger demonstrated using QED calculations that this phenomenon is caused by electrons and muons emitting and reabsorbing a photon—a particle that transmits light. While at CERN,

Kinoshita saw experimental data corresponding to high-precision measurements of the muon *g*-factor.

"The best way of determining the accuracy of QED is to examine the *g*-factor value. This is because we can determine the *g*-factor at high precision both experimentally and theoretically as it represents a simple system involving the nature of a single particle," explains Kinoshita.

The deviation of the *g*-factor away from 2 is most influenced by the emission and reabsorption of one photon. Emission and reabsorption of photons can be visually represented by a Feynman diagram (Fig. 1, part A, inset). For electrons, a single Feynman diagram can depict the case for one photon, allowing the *g*-factor to be calculated manually. To obtain the *g*-factor value to a higher level of precision, it is necessary to determine how the *g*-factor is influenced when the number of photons to be emitted and reabsorbed increases to 2 or 3. For 2 photons, 7 different Feynman diagrams are available and the *g*-factor may still be manually calculated. However, with 3 photons, 72 different Feynman diagrams exist, making manual calculation quite difficult.

To obtain the *g*-factor value of the muon at the precision of the experimental data from CERN, it was necessary to calculate the influence of up to 3 photons. Therefore, Kinoshita began to derive the *g*-factor through QED-based large-scale computation. In 1974, he succeeded in determining the *g*-factor value of the electron to the precision of 1 part in 3 billion.

Kinoshita assumed that this research was finally complete, but soon learned that researchers at the University of Washington had begun new experiments to measure the electron's *g*-factor. "[The researchers] were seeking to obtain measurements to the level of precision of 1 part in 100 billion—three digits greater than the precision of conventional experiments. So I could not help but continue my research."

Understanding ultimate theories with QED

To further increase the precision of his measurements, Kinoshita began to determine the influence of 4 photons. This required the calculation of 891 different Feynman diagrams. "I created a prototype method for large-scale computation when I was attempting to calculate the influence of 3 photons. I then began to use an expanded version of this prototype in my calculations," says Kinoshita. "When the computing process reached its final stage, I asked Makiko Nio, who had been a student at my laboratory at Cornell, to join the project."

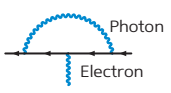
In 2007, Kinoshita, Nio and colleagues succeeded in calculating the influence of 4 photons and deriving the *g*-factor value of the electron to the precision of 1 part in 1 trillion. However, the following year, researchers at Harvard University announced that they had obtained measurements to the precision of 1 part in 3.6 trillion. So, to increase the precision of Kinoshita's calculations even further, 12,672 different Feynman diagrams representing the influence of 5 photons had to be computed (Fig. 1, part A).

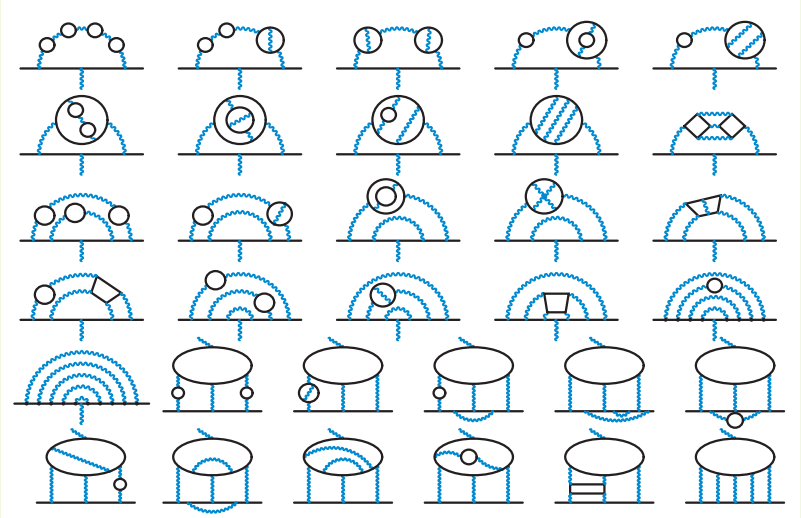
Kinoshita's team executed the enormous calculations using RIKEN's supercomputers—then the RIKEN Super Combined Cluster (RSCC), and the RIKEN Integrated Cluster of Clusters (RICC)—over a nine-year period. In 2012, they announced their success in deriving the electron's *g*-factor to the precision of 1 part in 1.3 trillion (Fig. 1, part B). To date, this is the most precise theoretical calculation performed in the history of physics.

"Our calculation agreed with Harvard University's measurement. If it turns out that QED breaks down, there would surely be disagreement between QED-based calculations and the increasingly precise measurements that have been made, but up to now this has not happened," says Nio. "However, theory predicts that if the precision of experimental measurements is further increased by one digit or so, a disagreement may occur between QED-based calculations and these measurements."

"Ultimate theories that surpass the current standard theory—including QED—have now been proposed," continues Nio. "Disagreement between calculated and measured *g*-factor values

A: Feynman diagrams
A Feynman diagram depicting a single photon influencing an electron's *g*-factor (left) and examples of some of the 12,000 Feynman diagrams depicting the influence of five photons (below).





B: Calculation of an electron's *g*-factor obtained using quantum electrodynamics (QED)

$$g/2 = 1.001\ 159\ 652\ 181\ 78 \pm 0.000\ 000\ 000\ 000\ 77$$

Figure 1: Calculating the electron's *g*-factor

will therefore provide an important clue to the determination of which theory is right. Even so, it is quite difficult to increase precision by one digit both experimentally and theoretically." Hence, Nio is working with Kinoshita to further increase the precision of such calculations to the point where the theories of QED, as established by Tomonaga and others, fail.

Stability of the atomic nucleus

At present, Hatsuda and his colleagues are pushing the boundaries of the research developed by Yukawa. "Atomic nuclei consist of protons and neutrons (Fig. 2, part A). Forces exerted between protons or neutrons are called nuclear forces. In 1935, Yukawa proposed the theory that as protons and neutrons exchange the then-unknown π mesons, an attractive force emerges to form atomic nuclei," explains Hatsuda.

In the mid-1940s, observations of cosmic rays led to the discovery of the π meson as predicted by Yukawa, verifying his meson theory. "However, a great puzzle remains to be solved with regard to nuclear forces," Hatsuda notes. "If attractive forces are the only type of nuclear force, the atomic nucleus should collapse because the attraction increases as protons and neutrons approach each other. If so, the current Universe, which is full of galaxies and stars, could not exist stably. Why do atomic nuclei not collapse? This is one of the major enigmas of nuclear physics."

Later experiments showed that a repulsive force is exerted when protons and neutrons approach each other at distances shorter than a given level, and in the 1960s, they were found to each comprise three particles called quarks (Fig. 2, part A). "To obtain insight into nuclear forces, we must understand the forces exerted between quarks, which are known as 'the strong interaction'. A theory of the strong interaction was announced by Nambu in 1966," says Hatsuda.

Quantum chromodynamics (QCD), the quantum field theory of elementary particles such as quarks and gluons, was established through the efforts of many researchers across a broad range fields and is able to explain the strong interaction. Experiments in the mid-1970s, established the validity of QCD. "QCD is one of the most beautiful and most complex theories that has been discovered by mankind," says Hatsuda, enthusiastically.

Successfully calculating nuclear forces

To accurately determine nuclear forces with QCD, enormous calculations are needed. It was therefore necessary to both increase computing speeds and develop a new method of computing. "It was only at the start of the twenty-first century that we became able to accurately calculate the properties of protons and neutrons comprising three quarks using QCD," says Hatsuda.

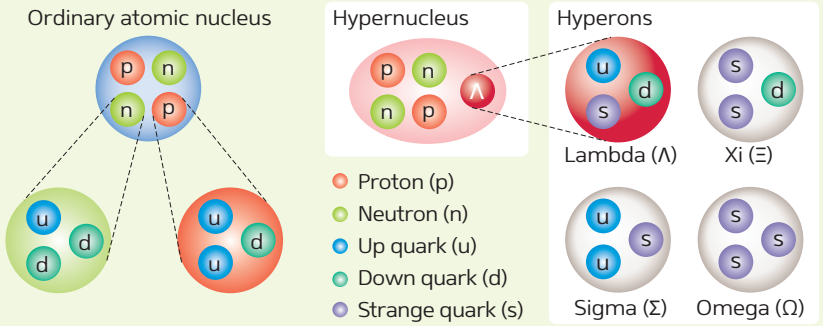
In 2005, Hatsuda and his collaborators began to accurately calculate nuclear forces using QCD. The following year, he received the first result from one of his fellow researchers. "The moment I watched it, I felt my body shake because the calculation definitively showed that nuclear forces became not only attractive forces at long distances, but also repulsive forces at short distances (Fig. 3)," he recalls. "I was so excited that I could not help but email many people to inform them of the calculation, including the US physicist Frank Wilczek (the 2004 Nobel Laureate in Physics), who contributed to the establishment of QCD. Wilczek later spoke about our work at the meeting held at Kyoto University in January 2007 to celebrate the centenary of the births of Yukawa and Tomonaga."

Hatsuda explains how he was able to obtain accurate calculations of nuclear forces using QCD. "Because I was conducting research into matter at the initial stage of the Universe, I knew a lot about both quarks and atomic nuclei. I was therefore able to mathematically define nuclear forces using QCD. Once defined, the forces could be calculated by large-scale numerical computation." He and his collaborators performed their calculations with a supercomputer that had just become available at KEK, the High Energy Accelerator Research Organization in Japan.

Entering the next stage using RIKEN's K computer

Nuclear forces work as repulsive forces over short distances due to the Pauli exclusion principle, a basic principle in quantum physics, says Hatsuda. Particles in the microscopic world can be categorized into one of two types: fermions and bosons. Quarks and electrons are fermions, whereas photons are bosons.

A: Ordinary atomic nucleus and a hypernucleus
An ordinary atomic nucleus consists of protons and neutrons. Each particle consists of up and down quarks (left). A particle made of three quarks including at least one strange quark is called a hyperon, and an atomic nucleus containing a hyperon is known as a hypernucleus (right).



B: H-dibaryon
The H-dibaryon, a six-quark hadron, has been theoretically predicted to consist of two up quarks, two down quarks and two strange quarks.

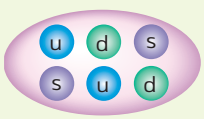


Figure 2: **An ordinary atomic nucleus and hyperon, and the H-dibaryon**

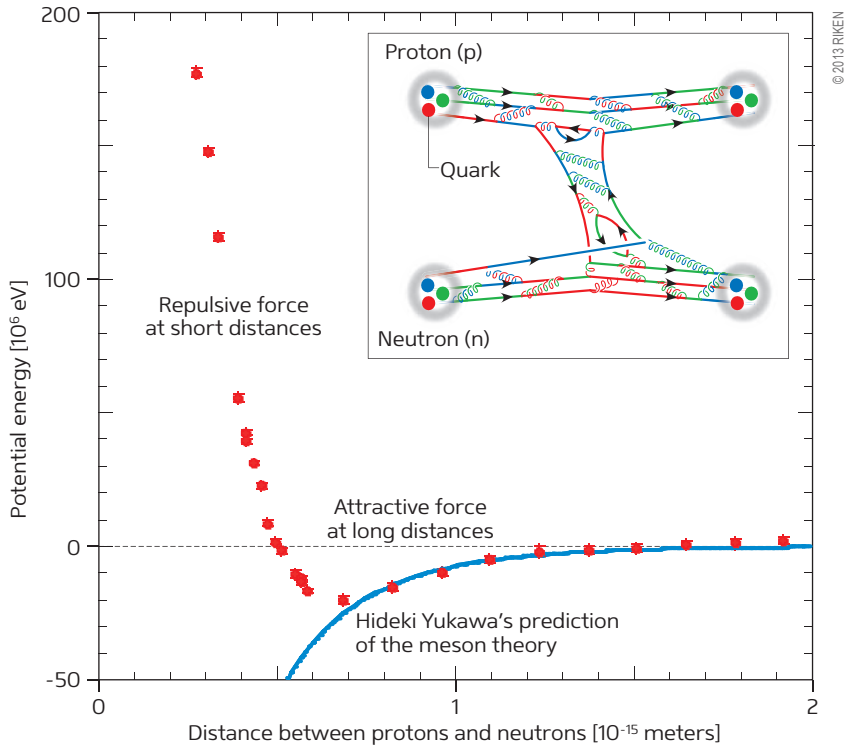


Figure 3: Nuclear forces derived by accurate computing based on quantum chromodynamics (QCD)

Although any number of bosons can occur concurrently at the same position, fermions of the same type cannot—the Pauli exclusion principle.

"There are six types of quark, including up, down and strange," explains Hatsuda. "Protons and neutrons each comprise a combination of up and down quarks. When protons and neutrons are forced to approach each other within a given distance, up and down quarks in the same state overlap each other at the same position, where repulsive forces work according to the Pauli exclusion principle. The mechanism behind such repulsive forces cannot be elucidated in detail unless the precision of QCD-based computation of nuclear forces is improved."

To this end, Hatsuda and his colleagues started to calculate nuclear forces using RIKEN's supercomputer—the K computer—in 2012. "I expect the calculations to be completed in 2014. The answer to the long-asked question of why atomic nuclei do not collapse will finally be found," he says.

There are now around 3,000 known atomic nuclei. At the RNC, experiments using the Radioactive Isotope Beam Factory (RIBF)—RIKEN's advanced particle accelerator facility—are being conducted to create a further 4,000 different unstable atomic nuclei, and to examine their properties in detail. The aim is to elucidate the processes by which the atomic nuclei of heavy elements form as a result of supernova explosions.

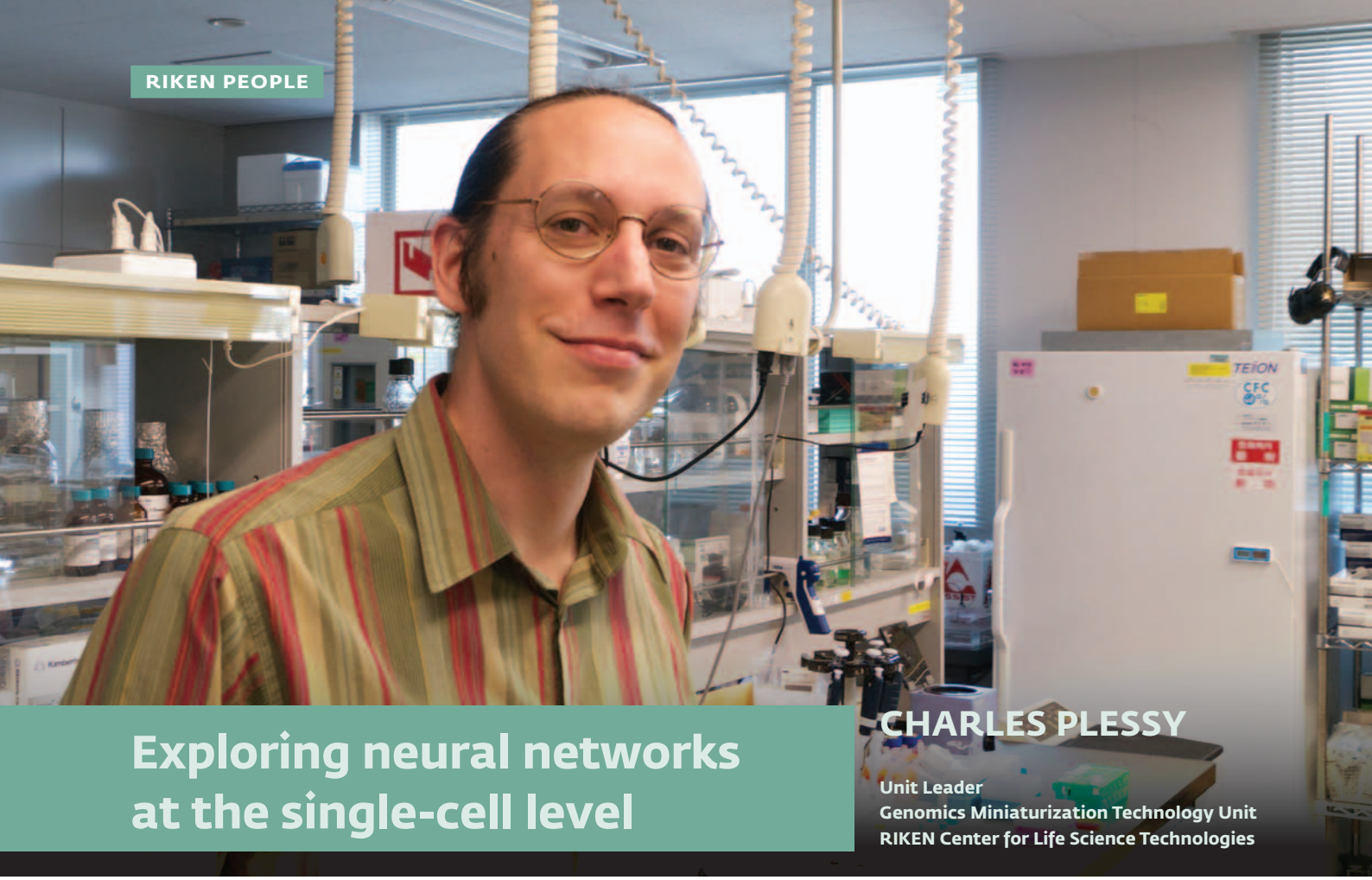
"Apart from protons and neutrons, each of which consists of up and down quarks only, there are other particles comprising three quarks that include at least one strange quark, known as the hyperons," notes Hatsuda. "New types of atomic nuclei, known as hypernuclei, are thought to comprise an assembly of hyperons, protons and neutrons (Fig. 2, part A)," he continues. "However, the properties of the nuclear forces of hyperons remain unclear."

Hatsuda says that researchers at the Japan Proton Accelerator Research Complex (J-PARC), built jointly by KEK and the Japan Atomic Energy Agency, are planning to create many different hypernuclei that contain at least one type of hyperon, such as a lambda (Λ) particle. "Theoretical description of the nuclear forces for hyperons will be essential for advancing the physics of hypernuclei. We aim to carry out such theoretical analysis by accurately calculating the nuclear forces for hyperons from QCD."

Looking to the future, Hatsuda is planning to focus his research on multi-quark hadrons, which are particles composed of more than four quarks. To date, hadrons comprising two or three quarks have been found, but none of four or more. "Although no definitive evidence has been obtained, it is theoretically possible for such multi-quark hadrons to exist. At J-PARC, researchers are preparing to create a type of hadron—known as the H-dibaryon—comprising six quarks that include two up quarks, two down quarks and two strange quarks (Fig. 2, part B). An important goal of our research is to determine the properties of H-dibaryons through QCD by making accurate calculations with the K computer," says Hatsuda of the next stage of his team's research.

ABOUT THE RESEARCHER

Tetsuo Hatsuda was born in Osaka, Japan, in 1958. He graduated from the Physics Department at Kyoto University in 1981 and obtained his PhD in 1986 from the same university. After four years as a postdoctoral fellow, including periods spent at KEK, the State University of New York at Stony Brook in the United States and CERN, Switzerland, he was appointed as research assistant professor then assistant professor at the University of Washington in the US. Hatsuda returned to Japan in 1993, becoming a professor at the University of Tokyo in 2000. In 2011 he joined the RIKEN Nishina Center for Accelerator-Based Science, becoming deputy director of the RIKEN Nishina Center in 2012. Hatsuda's research focuses on quantum many-body problems, matter under extreme conditions, the structure of neutron stars and numerical simulation of lattice quantum chromodynamics.



Exploring neural networks at the single-cell level

CHARLES PLESSY

Unit Leader
Genomics Miniaturization Technology Unit
RIKEN Center for Life Science Technologies

How did you join RIKEN?

I first came to RIKEN for an interview in the spring of 2004, a time when all the cherry blossoms were in bloom. When I was moving to Japan after taking up the position, I bought a one-way ticket and put all of my belongings into two big bags, ready to begin what has become an open-ended adventure for me.

Why were you drawn to RIKEN?

When I saw an advertisement for a postdoctoral researcher at RIKEN, the line "the gene network in the neural network" fascinated me.

I was impressed by the level of professionalism that I encountered during my recruitment process, and the discussions I had with the researchers I met at RIKEN further inspired me to become part of the organization. I knew that RIKEN had a culture of great diligence, and that did not deter me at all.

What is your current field of research?

I still focus on the gene network as part of the bigger neural network. Over the years, we have significantly improved our technology to be able to monitor gene activity and infer regulatory networks, allowing us to elucidate the interactions between specific factors. Now that my team is gaining an insight into such networks at the single-cell level, we are looking to apply our technology to brain samples.

What is the best thing about working at RIKEN?

RIKEN is a research center where one can dedicate themselves to science with minimal distraction. The work environment is really conducive to research. I have access to excellent facilities and equipment, and other forms of support such as assistance with administrative duties. Furthermore, there is no requirement for me to undertake teaching duties, meaning that I have more time and energy to focus on research.

What has been the highlight of your time at RIKEN so far?

Undoubtedly, it was winning the 2011 RIKEN Research Incentive Award and receiving it from Ryoji Noyori, president of RIKEN.

Incidentally, that year also brings back many poignant memories of the Great East Japan Earthquake. I am full of respect and admiration for the patience and resilience demonstrated by the people of Japan during that difficult time. The solidarity and support shown by people from around the world was also very heartening.

What would you say to other people considering joining RIKEN?

RIKEN provides plenty of support to foreign researchers, allowing them to begin their new life in Japan with ease.

When I first arrived, the International Cooperation Office provided me with temporary accommodation at International House on the Wako campus, giving me ample time to find a permanent place to live. Everyone I have met at RIKEN has been friendly and encouraging, and it helps tremendously that all discussions and research are conducted in English.

I would say that RIKEN is definitely a place like no other in which to fulfill your hopes and vision, and to imagine and realize projects that would be hard to do elsewhere. If you are considering becoming a part of RIKEN, do not be afraid to think big!

CONTACT INFORMATION

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RIKEN summer schools in 2013

Summer offers us the chance to work toward acquiring new skills by attending summer schools or training programs. This year, a number of RIKEN centers are involved in the organization of such workshops.

In July, the second RIKEN BioResource Center–Nanjing University International Summer Intensive Course of the Mouse will be held at the Model Animal Research Center of Nanjing University in China. This summer school presents a short but intensive educational program on the use of laboratory mice for biomedical research and developmental biology, and runs from 29 to 31 July 2013.

Later on in the summer, the RIKEN SPring-8 Center in Harima will host the Cheiron School, which is organized annually by the Asia–Oceania Forum for Synchrotron Radiation Research (AOFSRR). Taking place between 24 September and 3 October 2013, the school will introduce the science and technology of synchrotron radiation to graduate students and young researchers. In addition, all participants will attend and present a poster at the preceding 7th AOFSRR Workshop—to be held in nearby Himeji on 21–24 September.

For more information about both schools, visit: www.riken.go.jp/en/pr/events/lectures. ■

Participants of the ten-day Cheiron School will learn about synchrotron radiation science and technology at the RIKEN SPring-8 Center.

Plant genetics resource database upgrade

The RIKEN BioResource Center (BRC) has recently made an upgraded version of its plant genetics resource database, SABRE2, available to the scientific community.

The SABRE2 database allows researchers to easily search and compare the genes of crops and model plants.

SABRE2 integrates resources from the BRC and other Japanese research institutes that form part of the Ministry of Education, Culture, Sports, Science and Technology (MEXT)'s National BioResource Project. The improved database enables researchers to search and compare the genes of crops and model plants. SABRE2 also makes information on the model plant *Arabidopsis* readily accessible to researchers studying crops. ■

RIKEN and Agricultural Genetics Institute of Vietnam sign Memorandum of Agreement

On 22 May 2013, a delegation from Vietnam, headed by Deputy Prime Minister Nguyen Thien Nhan, visited RIKEN's campus in Yokohama to sign a Memorandum of Agreement (MoA) on collaboration between the RIKEN Center for Sustainable Resource Science and the Agricultural Genetics Institute of Vietnam. The MoA will promote closer cooperation on research into the molecular breeding of useful varieties of cassava, an important source of food in tropical regions. ■

Deputy Prime Minister Nguyen Thien Nhan spoke at the signing of a Memorandum of Agreement between RIKEN and the Agricultural Genetics Institute.

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