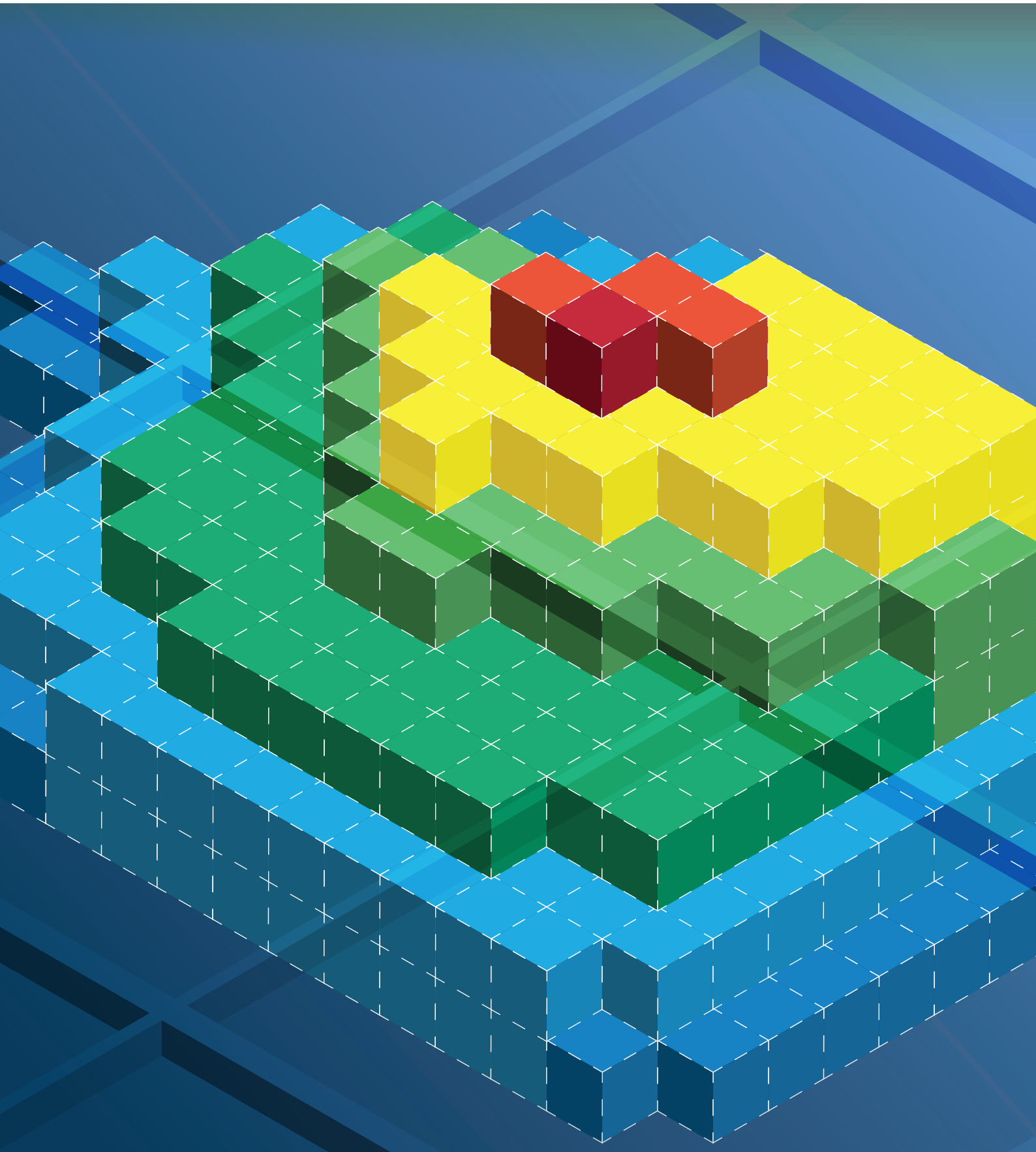
RIKEN RESEARCH

MARCH 2014
VOLUME 9 NUMBER 3

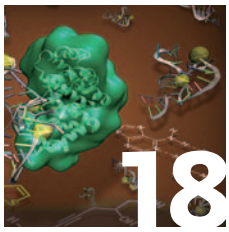


Synthetic nuclear physics

Accelerating toward the island of stability

Neural Circuit Genetics Research Building in Wako

Table of contents



RIKEN RESEARCH

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

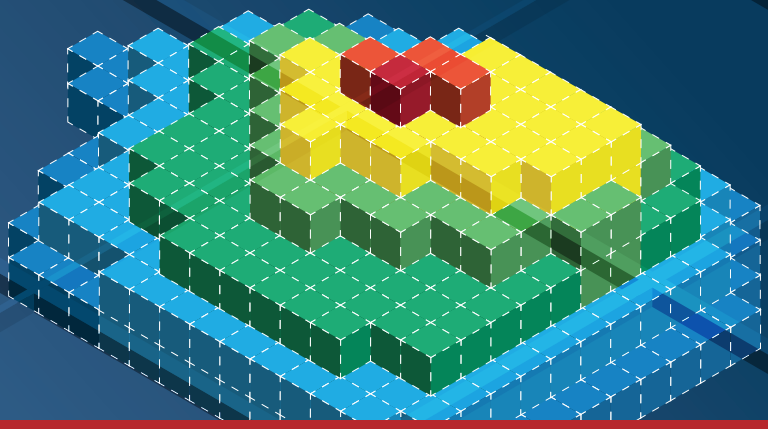
Initially established as a private research foundation in Tokyo in 1917, RIKEN became an independent administrative institution in 2003.

RIKEN RESEARCH is a website and print publication intended to highlight the best research being published by RIKEN. It is written for a broad scientific audience and policy makers interested in science and aims to raise global awareness of RIKEN and its research.

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

RIKEN Global Relations and Research Coordination Office
2-1, Hirosawa, Wako, Saitama, 351-0198, Japan
TEL: +81 48 462 1225
FAX: +81 48 463 3687
E-Mail: rikenresearch@riken.jp
URL: www.rikenresearch.riken.jp
www.riken.jp

RIKEN supports research at nine sites across Japan



Synthetic nuclear physics

The magic of nuclear physics

HIDETO EN'YO
Director
RIKEN Nishina Center for Accelerator-Based Science

Except for hydrogen, all other chemical elements originated from violent nuclear processes in stars. Powerful machines known as accelerators allow physicists to study how these elements form and also to create new elements and atomic isotopes. As such, employing accelerators to study atoms not only improves our understanding of the Universe, but also produces synthetic isotopes useful in applications that include medical diagnostics.

Shortly after the Big Bang, the Universe consisted only of hydrogen—the smallest of the chemical elements—and its electrically uncharged partner, the neutron. The diversity of chemical elements that have come into existence since then resulted from nuclear processes taking place within stars. The element helium soon followed hydrogen, forming from the fusion of two hydrogen atoms, and then came carbon, which forms upon the fusion of three helium atoms. Heavier elements, including iron—the most energetically stable element—are created at the end of the life of a star. And elements heavier than iron appear only as a product of catastrophic stellar processes such as supernovae.

Nuclear physicists are using powerful accelerators to shed light on the mechanisms underpinning the Universe's violent beginnings. Such accelerators can create new atoms with exotic, unstable nuclei that had vital roles in the creation of heavier elements during the explosion of stars.

The dawn of synthetic nuclear physics

Nuclear physics came of age as a research field in the 1920s, when nuclear particles, such as the proton, were discovered. Initial interest focused on understanding the properties of these particles and their involvement in the fundamental nuclear processes that were also discovered at the time: fission and fusion.

Yoshio Nishina was a pioneer of nuclear research in Japan. Following his education at the University of Tokyo and research stints in Europe in the 1920s, Nishina became a chief scientist at RIKEN in 1931. His mission was to establish a nuclear physics laboratory.

One of Nishina's key research areas—and still the subject of ongoing research—was the study of different nuclear isotopes. Atomic cores consist of two types of particles: the proton, the number of which determines the identity of a chemical element; and the neutron, which is electrically neutral and determines an element's isotopes. Hydrogen, for example, has one proton and three different isotopes; but heavier chemical elements, such as uranium, can have many different isotopes.

The stability of an isotope strongly depends on its mix of neutrons and protons, and this mix also influences how one element transforms into another during a nuclear reaction. A radioactive element, such as uranium, can follow many different reaction pathways to become a more stable isotope. Even the radioactive decay rates of an element can vary greatly between isotopes, ranging from billions of years to a fraction of a second.

An ideal way to study isotope properties is by synthesizing isotopes in the laboratory with the help of accelerators. These machines accelerate and smash atoms together. At sufficiently high energy, the collision of atoms creates new isotopes, or even new elements. Using the first Japanese accelerator—a cyclotron—Nishina discovered a new isotope of uranium, uranium-237, which differed from the form typically found in nature, uranium-238 (containing 92 protons and 146 neutrons).

Nishina built two cyclotrons at RIKEN but both were destroyed after the Second World War. New cyclotrons followed at RIKEN and the latest, the ninth cyclotron, is the world's most powerful superconducting ring cyclotron (SRC). The SRC can accelerate heavy atomic nuclei to speeds of up to 70 per cent of the velocity of light and has a beam intensity that is 100 times greater than any other accelerator in the world.

The SRC is part of the nuclear research facility at RIKEN that now bears Nishina's name—the RIKEN Nishina Center for

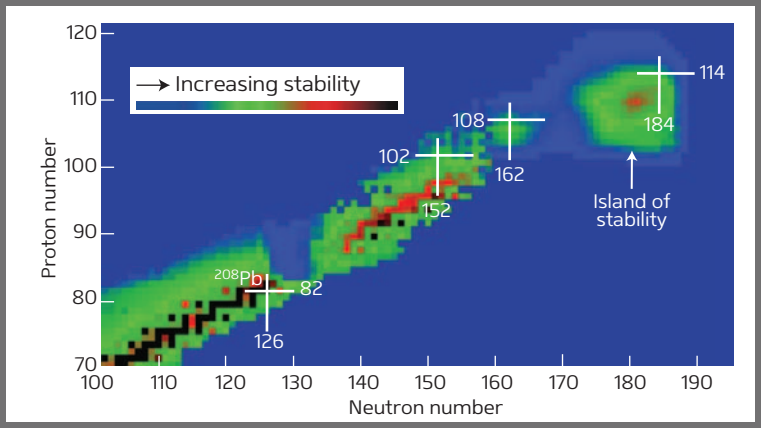


Figure 1: The island of stability

The white crosses indicate isotopes with 'magic' proton or neutron numbers, for example lead-208 (^{208}Pb), which consists of two magic numbers—82 protons and 126 neutrons—making it especially stable. While very heavy atoms with proton numbers in the hundreds are highly unstable, nuclear physicists predict that even some of the heaviest atoms have magic numbers and form an 'island of stability'. The as-yet-undiscovered 'doubly magic' nucleus with 114 protons and 184 neutrons is presumed to be located on this island.

Accelerator-Based Science (RNC). Inaugurated in 2006, the center hosts some 200 full-time scientists and collaborates with many international institutions.

Demystifying 'magic numbers'

Experiments using the RNC's latest generation of cyclotrons are pushing frontiers in nuclear research, particularly in the search for new isotopes with so-called 'magic numbers'. These isotopes have a set number of protons or neutrons in their nuclei and are surprisingly stable in comparison to other isotopes. Calcium-54, for example, consists of 20 protons and 34 neutrons. This isotope is very unusual: typically, 34 neutrons do not constitute a 'magic' isotope; and, its magic properties were theoretically proposed shortly before their experimental discovery by researchers at RIKEN[1].

Explaining how elements as heavy as uranium could have resulted from stellar explosions hinges on understanding magic numbers and the stability of their associated elements.

The intense beams of atomic isotopes generated by RIKEN's cyclotrons are useful in the search for very heavy elements. As heavy isotopes are accelerated and collide with each other, they re-assemble into different atoms, allowing the discovery of new chemical elements. Such research led to the discovery in 2012 of element 113 at the RNC[2], after more than nine years of thorough searching.

The synthesis of new chemical elements could lead to a new understanding of nuclear physics. Very heavy atoms are highly unstable and have a very short life, making it difficult to prove their existence following high-energy collisions. However, nuclear physicists predict that even some of the heaviest atoms have magic numbers and could survive for longer after a collision. They are predicted to form a so-called 'island of stability' within the short-lived heavy elements, which is typically the domain of elements with approximately 120 protons (Fig. 1).

Since existing accelerators lack the power necessary to reveal new elements within this island of stability, RIKEN plans to replace its synchrotrons 5 and 6 with a new superconducting accelerator. This powerful accelerator would produce beam intensities some 100 times greater than presently possible, securing RIKEN's leadership in the study of heavy isotopes.

Nuclear research for better living

Atomic accelerators also have application beyond fundamental physics. In medicine, for example, certain radioactive isotopes are used as markers in diagnostic experiments because their distribution within the body can be precisely measured. Many of these isotopes are a by-product of uranium fission in nuclear reactors. As research reactors are being decommissioned, alternative methods to produce radioactive isotopes, including accelerators, have become increasingly important.

The use of high-energy ion beams further extends to agriculture. Naturally occurring radioactivity is one of the causes of mutations in plants. Radioactive rays can destroy DNA or cause small changes to the genome. Over many generations, such changes can accumulate and influence an organism's evolution.

With ion beams, this process can be sped up by introducing mutations at a faster rate. Careful selection of useful mutations in each generation can produce better plants for cultivation. Developing rice that is tolerant to salty water, or even mixtures containing up to 50 per cent seawater, is one example. Controlled mutations could become increasingly important in ensuring a sufficient food supply for an ever-increasing global population.

After more than 80 years of nuclear physics research at RIKEN, much work is still required to better understand the properties of magic isotopes, the formation of heavy elements, the interplay of protons and neutrons in the nucleus and the internal structure of protons and neutrons. Attaining this understanding will require accelerators with even higher energies and intensities than are presently available. In that quest, the RNC will continue to play an important role. The new generation of accelerators being planned will help us to understand how the elementary particles in an atom interact with each other to form the world around us.

1. Steppenbeck, D., Takeuchi, S., Aoi, N., Doornenbal, P., Matsushita, M. *et al.* Evidence for a new nuclear 'magic number' from the level structure of ^{54}Ca. *Nature* **502,** 207–210 (2013).
2. Morita, K., Morimoto, K., Kaji, D., Haba, H., Ozeki, K. *et al.* New Result in the production and decay of an isotope, ^{278}113, of the 113th Element. *Journal of the Physical Society of Japan* **81,** 103201 (2012).

Physics

Twin x-ray pulses light up matter

The SACLA free electron laser can now deliver pairs of high-intensity x-ray pulses with different wavelengths

X-ray beams are invaluable tools for scientific research. The patterns they create after diffracting through a crystal can reveal the atomic structure of the material, and blasting a sample with pulses of x-rays can help to identify the sample's composition or capture snapshots of the atomic processes underlying incredibly fast chemical reactions.

Toru Hara and colleagues from the RIKEN SPring-8 Center and the Japan Synchrotron Radiation Research Institute have now developed a technique that can produce pairs of very short x-ray pulses in which each pulse has a different wavelength[1]. The system promises to open up a new frontier in x-ray science, allowing researchers to probe the atomic world in unprecedented detail.

The x-ray pulses are produced at SACLA (SPring-8 Angstrom Compact Free Electron Laser), which forms part of the SPring-8 (Super Photon Ring-8 GeV) synchrotron radiation facility in Harima. The SACLA x-ray free electron laser (XFEL) accelerates a stream of electrons to close to the speed of light and passes it through a series of five-meter-long undulator channels containing alternating magnetic fields. This path causes the electrons to 'wiggle' from side to side, forcing them to eject some of their energy by emitting x-rays (Fig. 1). These tightly bunched x-rays are all in phase, just like a coherent laser beam, and normally have the same wavelength, which is determined by the size of the gap between the undulator magnets. The energy of the x-ray pulses ranges from 5 to 15 kiloelectronvolts, and the pulses are typically less than 10 femtoseconds in length. The SACLA XFEL is one of only two facilities in the world where researchers can use such powerful, ultrashort x-ray pulses to study matter.

Generating a double pulse

The new system generates twin pulses of different wavelengths by introducing a detour section between the eighth and ninth undulators. This magnetic chicane does not affect the x-rays generated in the first eight undulators, which continue on their course unimpeded. However, it forces the electrons to take a longer route, slightly delaying their arrival at the ninth and subsequent undulators. This second leg has a different magnetic gap, causing the production of x-rays of a different wavelength. The second x-ray pulse trails the first by up to 40 femtoseconds, and the delay can be fine-tuned to a fraction of a femtosecond. The resultant paired x-ray pulse train is referred to as a two-color double-pulse (TCDP) XFEL.

The intensity of each pulse can be adjusted by changing the number of operational undulators, and the two pulses can be made to hit a sample from slightly different directions by repositioning the second set of undulators. These features should make the x-rays

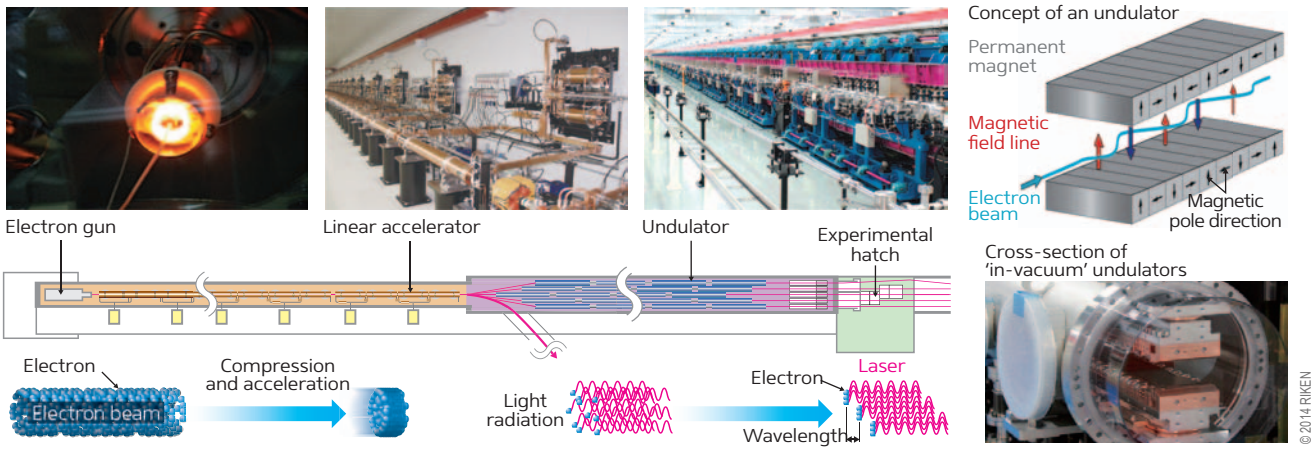


Figure 1: When bunches of electrons moving close to the speed of light are forced to wiggle through undulators, they produce coherent x-ray pulses.

Figure 2: SACLA (SPring-8 Angstrom Compact Free Electron Laser) can generate intense pulses of x-rays to probe the atomic structure of matter.

useful for answering many different types of scientific questions, says Hara.

A new light source with many uses

The TCDP system has many potential applications in research. In imaging experiments, for example, the pulses could be used to produce complementary diffraction patterns that help to refine crystal structures with greater accuracy. "Taking images of a three-dimensional structure from different angles provides more information," explains Hara.

When low-intensity x-rays are used, samples can be rotated and images taken from several angles. "In the case of XFELs, however, the sample is destroyed by just one intense pulse in most instances. So, many identical samples are prepared and exchanged for each XFEL shot," notes Hara. Being able to take two snapshots at once would ease the burden of sample preparation.

The TCDP system could also be used to capture extremely fast events, such as structural changes in nanoparticles. Each x-ray pulse would produce a snapshot of the structure, and reveal how it evolves over a femtosecond timescale. This sort of technique could uncover the physical properties of materials at the atomic scale or track the course of chemical changes—vital information for researchers designing new materials and catalysts or trying to understand biological processes such as protein folding.

The two pulses could also serve different functions. The first could excite electrons in the target and the second could record the outcome of that process immediately after using a 'pump–probe' approach. "In this case, the two pulses would need to have different wavelengths," says Hara. "If the two pulses had the same wavelength, we would not be able to distinguish whether the observed signal was due to the pump pulse or the probe pulse."

Greater separation makes the difference

Although other groups of researchers have also recently produced TCDP x-rays, the wavelengths of their two pulses only differ by a tiny percentage. "A separation of a few per cent is not enough to clearly separate the signals," says Hara. "Also, it limits the range of target processes that can be observed, since some processes need a large gap between the two wavelengths."

In comparison, the wavelengths of the two x-ray pulses at the SACLA facility (Fig. 2) can be separated by more than 30 per cent. The wavelengths are also much shorter than achieved by other TCDP systems, providing better spatial resolution and revealing finer atomic details. The pulses have enough energy to rip electrons from the inner shells of atoms, triggering a cascade of other electrons that emit more x-rays in a characteristic spectrum that can be used to identify the atom—a technique known as energy-dispersive x-ray spectroscopy.

Researchers are now putting the system through its paces. "The two-color XFEL has already been used in experiments, but these are ongoing and the results have not yet been published," says Hara. "I hope new ideas for experimental methods will emerge from these experiments in the future."

1. Hara, T., Inubushi, Y., Katayama, T., Sato, T., Tanaka, H., Tanaka, T., Togashi, T., Togawa, K., Tono, K., Yabashi, M. & Ishikawa, T. Two-colour hard x-ray free-electron laser with wide tunability. *Nature Communications* **4,** 2919 (2013).

ABOUT THE RESEARCHER

Toru Hara was born in Tokyo, Japan, in 1966. He graduated from the Faculty of Engineering at the University of Tokyo in 1989 and received his PhD from the University of Paris XI in France in 1995. He entered RIKEN as a research scientist for the JAERI–RIKEN SPring-8 Project Team, later joining the RIKEN SPring-8 Center. At the center, Hara contributed to SACLA (SPring-8 Angstrom Compact Free Electron Laser) and its precursor, the SPring-8 Compact SASE Source (SCSS), developing insertion devices and accelerator optics design as well as contributing to the operation of the free electron lasers. In 2011, Hara became team leader of the Beam Dynamics Team at the RIKEN SPring-8 Center. His current research focuses on the beam dynamics of linear accelerators and free-electron-laser physics.

The benefits of a spotless mind

A cell process that normally clears debris is also responsible for the secretion of a neurotoxic molecule that aggregates in the brain of individuals with Alzheimer's disease

Alzheimer's disease is an age-related memory disorder characterized by the accumulation of clumps of the toxic amyloid-β (Aβ) protein fragment in the extracellular space around neurons in the brain. Drugs that help to 'clean up' cells by inducing autophagy—the degradation of unnecessary cellular components—are known to lower Aβ levels within cells and have been shown to rescue memory deficits in mice. A team of researchers including Per Nilsson and Takaomi Saido from the RIKEN Brain Science Institute have now found that autophagy also plays an important role in secreting Aβ from the cell into the extracellular space[1].

The researchers set out to investigate what would happen to extracellular Aβ aggregates, called plaques, when genetic methods were used to eliminate the autophagy process. They started with transgenic mice commonly used as a model for Alzheimer's disease. These mice have high levels of Aβ and Aβ plaque accumulation in their brains, and display learning and memory deficits. Surprisingly, in genetically engineered variants of these mice lacking *autophagy-related gene 7* (*Atg7*), which is required for normal autophagy, the researchers found fewer extracellular Aβ plaques in the brain; instead, the Aβ seemed to accumulate inside the neurons. Conversely, increasing the expression of the Atg7 protein in neurons grown in cell culture resulted in an increase in the release of Aβ from the cells into the tissue culture medium. The findings suggest that autophagy is required for the secretion of Aβ from neurons into the extracellular environment.

Mice with an elevated expression of Aβ but defective autophagy seemed to have degenerated brain structures (Fig. 1), as well as sicker neurons—as defined by their expression of markers of cell death—and poorer learning and memory functions than mice with high Aβ expression but normal autophagy. This result indicates that autophagy is important for maintaining normal neuronal function and cognition in Alzheimer's disease. Moreover, because autophagy lowers Aβ levels within the cell, the researchers deduced that intracellular Aβ may be more toxic than extracellular Aβ with respect to inducing neuronal dysfunction and memory impairment.

The findings suggest that the effectiveness of therapeutic strategies for Alzheimer's disease may be improved by targeting the elimination of intracellular Aβ deposits rather than extracellular plaques. "Intraneuronal Aβ accumulation is seen in early Alzheimer's disease in humans, similar to what we found upon autophagy deletion in mice," explains Nilsson. "Targeting this pool of Aβ may therefore offer a potential treatment for Alzheimer's disease," he says.

1. Nilsson, P., Loganathan, K., Sekiguchi, M., Matsuba, Y., Hui, K., Tsubuki, S., Tanaka, M., Iwata, N., Saito, T. & Saido, T. C. Aβ secretion and plaque formation depend on autophagy. *Cell Reports* **5,** 61–69 (2013).

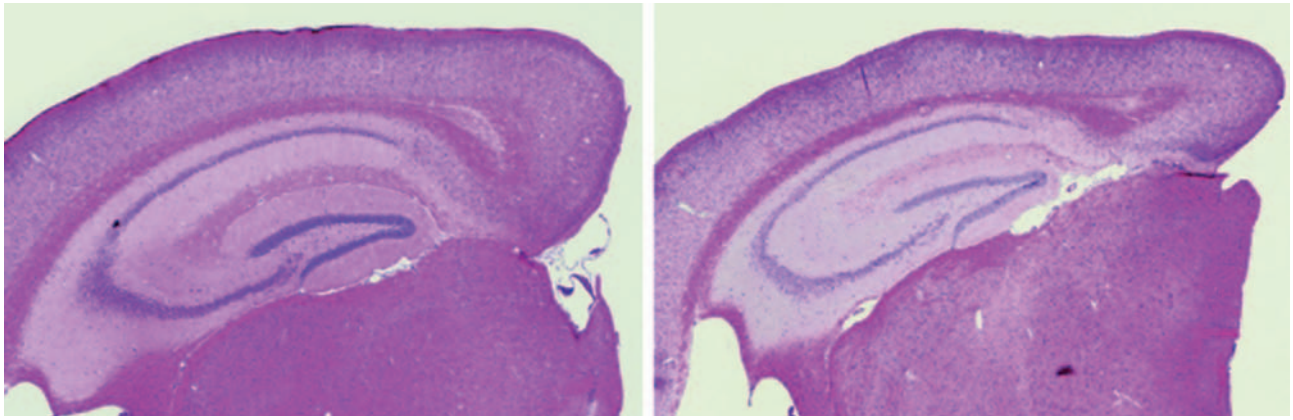


Figure 1: Mice lacking autophagy and with high levels of Aβ (right) have degenerated brain structures compared with normal mice (left).

Self-healing hydrogels ease into production

Mechanically tough hydrogels that repair themselves within seconds are now easier to manufacture thanks to novel polymer binding agents

Hydrogels are semi-solid materials formed by polymer chains that trap water molecules in three-dimensional gels. They are used in a variety of applications, including soft contact lenses, but the fragile nature of the materials means that their utility has remained limited. Yasuhiro Ishida, Takuzo Aida and colleagues at the RIKEN Center for Emergent Matter Science are challenging this limitation by developing strong hydrogels, or 'aqua materials', that could outperform even conventional plastics. As part of an international collaboration, the team has now improved the production of a robust, moldable hydrogel that heals itself rapidly after being sliced open[1].

The RIKEN team previously developed a hydrogel containing three primary ingredients: tiny flakes or 'nanosheets' of anionic clay, an exfoliating chemical that keeps the nanosheets from agglomerating, and a polymer binder containing positively charged guanidinium cations. Mixing small amounts of these substances into a beaker containing water causes a self-standing gel to form within seconds due to cross-linking interactions between the clay nanosheets and the polymer binder (Fig. 1). Since the hydrogel is held together by hydrogen bonding and electrostatic forces instead of permanent chemical bonds, if cut open it can be repaired by simply pressing the gel back together.

Developing practical applications for this hydrogel proved difficult, however, because the polymer binder contains dendritic units—multiply branched, star-shaped molecular chains that can only be synthesized through time-consuming procedures. Unfortunately, binding agents made from more traditional acrylic polymers severely affect the performance of the self-healing aqua materials.

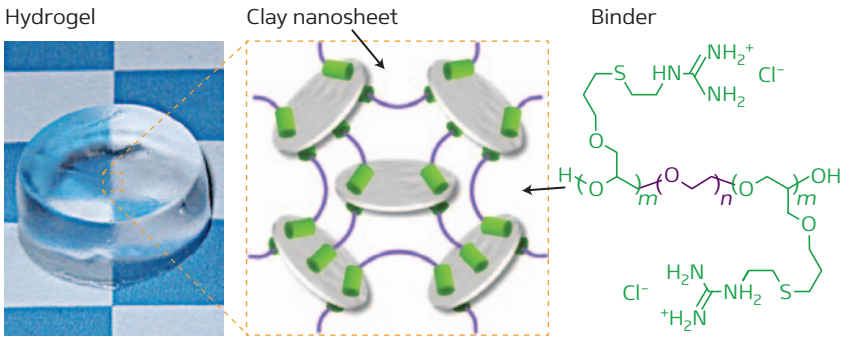


Figure 1: Free-standing hydrogels formed by the interaction between clay nanosheets and polymer binding agents may find future use in biomedical applications.

To devise a solution, the RIKEN team collaborated with Craig Hawker and colleagues from the University of California, Santa Barbara, in the United States to investigate the possibility of using advanced polymers known as 'ABA triblock copolyethers' that link adhesive ionic end-units (A blocks) and a flexible poly(ethylene oxide) core (B block) into a linear chain. This type of polymer mimics the essential attributes of dendritic binding agents but can also be easily synthesized.

Experiments demonstrated that the ABA triblock copolyethers cross-linked with the clay nanosheets as well as the original dendritic polymer binder. After optimizing the chain lengths of each ABA triblock segment, their new polymer binder rapidly generated a hydrogel with comparable mechanical strength and self-mending capabilities. The hydrogel also displayed an intriguing 'shape memory' behavior that enabled it to retain its structure after drying and re-wetting with water and organic or ionic liquids. "With these advantageous features, the hydrogel could find applications in biomedical treatments and surgical operations, including use as anti-adhesive materials," notes Ishida.

1. Tamesue, S., Ohtani, M., Yamada, K., Ishida, Y., Spruell, J. M., Lynd, N. A., Hawker, C. J. & Aida, T. Linear versus dendritic molecular binders for hydrogel network formation with clay nanosheets: Studies with ABA triblock copolyethers carrying guanidinium ion pendants. *Journal of the American Chemical Society* **135,** 15650–15655 (2013).

Building blocks help silence genes

Head-to-tail connections in gene-repressing complexes maintain proper DNA regulation during embryo and tumor formation

Polycomb-group (PcG) proteins play an important role in controlling gene expression. Complexes containing PcG proteins are thought to inhibit or 'silence' gene activity by localizing to specific targets in the genome and remodeling how DNA is wound up into chromosomes, but the exact mechanism by which these complexes repress gene activity remains poorly understood. Kyoichi Isono, Haruhiko Koseki and colleagues from the RIKEN Center for Integrative Medical Sciences have now pinpointed the part of a critical PcG protein complex that is essential for maintaining a robust, yet reversible, gene repression program during both mammalian development and cancer progression[1].

Isono, Koseki and their colleagues set out to identify the formation mechanism of a cluster of PcG proteins known as Polycomb-group repressive complex-1 (PRC1). They focused their attention on a particular domain within one of the molecules in the PRC1 complex: the sterile alpha motif (SAM) of polyhomeotic-like protein 2 (Phc2). The SAM domain helps to keep Phc2 proteins in the same orientation, facilitating head-to-tail, building-block-like linking of repeated copies of the PRC1 complex (Fig. 1).

The team created human cells designed to express Phc2 carrying a mutation in the SAM domain. This mutation prevented PRC1 binding but did not affect the basic assembly of each complex. Nonetheless, the researchers observed a substantial reduction in PRC1 cluster or 'body' formation in the cell nucleus, indicating that the construction of linked repeats of PRC1, driven by SAM domain polymerization, may be necessary for the proper functioning of the complex. In embryonic mice genetically engineered to possess a mutant SAM domain, Isono, Koseki and their team observed defects in the developing skeleton, further supporting the notion that the aberrant PRC1 bodies were not maintaining proper gene regulation in the absence of the SAM domain.

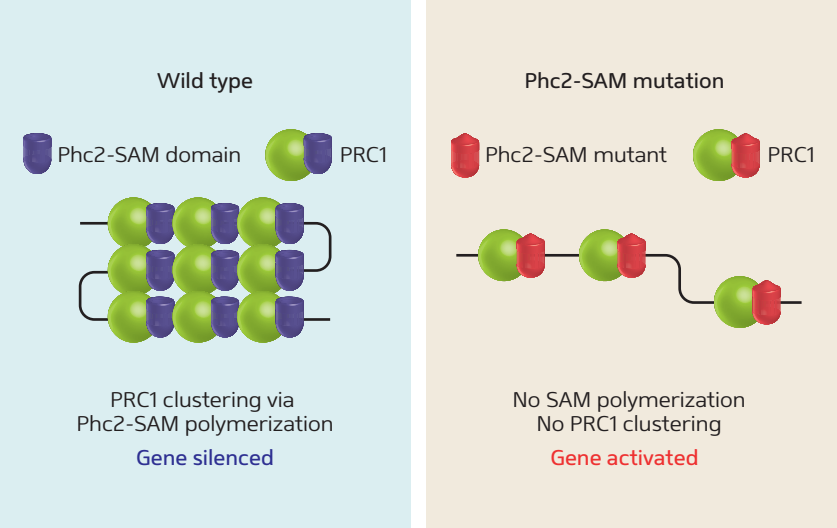


Figure 1: A functional SAM domain is needed to facilitate PRC1 clustering and PcG-mediated gene silencing.

The findings illuminate a key aspect of mammalian embryo formation. According to Isono, however, the results could also have implications that go well beyond the understanding of basic developmental processes. "The repressive function of PRC1 has a strong impact on not only pluripotency and differentiation of stem cells but also tumorigenesis," he says. "The mechanism that underlies SAM polymerization and depolymerization could be useful for regenerative medicine and cancer therapy."

A particularly exciting potential therapeutic target might be compounds that destroy the interactions between SAM domains, which could halt tumor growth in cancer patients. "I believe we can develop such drugs by conducting microscopic screenings for PcG body morphology," Isono says.

1. Isono, K., Endo, T. A., Ku, M., Yamada, D., Suzuki, R., Sharif, J., Ishikura, T., Toyoda, T., Bernstein, B. E. & Koseki, H. SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. *Developmental Cell* **26,** 565–577 (2013).

A critical theory in brain development

A new theory explains how 'critical periods' are triggered during development of the nervous system

Experiments performed in the 1960s showed that rearing young animals with one eye closed dramatically altered brain development such that the parts of the visual cortex that would normally process information from the closed eye instead process information from the open eye. These effects can be induced only within a specific period of time—a 'critical period' during which the developing nervous system is particularly sensitive to its environment.

Subsequent work has shown that the onset of the critical period in the primary visual cortex requires the maturation of circuits containing neurons that synthesize and release an inhibitory neurotransmitter called gamma-aminobutyric acid (GABA). Now, Taro Toyoizumi and colleagues from the RIKEN Brain Science Institute have presented a theory that explains how this inhibition triggers the critical period[1].

The theory is based on a computer model of the primary visual cortex containing neurons that receive and process information from the eyes. The model incorporates spontaneous and visually evoked neuronal activity as reported in earlier studies. The simulation also incorporates an activity-dependent form of synaptic plasticity—the process by which connections between neurons are strengthened or weakened in response to neuronal activity.

During early development, spontaneous activity accounts for the majority of activity in the primary visual cortex. With time, however, spontaneous neuronal activity decreases whereas activity evoked by visual experiences increases. The new theory states that the critical period is triggered by the maturation of inhibitory neuronal circuitry, which suppresses the spontaneous activity in the primary visual cortex relative to the activity driven by incoming visual information.

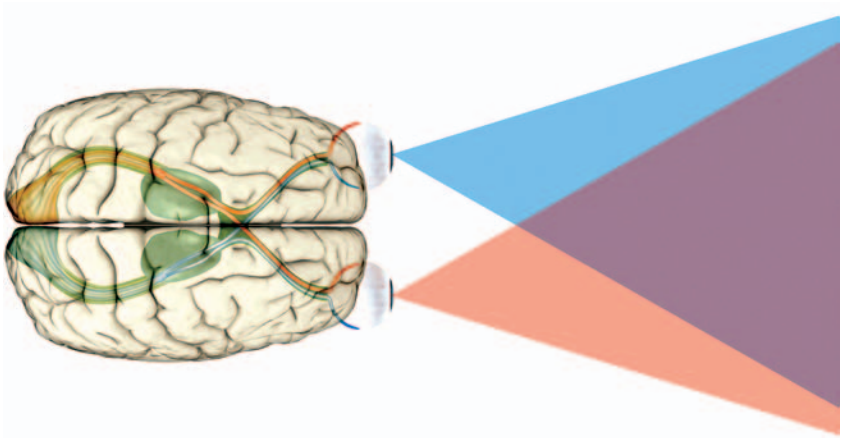


Figure 1: Closing one eye during the 'critical period' for ocular dominance plasticity can induce experience-dependent rewiring of the visual cortex.

The researchers turned to mice to find evidence to support the theory. Using electrodes to record primary visual cortex activity in freely moving mice, they showed as predicted by theory that the anti-anxiety drug diazepam, which enhances inhibitory activity, lowered the ratio of spontaneous to visual activity in mutant mice with weak inhibition—those lacking the gene encoding glutamic acid decarboxylase-65, an enzyme for synthesizing GABA. Such mice are known not to enter the critical period even in adulthood, but can be induced to do so by administration of diazepam.

Importantly, the theory explains distinct experience-dependent plasticity that takes place before the onset of the critical period, which has been observed in experiments but not explained by other theories. "In the future," says Toyoizumi, "it would be useful to be able to control developmental plasticity stages by manipulating spontaneous activity in specific brain areas, which could have therapeutic applications."

1. Toyoizumi, T., Miyamoto, H., Yazaki-Sugiyama, Y., Atapour, N., Hensch, T. K. & Miller, K. D. A theory of the transition to critical period plasticity: Inhibition selectively suppresses spontaneous activity. *Neuron* **80,** 51–63 (2013).

The magical stability of nuclei

Experiments on neutron-rich atomic nuclei could help scientists to understand nuclear reactions in exploding stars

Almost all the mass of an atom is concentrated in its nucleus in the form of protons and neutrons. Some nuclei are intrinsically unstable, existing for only a short time before decaying into stable nuclei. Understanding why some nuclei are stable and others are not could help to explain the presence of matter in the Universe. Hiroshi Watanabe at the RIKEN Nishina Center for Accelerator-Based Science and an international team of scientists have now shown experimentally that nuclei with many neutrons can be more stable than might be expected, but only if the number of neutrons is just right[1].

The number of protons in an atom is fixed for each element, but the number of neutrons can vary. Palladium nuclei have 46 protons, and in naturally occurring palladium nuclei on Earth, the number of neutrons can vary between 56 and 64, resulting in a series of 'isotopes' of palladium. In the lab, scientists can create short-lived nuclei with many more neutrons, known as 'heavy' isotopes.

Using the Radioactive Isotope Beam Factory (RIBF), Watanabe and his colleagues created palladium nuclei with 80 and 82 neutrons—labeled palladium-126 (46 protons plus 80 neutrons) and palladium-128, respectively. "Only palladium isotopes with up to 74 neutrons have been studied to date," explains Watanabe. "Our experiment reached 82 thanks to high-intensity uranium beams and a highly efficient gamma-ray detection system."

The experiment involved accelerating a beam of uranium ions toward a beryllium target. The collision created a wide variety of nuclei with different numbers of protons and neutrons, and the sophisticated ion selection system at the RIBF allowed specific heavy isotope nuclei to be isolated based on their physical and electromagnetic properties to create a pure, heavy ion beam. The team then assessed the stability of these artificially generated nuclei by observing gamma rays emitted from nuclear excited states using an array of sensitive detectors known as EURICA (Euroball-RIKEN Cluster Array) (Fig. 1).

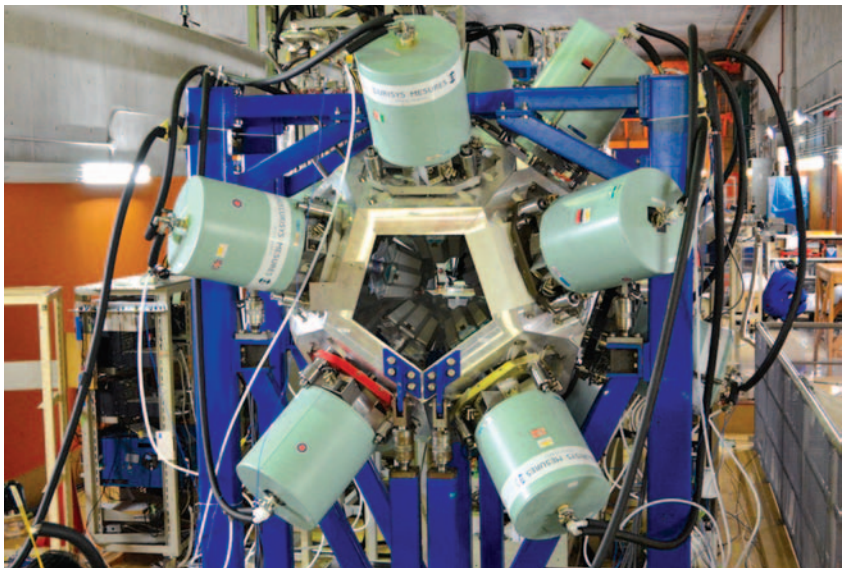


Figure 1: Gamma rays emitted during the decay of neutron-rich palladium nuclei were detected using the EURICA array of germanium detectors.

Nuclei with 2, 8, 20, 28, 50, 82 or 126 neutrons or protons are empirically known to be stable, representing the 'magic numbers' of nuclear particles. Yet some studies have suggested that these conventional magic numbers disappear if the number of protons and neutrons is highly unbalanced. The results from the RIBF research, however, show that the magic number of 82 neutrons is fairly robust, making palladium-128 relatively stable. "Next, we want to see if 82-neutron nuclei sustain their magical stability when the proton and neutron ratio is even more extremely unbalanced," says Watanabe.

1. Watanabe, H., Lorusso, G., Nishimura, S., Xu, Z. Y., Sumikama, T., Söderström, P.-A., Doornenbal, P., Browne, F., Gey, G., Jung, H. S. *et al.* Isomers in ^{128}Pd and ^{126}Pd: Evidence for a robust shell closure at the neutron magic number 82 in exotic palladium isotopes. *Physical Review Letters* **111,** 152501 (2013).

The pauses that refresh the memory

Certain symptoms of schizophrenia may arise from uncontrolled activation of neurons that help to build memories during periods of rest

Sufferers of schizophrenia experience a broad gamut of symptoms, including hallucinations and delusions, as well as disorientation and problems with learning and memory. This diversity of neurological deficits has made schizophrenia extremely difficult for scientists to understand, thwarting the development of effective treatments. A research team led by Susumu Tonegawa from the RIKEN–MIT Center for Neural Circuit Genetics has now revealed disruptions in the activity of particular clusters of neurons that might account for certain core symptoms of this disorder[1].

Tonegawa's laboratory previously found that mice lacking the protein calcineurin in certain regions of the brain exhibit many behavioral deficits that are characteristic of schizophrenia. In their most recent study, the researchers sought out physiological alterations at the single-cell or circuit level that could connect the absence of the calcineurin protein in the brain with these behavioral impairments.

Their study focused on the hippocampus, a region of the brain associated with memory and spatial learning. Within the hippocampus, specialized 'place cells' switch on and off as an animal explores its environment. During subsequent periods of wakeful rest, these place cells continue to fire in patterns that essentially 'replay' recent wanderings, allowing the brain to build memories based on these experiences. The researchers used precisely positioned electrodes to measure differences in brain activity in these cells for normal mice and the calcineurin-deficient mouse model of schizophrenia.

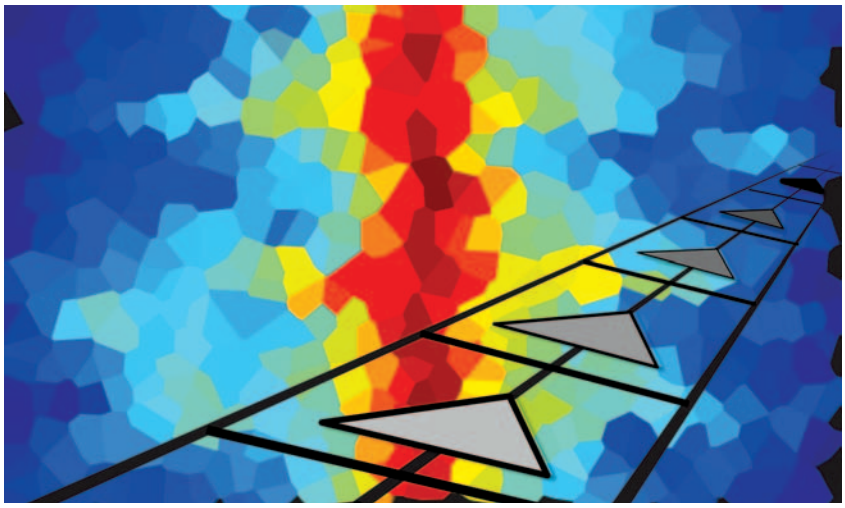


Figure 1: A map of neuronal activity during wakeful rest in calcineurin-deficient mice. In normal mice, the map reveals complex firing patterns, which are absent in this mouse model of schizophrenia.

Remarkably, essentially identical place cell activity patterns were observed for both sets of mice during active exploration. Once the animals were at rest, however, the calcineurin-deficient mice displayed a dramatic increase in place cell activity. In the normal hippocampus, the resting replay process depended on sequential activity from place cells corresponding to specific, real-world spatial coordinates. In contrast, this correlation was all but lost in the calcineurin-deficient mice (Fig. 1). Instead, these neurons often seemed to fire indiscriminately, creating high levels of 'noise' that overwhelmed actual location information and thwarted memory formation.

"Our study provides the first potential evidence of disorganized thinking processes in a schizophrenia model at the single-cell and circuit level," says Junghyup Suh, a member of Tonegawa's research team. These findings fit with an emerging model that suggests that schizophrenic symptoms may arise from excess activation of brain regions within a 'default mode network'—which includes the hippocampus—during wakeful rest. "Neurobiological approaches that can calm down the default mode network may therefore open up new avenues to alleviating symptoms or curing this mental disorder," says Suh.

1. Suh, J., Foster, D. J., Davoudi, H., Wilson, M. A. & Tonegawa, S. Impaired hippocampal ripple-associated replay in a mouse model of schizophrenia. *Neuron* **80,** 484–493 (2013).

Quarks and gluons go with the flow

Calculations suggest that smashing protons into ions of lead creates a quark–gluon plasma that behaves like a liquid

The Large Hadron Collider (LHC) at CERN (European Organization for Nuclear Research) in Switzerland is best known for its discovery of the Higgs boson, formed during collisions between bunches of protons traveling close to the speed of light. However, it has also been smashing protons into ions of lead to generate clouds of quarks and gluons—the fundamental particles inside the protons and neutrons of the atomic nucleus.

Experiments at the LHC have recently shown that this quark–gluon plasma is more liquid than physicists had expected. Adam Bzdak from the RIKEN BNL Research Center at Brookhaven National Laboratory in the United States and colleague Vladimir Skokov of Western Michigan University now offer an explanation for the effect[1].

When two lead ions collide, the quark–gluon plasma they create flows like a liquid. This hydrodynamic flow carries other particles created in the collision, like boats drifting along a fast-flowing river.

Swapping one of the colliding ions for a proton should have made this hot soup behave less like a liquid because there would be fewer particles involved. Therefore, it came as a surprise when scientists at the LHC found that ejecta from these collisions were also carried along on a wave of plasma.

Bzdak and Skokov calculated what should have happened to the pions, protons and kaons generated in the collisions had the quarks and gluons acted independently, without hydrodynamic interactions. They then compared their results from this 'wounded nucleon' model with the LHC's data for the collisions. They found that pions—the lightest particles—behaved very nearly as forecast by the model, whereas the heavier kaons and protons had more momentum than predicted. The more massive particles received a greater momentum boost from hydrodynamic effects, says Bzdak. "It's an indication of hydrodynamics."

Figure 1: Inside the Compact Muon Solenoid detector at the Large Hadron Collider.

Photograph courtesy of Michael Hoch © 2013 CERN

Bzdak and Skokov's calculations could help to refine scientists' understanding of the quark–gluon plasma that filled the Universe during its first moments. Pinning down how quarks and gluons interact at different energies would also help to refine the quantum theories that describe their behavior.

Bzdak notes that there is an alternative explanation for the LHC's observations, called the color glass condensate model. The model predicts that at very high energies, protons become saturated with a seething mass of extra gluons, which explains the extra momentum gained by more massive particles spraying from the collision. The next challenge for physicists, says Bzdak, is to test other experimental predictions of the two models to work out which of them offers the best description of the quark–gluon plasma.

1. Bzdak, A. & Skokov, V. Average transverse momentum of hadrons in proton–nucleus collisions in the wounded nucleon model. *Physics Letters B* **726,** 408–411 (2013).

Mapping objects in the brain

A brain region that responds to a particular category of objects is found to consist of small clusters of neurons encoding visual features of these objects

The ability to recognize objects in the environment is mediated by the brain's ability to integrate and process massive amounts of visual information. A research group led by Takayuki Sato and Manabu Tanifuji from the RIKEN Brain Science Institute has now discovered that in macaque monkeys, this remarkable ability is supported by mosaic-like structures in the anterior inferior temporal (IT) cortex, where localized clusters of neurons encode different visual features in an organized hierarchy[1].

Two competing models have been proposed to explain the functional organization of brain regions that underlies object recognition in primates. One model states that discrete brain 'modules' process stimuli from particular categories, such as faces, with object recognition arising from communication among the modules. The other model postulates that the visual cortex extracts generic features, which are then composited to recognize specific objects. Since both models are based on measurements of functional signals produced by metabolic changes associated with neural activity rather than measurements of the neuronal activity itself, the precise underlying mechanism responsible for object recognition has remained unclear.

To resolve this debate, the researchers undertook dense electrophysiological mapping of neural activity in anesthetized macaque monkeys exposed to a series of color images from different object categories: faces, hands, bodies, food and various other objects. Sato and his colleagues directly recorded neuronal activity from multiple locations within the anterior IT cortex, which allowed them to track the location of neurons that responded to a particular object category.

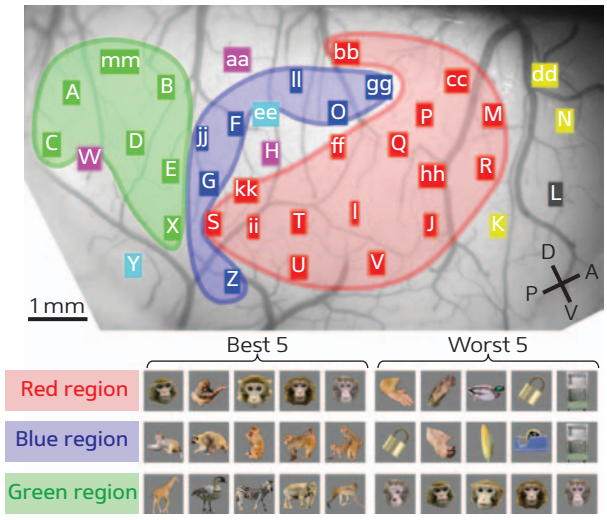


Figure 1: Neuronal activity during exposure to various images reveals distinct spatial groupings. The red region, for example, responds well to face stimuli.

The team found that some regions responded best to faces and others to monkey bodies (Fig. 1). While there were also regions that responded worst to faces, none appeared to respond preferentially to hands, food or manufactured items.

Interestingly, small neuron clusters within a region appeared to be selective to different facial features, responding differently to human and monkey faces and to scrambled and normal faces. This indicates that a region in the anterior IT cortex that is selective for an object category consists of smaller-scale neuron clusters that are selective for particular visual features.

"The cortical mosaics that encode visual information seem to be efficient functional structures where object-category information and information about constituent features are represented within the limited space of the brain," explains Sato. "This could also be the way that the brain organizes information in other sensory modalities, such as hearing." If the results are also found to extend to humans, they may offer insight into the visual recognition of objects and the development of language.

1. Sato, T., Uchida, G., Lescroart, M. D., Kitazono, J., Okada, M. & Tanifuji, M. Object representation in inferior temporal cortex is organized hierarchically in a mosaic-like structure. *The Journal of Neuroscience* **33,** 16642–16656 (2013).

Keeping it clear

Vanadium dioxide 'smart glass' can be activated to block infrared light while remaining transparent to visible light

'Smart glass' can switch from transparent to opaque at the flick of a switch and is increasingly used in cars, aircraft and homes to reduce the Sun's glare and filter out infrared light and heat. Masaki Nakano and colleagues from the RIKEN Center for Emergent Matter Science have now used vanadium dioxide to make a transparent material that can be activated to block infrared light without affecting its transparency for visible light[1].

Vanadium dioxide is a well-known thermochromic material that is transparent below about 30°C and reflects infrared light above 60°C. This transition is related to a change in crystal structure that also results in a shift from electrically insulating properties at lower temperatures to conductive properties at higher temperatures.

For the first time, Nakano and colleagues have been able to trigger this change using a static voltage rather than heat. Previous attempts were unable to create a large enough electric field to completely switch the material from an insulator to a conductor.

The researchers developed a type of field-effect transistor in which a voltage at a 'gate' terminal controls the conductivity of a vanadium dioxide channel running between 'source' and 'drain' terminals (Fig. 1). In their electric double-layer device, the gate terminal lies not on top of the channel but next to it, which ensures that it does not get in the way of light passing through the vanadium dioxide.

The channel and terminals are covered by an ionic liquid, which contains positive and negative molecules. When a voltage is applied, positive molecules accumulate in a nanometer-thick layer on top of the channel, creating a very high electric field. This triggers a metal–insulator phase transition throughout the 50 nanometer-thick layer of vanadium dioxide, causing it to become a conductor and block infrared light.

Changing the gate voltage from 1 volt to 3 volts caused the same change in conductivity in vanadium dioxide film as an increase in temperature from 30°C to 77°C. While this roughly halved the transmittance of infrared light, visible light was not affected.

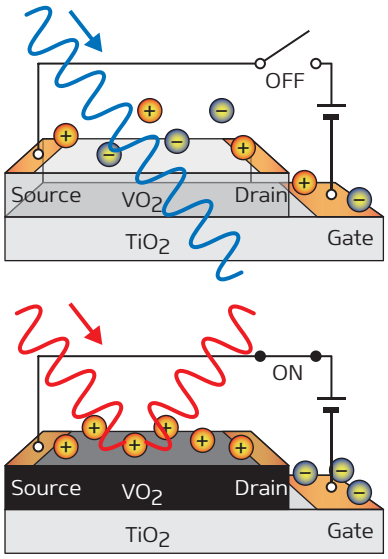


Figure 1: In its 'OFF' state (top), the vanadium dioxide (VO_2) film is transparent to visible and infrared light. When the transistor is switched 'ON' (bottom), it draws positively charged molecules across the vanadium dioxide film, changing its electronic state and crystal structure so that the material reflects infrared light but allows visible light to pass through.

The device draws almost no dissipative current, which keeps its power consumption very low. "We think that this would be very promising for a future low-energy consumption society," says Nakano. The researchers are now trying to improve the contrast in infrared transmittance between the device's 'ON' and 'OFF' states. "For practical applications," adds Nakano, "demonstrating similar performance in a large-area device is essential."

1. Nakano, M., Shibuya, K., Ogawa, N., Hatano, T., Kawasaki, M., Iwasa, Y. & Tokura, Y. Infrared-sensitive electrochromic device based on VO_2. *Applied Physics Letters* **103,** 153503 (2013).

Insights into a cellular security system

A bacterial defense mechanism against viral invasion shows a surprising degree of functional diversity

Bacteria may lack a true immune system, but this does not leave them defenseless against bacteriophage viruses and other pathogens. A system of genomic sequence elements called clustered regularly interspaced short palindromic repeats (CRISPR) and various CRISPR-associated proteins (Cas) help to recognize and destroy foreign genetic material delivered by such invaders.

An international research group led by Akeo Shinkai from the RIKEN SPring-8 Center and John van der Oost of Wageningen University in the Netherlands has now dissected one such CRISPR-Cas pathway, revealing functional insights that also highlight important differences in how these systems operate across bacterial species[1].

The researchers focused their attention on *Thermus thermophilus*, a bacterium that thrives at high temperatures and features a relatively simple and compact genome, making it amenable to experimental work. Of the bacterium's multiple CRISPR-Cas pathways, the researchers explored the pathway known as subtype III-B, which targets foreign RNA rather than DNA.

Every CRISPR element contains multiple repeats of gene sequences separated by unique spacer sequences, each of which corresponds to a potential CRISPR-Cas target. Importantly, the CRISPR-Cas system also collects 'trophies' from novel invaders, incorporating their sequence information into new CRISPR-spacer elements, thus enabling future recognition of the same pathogen. These CRISPR genes are transcribed to produce spacer-specific CRISPR RNAs (crRNAs), which combine with a collection of Cas proteins known as the Cmr complex. Shinkai and van der Oost were able to isolate the Cmr complex and identified an elongated structure that they describe as resembling a 'sea worm', with a channel that could potentially accommodate the crRNA strand (Fig. 1).

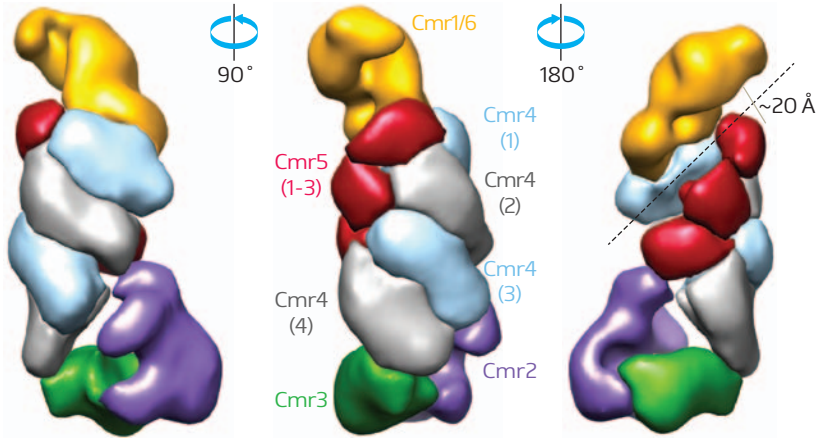
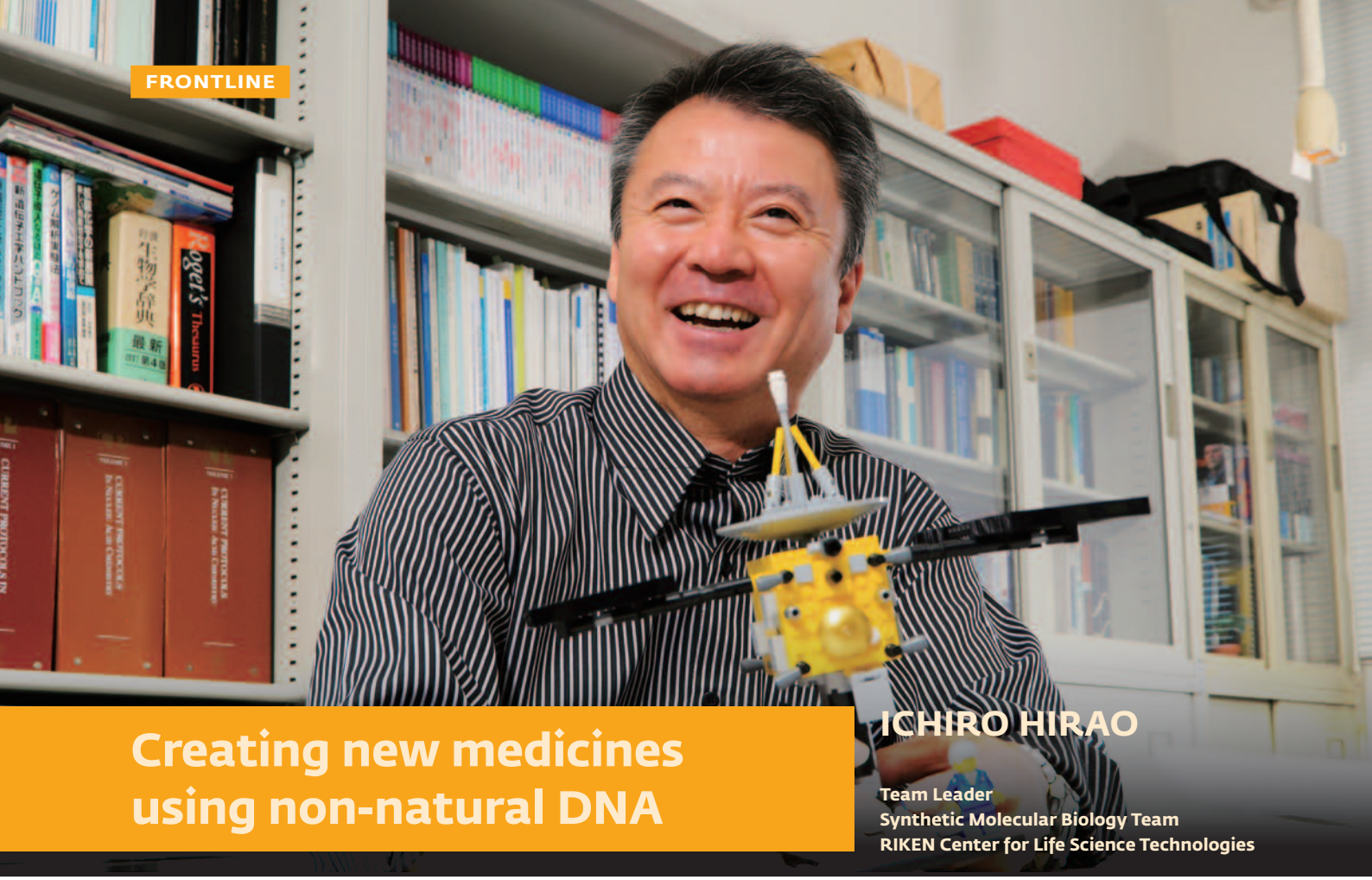


Figure 1: Structure of the Cmr complex, which contributes to bacterial antiviral defense. Each color represents a different protein component and the dashed line indicates a channel where the crRNA is likely to reside.

The researchers were also able to isolate these associated crRNAs and determined that Cmr predominantly uses spacer sequences from just 4 of the 11 CRISPR loci in the *T. thermophilus* genome. They also identified a surprising mechanism for Cmr-induced cleavage, where the complex cuts at multiple sites at fixed distances along the target, as opposed to the sequence-specific or single-site cleavage mechanisms identified in other CRISPR-Cas pathways. "The *T. thermophilus* Cmr complex may have evolved to kill bacteriophages as quickly as possible," says Shinkai. "This demonstrates the diversity of the CRISPR-Cas system."

Shinkai and his colleagues now hope to probe the three-dimensional structure of the complex in more depth and to embark on similar analyses for the other CRISPR-Cas pathways active in *T. thermophilus*. "This will hopefully lead to a more systematic understanding of these systems within the cell and of the diversity of these systems in the microbial world," says Shinkai.

1. Staals, R. H. J., Agari, Y., Maki-Yonekura, S., Zhu, Y., Taylor, D. W., van Duijn, E., Barendregt, A., Vlot, M., Koehorst, J. J., Sakamoto, K. *et al.* Structure and activity of the RNA-targeting type III-B CRISPR-Cas complex of *Thermus thermophilus*. *Molecular Cell* **52,** 135–145 (2013).



Creating new medicines using non-natural DNA

ICHIRO HIRAO

Team Leader
Synthetic Molecular Biology Team
RIKEN Center for Life Science Technologies

Classically, DNA molecules contain four different 'bases'—chemical moieties that bind to each other in a specific pairwise manner. However, Ichiro Hirao and his colleagues have succeeded in developing a DNA molecule known as an 'aptamer' that can specifically bind to a target molecule, such as a disease-related protein, using 'non-natural' DNA that contains a fifth synthetic base. Aptamers are just one potential application of non-natural DNA, says Hirao, who is working to establish a new technical basis for the life sciences using synthetic bases.

Expanding the function of DNA

In the science fiction film *Evolution* (2001), a unicellular extraterrestrial life form transported to Earth on a meteor very quickly evolves into a multicellular creature. The film postulates that the presence of ten different bases in the DNA of the extraterrestrial life form could facilitate such rapid evolution.

"In real life, the American researcher Alexander Rich hypothesized in 1962 that the information carried by DNA and its function can be increased by expanding the variety of DNA bases," says Hirao.

The DNA of all organisms on Earth consists of four bases: adenine (A), thymine (T), guanine (G) and cytosine (C). Two strands of DNA are joined by pairings between A–T and G–C to form a double helix structure (Fig. 1). The alignment of the bases, known as the base sequence, is converted into RNA during the process of transcription and proteins are subsequently biosynthesized during translation.

Nucleic acids, DNA and RNA, work as 'information molecules' to produce proteins, which in turn serve as the 'functional molecules' that take responsibility for the chemical reactions required to absorb nutrients from the external environment to generate energy and produce body-forming substances—the basis of life. "Proteins are biosynthesized based on information stored in DNA but proteins are also required to produce DNA. So the question arises: when life emerged on primitive Earth, which was produced first—DNA or protein?" notes Hirao. "In the early 1980s, ribozyme was discovered as a unique RNA that functions like an enzyme—a protein that catalyzes chemical reactions—leading to the hypothesis that the first life form was an RNA-based entity."

Much research has been performed to increase the functional variation of RNA. Molecules that bind specifically to disease-causing proteins, cancer cells and other targets, such as the protein antibodies that defend the body from foreign entities, have the potential for use in therapeutics and diagnostics. Researchers later discovered that DNA has similar traits. Fragments of RNA or DNA that bind to target molecules are called nucleic acid aptamers (Fig. 2).

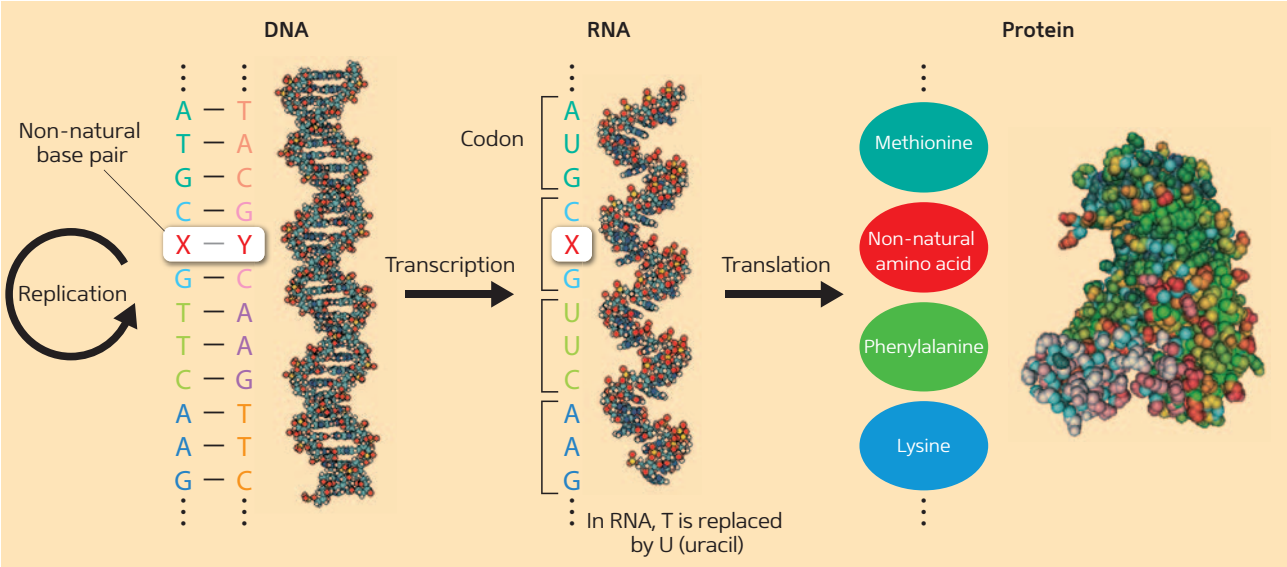


Figure 1: Expanding the DNA replication, transcription and translation system through the addition of a non-natural base pair

By adding a non-natural base pair (X–Y) to DNA and allowing it to be transcribed, non-natural bases can be incorporated into RNA to increase the variety of codons. Allocating the extra codons to non-natural amino acids and translating them into amino acids allows a protein comprising many non-natural amino acids to be created.

Scientists usually require a long period of time in order to prepare antibodies because they must be produced in an animal. In contrast, an aptamer can be quickly prepared by using an *in vitro* evolutionary engineering approach. Once obtained, an aptamer can be mass-produced by chemical synthesis according to its base sequence. Furthermore, while the administration of an antibody often stimulates the body's immune system to eliminate it as a foreign substance, this rejection seldom occurs with a nucleic acid aptamer. Therefore, nucleic acid aptamers have attracted much attention for their potential use as new medicines.

In order to bind, an aptamer must 'fit' the shape of its target just like a key fits in a keyhole. To create an aptamer, bases are randomly arranged to build a library of nucleic acid molecules of various shapes. When a target is placed in the library, some of the nucleic acid molecules will bind to it and can be 'fished' from the pool. After amplification, the nucleic acid molecules are exposed to increasingly severe fishing conditions, allowing the selection of nucleic acid molecules with increasingly higher bindability. In evolutionary engineering this process, which is like natural selection, is repeated to select desired aptamers.

However, the only pharmaceutical aptamer to have thus far been developed is pegaptanib, or 'Macugen', a therapeutic drug developed to treat age-related macular degeneration, a disease that affects the retina. Macugen is a modified RNA aptamer that binds to vascular endothelial growth factor (VEGF) and inhibits its function.

A non-natural DNA base

The availability of just a single aptamer-based drug indicates that drug discovery using nucleic acid aptamers is a difficult process. "An antibody-forming protein can consist of up to 20 different amino acids. This wide variety accounts for the morphological and chemical diversities of antibodies," explains Hirao. Antibodies are estimated to be present in a vast number of shape variations, which imparts them with the ability to bind to all foreign substances in the body, offering protection against these foreign substances.

"On the other hand, because there are only four variations of the bases that constitute RNA or DNA, the variations in their morphology and chemical properties are limited, making it difficult to develop aptamers that bind strongly to targets," continues Hirao. "By increasing the kinds of bases available, it may be possible to broaden the morphological variation and hence to create strongly binding aptamers. Accordingly, a project to create DNA containing a non-natural base was launched in Japan in 1996 and I returned home to start it."

To repeat selection using the evolutionary engineering approach, researchers must be able to amplify DNA that contains non-natural bases. In 2009, Hirao and his colleagues were the first to successfully develop the non-natural base pair 'Ds–Px', which can be replicated with high accuracy using the traditional PCR technique for amplifying DNA *in vitro* (Fig. 3).

Subsequently, Hirao and his team investigated whether the functional variation of a non-natural DNA could be increased by expanding the variation of bases contained within. First, they built a library containing between 100 trillion and 1 quadrillion different non-natural DNA fragments, each consisting of about 40–50 randomly arranged combinations of five different bases: A, T, G, C plus the non-natural base Ds. Next, they performed experiments to create a DNA aptamer that bound to VEGF and interferon γ, both of which are implicated in a wide variety of diseases.

"We performed seven rounds of target fishing and PCR amplification to select a non-natural DNA aptamer, which we found to have a binding power more than 100 times that of natural DNA aptamers," says Hirao. Through their efforts, Hirao and his team were able

to demonstrate the hypothesis that increasing the kinds of component bases expands the functional variation of DNA was correct.

Exploiting non-natural DNA in drug discovery

The non-natural DNA aptamer created by Hirao and his team possesses a greater binding capacity than conventional nucleic acid aptamers and protein antibodies. With its strong ability to bind, the aptamer could be used as a drug that securely captures the target and is highly effective, even at low doses.

Hirao was keen to discover why the mere addition of the non-natural base Ds increased the bindability of the aptamer to such a great extent. "When we examined the non-natural DNA aptamer, only 2 or 3 of the 40–50 aligned bases were found to contain the non-natural base Ds," he says. "We conjecture that the aptamer's affinity for protein was increased because without the addition of Px—to serve as the partner base of Ds—structural diversity was introduced due to the protruding synthetic base, and also because Ds is highly hydrophobic."

Proteins are composed from 20 amino acids, some of which are readily soluble in water (hydrophilic) and others of which are hardly soluble in water (hydrophobic). Hence, proteins contain hydrophobic regions. On the other hand, nucleic acids containing only natural bases are highly hydrophilic. Since hydrophilic and hydrophobic substances are unlikely to bind to each other, hydrophilic nucleic acid aptamers composed of natural bases are unsuitable for binding to the hydrophobic regions of proteins. However, the addition of Ds, a hydrophobic base, is thought to overcome this drawback to increase the bindability of the non-natural aptamer.

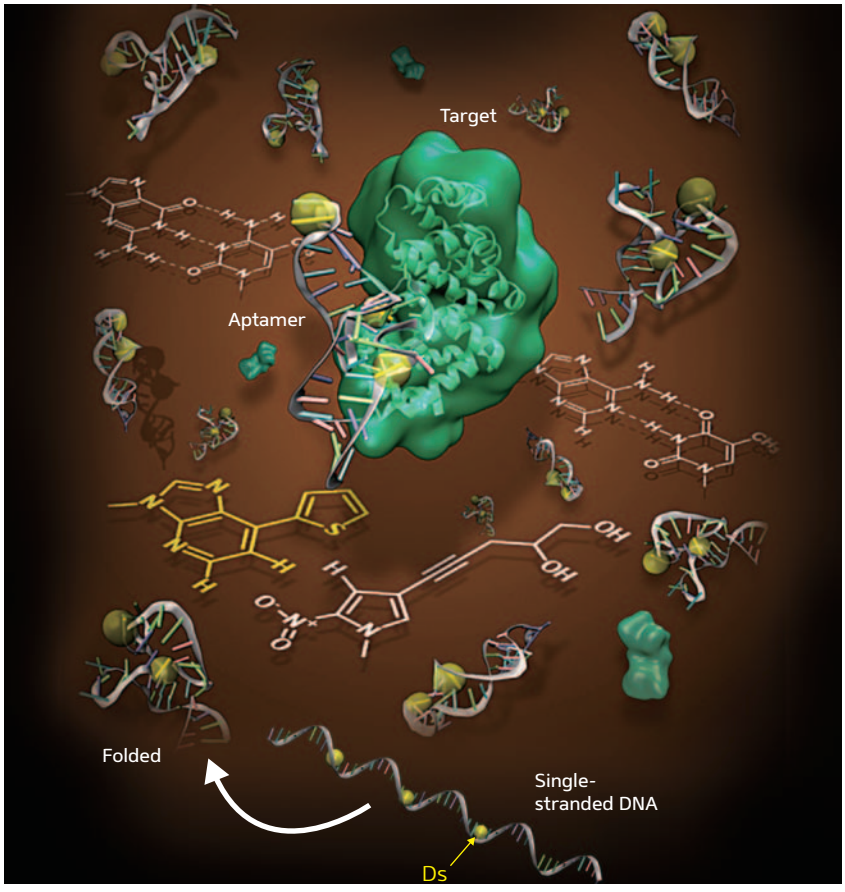


Figure 2: A non-natural DNA aptamer that binds to a target protein

A single-stranded molecule of DNA, known as an aptamer, composed from the four natural bases plus the non-natural base Ds (yellow sphere) is able to bind to a protein target. In the absence of the non-natural base Px to serve as the pair partner of Ds, the protrusion of hydrophobic Ds increases structural and chemical variation, resulting in the formation of an aptamer that binds strongly to the target.

Of concern to scientists is the potential for inducing adverse reactions in the body if a therapeutic DNA aptamer binds to a non-target protein or other substance. Therefore, Hirao and his colleagues made sure to select an aptamer that possessed both strong bindability and extremely high selectivity, and thus predominantly binds to the target.

In addition, a nucleic acid aptamer can only serve as a medicine if it reaches the target without being degraded. While DNA is chemically more stable than RNA, DNA-degrading enzymes are also present in the body. "Previously, I discovered that the addition of a certain short base sequence makes DNA unlikely to be degraded by such enzymes," explains Hirao. "Currently, we are conducting an experimental study to increase the stability of a non-natural DNA aptamer by attaching this short base sequence."

The final stage of drug discovery requires that the drug under development is administered to human subjects, allowing for the examination of the pharmacological effects and adverse reactions. "Fortunately, we received a proposal from an overseas research institution to use our non-natural DNA aptamer in such a study."

Hirao's team also receives many requests for preparing non-natural DNA aptamers that bind to new targets. "While it takes about half a year to make an organism that produces an antibody against influenza virus, in contrast, we are able to prepare a new non-natural DNA aptamer in around two weeks to a month." At the RIKEN Center for Life Science Technologies (CLST) Division of Bio-Function Dynamics Imaging, where Hirao's Synthetic Molecular Biology Team is based, researchers in the field of molecular imaging have been working to create radioisotope-labelled probes in order to assist in drug discovery and disease diagnosis. "Radioisotopes can be attached to our non-natural DNA aptamer," confirms Hirao. The radiolabelled aptamer can then selectively bind to its target biomolecule, allowing the biomolecule to be monitored using a

technique such as positron emission tomography (PET). "A PET probe is a suitable first practical application of a non-natural DNA aptamer. A joint research project for making such a probe is underway at RIKEN."

Non-natural DNA aptamers are also promising as a drug delivery system to transport drug molecules to targets with pinpoint accuracy. Hirao believes that it will be possible to create a non-natural DNA aptamer that binds selectively to a particular type of cancer cell, and could even enter the cell. "Throughout the history of drug discovery, many candidate molecules failed to be turned into medicines due to a lack of target bindability and selectivity," he notes. "Attaching a non-natural DNA aptamer may allow such molecules to be developed as medicines."

Allowing non-natural bases to function in cells

Initially, all experiments with non-natural bases were conducted *in vitro*. However, Hirao and his colleagues are aiming to both replicate and transcribe non-natural bases in biological cells. Techniques, such as fluorescently tagged proteins, have already been developed to allow the visualization of proteins within cells. However, there is no such technique for DNA and RNA. "We are developing a technique that will allow non-natural bases to emit light," says Hirao. "By achieving intracellular replication and transcription involving non-natural bases, we will be able to visualize the behavior of particular DNA and RNA molecules."

Intriguingly, researchers from the Division of Genomic Technologies at the CLST have obtained mounting evidence that RNA not only serves as a mediator for protein biosynthesis by transcribing information stored in DNA but also controls gene expression. "If we can realize non-natural base transcription in living cells, we will be able to make a particular RNA emit light, and subsequently monitor its behavior and explore its function," Hirao explains.

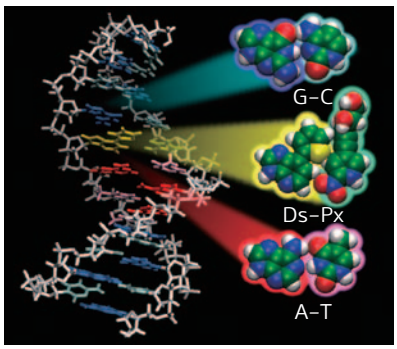


Figure 3: DNA consisting of 6 different bases, including the non-natural base pair Ds–Px

Meanwhile, Kensaku Sakamoto, who heads the Nonnatural Amino Acid Technology Team at the CLST, and his colleagues are conducting research to create non-natural proteins that function better than their natural counterparts through the addition of non-natural amino acids. However, only one or two non-natural amino acids can be integrated into a single protein because the number of 'instructions' that can be written in the RNA is limited. At translation, one amino acid is specified for each set of three RNA bases, known as a codon. The number of codons available when there are four different bases is thus $4 \times 4 \times 4 = 64$, to which the particular instructions for 20 different amino acids plus the completion of translation are allocated.

"If six different bases are available with the addition of the Ds and Px synthetic bases, the number of available codons will be $6 \times 6 \times 6 = 216$. By allocating non-natural amino acids to the extra codons, non-natural proteins incorporating many different non-natural amino acids may be created in an organism," says Hirao.

The ability to functionalize non-natural bases in living cells would create a new technique that surpasses currently available gene recombination technology.

"Cells incorporating a non-natural base could not survive without being continuously fed a food containing the non-natural base," notes Hirao. "These cells would never proliferate spontaneously in nature and thus could be biologically contained."

Exploring the origins of life

Hirao's interests also extend to the origins of life. "Before arriving at the Ds–Px paired synthetic bases, we made a wide variety of bases and selected those that were suitable for use as information-storing molecules. This process was similar to the history of evolution, during which life chose A, T, G and C."

In 2014, the Japan Aerospace Exploration Agency (JAXA) will launch the asteroid explorer Hayabusa-2, which has a mission to bring back samples containing organic matter from an asteroid. "The primary interest is whether molecules that could serve as a starting material for life are present," says Hirao. "However, the researchers need not focus on molecules being used by life on Earth, like A, T, G and C and the 20 natural amino acids. Instead, other molecules might be involved in biological function and information." Indeed, Hirao has high hopes that his non-natural base research will lead to exploratory studies of the variability of substances that can serve as genes for organisms that could exist elsewhere in the Universe.

ABOUT THE RESEARCHER

Ichiro Hirao was born in Shizuoka, Japan, in 1956. He graduated from the Numazu National College of Technology in 1976, and obtained his PhD in 1983 from Tokyo Institute of Technology. In 1984, he joined Kin-ichiro Miura's laboratory at the University of Tokyo, where he discovered unusual DNA mini-hairpin structures. To expand his research toward molecular biology and evolutionary engineering, in 1995 he moved to Andrew D. Ellington's laboratory at Indiana University. In 1997, he returned to Japan to begin work on the expansion of DNA's genetic alphabet. He now leads the Synthetic Molecular Biology Team at the RIKEN Center for Life Science Technologies, which focuses on non-natural base pair systems. He also serves as a visiting professor at Yokohama City University, Tokyo Institute of Technology and Hokkaido University.

Cracking the epigenetic code

AKI MINODA

Unit Leader
Epigenome Technology Exploration Unit
RIKEN Center for Life Science Technologies

What made you decide to become a scientist?

When I was young, I suffered from asthma. As a result, I always wanted to have a job where I could help other children who were suffering from diseases but never wanted to become a doctor. Then in high school, I read a book about the Ebola virus, which captivated me and drew me toward becoming a researcher.

Please describe your current research at RIKEN.

Our bodies are made up of several hundreds of different types of cells, for example hair cells and heart cells, but they all contain exactly the same DNA—the genome. How is this possible? It is achieved by a process called epigenetics—'epi-' meaning 'besides' or 'above' in Latin.

Each cell type knows which sections of the genome to utilize owing to a complex epigenetic marking system on and around the DNA. I am trying to crack this code—the 'epigenetic code'. Many diseases and cancers have an incorrectly written code; I hope to detect the incorrectly marked regions in order to develop biomarkers and therapeutic drugs.

How did you become interested in epigenetics?

During my PhD research, I studied yeast, a very simple single-celled organism and a huge contrast to the estimated 30 trillion cells that make up the bodies of humans. Nevertheless, even such simple organisms save energy by using an epigenetic code to read only the necessary parts of the genome. I found this very interesting and wanted to know more about how this is achieved.

What is the best thing about working at RIKEN?

The best thing about working at RIKEN is how international the organization is. My division in particular—the Division of Genomic Technologies—is one of the most international divisions at RIKEN and I am extremely proud to be a part of it. Having lived abroad for a long time, I feel very comfortable here.

Please tell us about your professional and personal goals.

I strive to continue to enjoy my research with as much passion as I have now, and for my work to have a meaningful impact on society and the individuals who are affected by various diseases. Furthermore, as a female researcher in Japan, I would like to be a role model for young female scientists, especially given that the ratio of female to male scientists in Japan is a lot lower than in the West.

How do you balance family life with your work at RIKEN?

My husband is American, and we moved together to Japan for my position at RIKEN. Consequently, he had to leave his job in the United States, which was a big decision for us to make. Fortunately, he is very understanding.

In general, I try to not work late during the week and to minimize working on the weekends. I would like others to know that it is possible to have a successful career as a female scientist and to also spend time with family on the weekends.

CONTACT INFORMATION

For details about working at RIKEN, please contact the RIKEN Global Relations and Research Coordination Office:
Tel: +81 48 462 1225
E-mail: pr@riken.jp

RIKEN and Universiti Sains Malaysia nurture fruitful partnership

On 16–20 December 2013, Todd Taylor from the RIKEN Center for Integrative Medical Sciences conducted a bioinformatics workshop at the Universiti Sains Malaysia (USM) School of Biological Sciences (SBS)—the first to result from the establishment of a formal relationship between RIKEN and USM.

Participants at the five-day workshop received practical introductory training in a wide range of fields, including the use of web-based resources, next-generation gene sequencing methods, gene prediction and functional analysis, RNA sequencing and analysis and metabolic-pathway reconstruction. "We hope that this bioinformatics workshop can be made into an annual event," remarked Amirul Al-Ashraf Abdullah, acting deputy dean of research and associate professor at the SBS, who was encouraged by the overwhelming response.

The workshop is the latest in a series of collaborations between RIKEN and USM. In 2011, the RIKEN–USM Joint Research Unit was created to isolate novel compounds from plants in southeast Asia for the development of novel drugs to treat tropical diseases. The team of Japanese and Malaysian researchers has since compiled a searchable and freely accessible online database of 200 plant species. The growing repository of chemical compounds will support the identification of biologically active compounds and the determination of target molecules for these compounds.

© 2013 Public Relations Office, Universiti Sains Malaysia

From left: Todd Taylor of the RIKEN Center for Integrative Medical Sciences, Sudesh Kumar, coordinator of the RIKEN–USM partnership and Amirul Al-Ashraf Abdullah, acting deputy dean of research of the USM School of Biological Sciences, at the bioinformatics workshop held in Malaysia.

Partnership between the two institutes extends to other areas as well, with over ten students from USM selected to conduct their PhD research at RIKEN as International Program Associates to date. "It is really an honor for USM to have a world-class research institute such as RIKEN as its partner," said Sudesh Kumar, a professor at USM and the coordinator of the RIKEN–USM partnership. "USM would like to strengthen this partnership with more joint activities, such as workshops, research training, technology transfer and other educational events," he added. ■

Katsuhiko Mikoshiba receives top French medal

Katsuhiko Mikoshiba, head of the Laboratory for Developmental Neurobiology at the RIKEN Brain Science Institute, was presented with the French Legion of Honor—the highest decoration in France—in a special ceremony held at the Embassy of France in Tokyo in December 2013.

© 2014 RIKEN

Katsuhiko Mikoshiba of the RIKEN Brain Science Institute received the French Legion of Honor—the highest decoration in France.

Mikoshiba received the distinction for his important contributions to the field of brain science and his service to cultural and scientific exchange between Japan and France. Among Mikoshiba's many notable achievements is the discovery in 1976 of the inositol 1,4,5-trisphosphate (IP_3) receptor, which plays an important role in many biological functions, including body development and brain plasticity. Mikoshiba's current research focuses on how the brain develops and the role of calcium in cellular communication.

The Legion of Honor was established in 1802 by the reigning French leader, Napoleon Bonaparte. Around 150 non-French citizens have since received the prestigious badge, 10 per cent of whom were Japanese—including two other individuals from RIKEN. ■

RIKEN welcomes the New Year with *Mochitsuki* festivals

On 17 January 2014, the RIKEN Mutual Benefit Society, or *Kyosaikai*, organized rice-cake-making festivals at RIKEN institutes in Wako and Yokohama to celebrate the New Year.

© 2014 RIKEN

RIKEN researchers and their families prepared rice cakes to welcome the New Year, following Japanese tradition.

Mochitsuki is a traditional event in Japan, where people gather to pound rice in a wooden mortar using a large wooden mallet. While one person pounds the rice, another person kneels to flip the rice cake, or *mochi*, taking care to avoid being hit by the mallet. The rice cakes are then served with sweet bean paste and other sweet or savory condiments.

The annual event at Wako and inaugural celebration in Yokohama were very popular among RIKEN researchers and their families, who enjoyed sharing in both the preparation and tasting of the rice cakes. ■

www.rikenresearch.riken.jp

RIKEN 2014-015