RIKEN RESEARCH

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Changing the guard

SPring-8 synchrotron radiation facility in Harima

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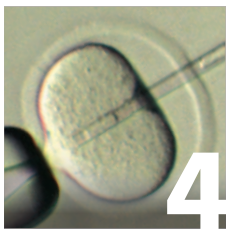

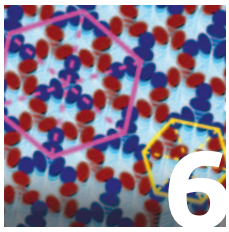

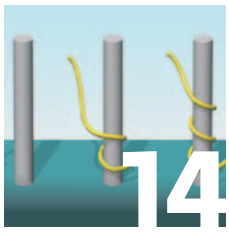

RIKEN RESEARCH

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Biology

Building an army of one

By boosting cloning efficiency, scientists unlock the potential to 'mass-produce' valuable animal strains for research and agricultural applications

Long before humans understood the principles of genes and heritability, they were already shaping animal evolution through selective breeding. The various domesticated species we cultivate today are the product of a centuries-long project of gradual selection and mating to generate strains with optimized physical and behavioral characteristics.

Nowadays, scientists have amassed considerable insight into animal genetics. Additionally, cloning technology theoretically offers the potential to replicate large numbers of livestock or research animals with identical, desirable traits. In reality, however, most efforts in this area have so far met with disappointment. Now, recent progress made by a research team led by Teruhiko Wakayama at the RIKEN Center for Developmental Biology suggests that multigenerational cloning may soon become a feasible option[1].

For over a decade, Wakayama's team has pursued the serial recloning of mice using a technique called somatic cell nuclear transfer (SCNT), which allows generation after generation of identical mice to be produced from a single-source donor. The mouse is an extremely well characterized animal model and should therefore be ideal for testing such strategies. However, Wakayama and his colleagues previously achieved only limited success. "Nobody had succeeded in continuous recloning for more than six generations," he says.

In general, SCNT is plagued by inefficiency, requiring a substantial investment to successfully obtain healthy cloned animals. Although steady progress in cloning technology has improved the odds, the root causes of failure in the recloning process remain obscure. Despite the difficulties, however, Wakayama was committed to overcoming this challenge. "I am always trying difficult studies where nobody has succeeded previously, and so I wanted to try this again," he explains.

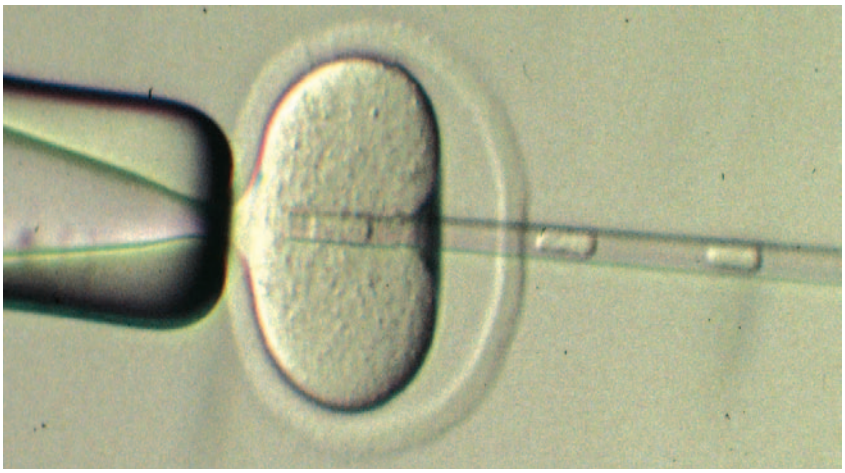


Figure 1: Cloning begins with the injection of the nucleus of a somatic cell from the animal being cloned into an oocyte from which the nucleus has been removed.

Growing an unusual family tree

SCNT involves extracting the nucleus from an adult donor cell and transferring it into a recipient oocyte from which the nucleus has been removed (Fig. 1). The environment of the oocyte induces a series of 'reprogramming' events in the donor nucleus, which subsequently behaves as if it were the nucleus of a newly fertilized egg. If successful, the resulting developmental process yields an embryo that is a clone of the donor animal.

The reprogramming process requires selective enzymatic addition of chemical modifications to targeted sites on the chromosomes, which in turn modulate expression of nearby genes through 'epigenetic' regulation. Certain other enzymes can undermine cloning success by removing these epigenetic marks, prematurely terminating the reprogramming process. Such enzymes can be inhibited by drugs like trichostatin A (TSA), and SCNT success rates can be boosted as much as fivefold using this TSA-facilitated strategy.

By employing TSA treatment, Wakayama and colleagues have been able to improve their SCNT success rate and achieve a breakthrough in serial recloning. Over the past eight years, the team has repeatedly cloned a single female donor mouse through

Figure 2: Mice obtained from generations 23, 24 and 25 of the serial recloning of a single donor animal.

25 generations and beyond (Fig. 2). From each subsequent generation, the researchers collected the nucleus from one of the 'cumulus cells' that surrounds the oocyte, and transferred it into a recipient oocyte. In this manner, they were able to successfully derive a new and healthy clone from every new generation.

The cloning success rate varied considerably across—and even within—generations: in some cases the rate was as low as 3% and in others as high as 20%. To identify factors that might be altering the rate, the researchers used a variety of control experiments and even accounted for the variability in experimental technique that would inevitably arise across an eight-year experiment. Ultimately, they were unable to identify any direct contributing factors. "There are too many possibilities," says Wakayama. "Even the season can affect the success rate."

Nevertheless, the cloned mice that were generated proved to be healthy, physiologically normal and exhibited typical lifespans. Indeed, a set of clones from generation 20 were each sufficiently fertile to yield normal-sized litters of healthy pups when bred. The animals proved equally healthy at the cellular level, with no evidence of accumulated genetic damage or abnormalities in gene expression relative to first-generation clones. Wakayama compares this achievement to duplicating a DVD versus a videotape. "Copying a videotape will decrease its quality, but copying a digital file does not," he says. "Thus, cloning by nuclear transfer is more similar to digital than analog copying."

A new beginning

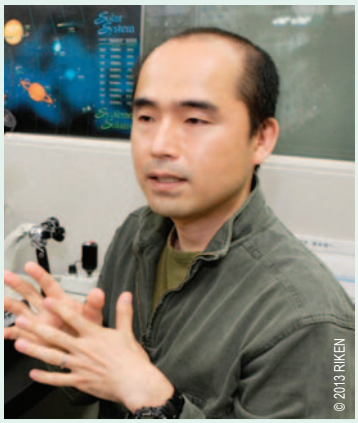
Following the publication of their findings, the team of researchers are continuing to generate clones from each new generation. Wakayama notes that they have reached generation 27 and are collecting data from these animals at present.

In principle there appears to be no inherent generational limit. As long as TSA is applied to facilitate proper nuclear reprogramming, each round of cloning is no less likely to succeed than the previous round. This suggests that earlier failures in serial recloning were simply the result of an extremely low base success rate, rather than the aggregate effect of cellular defects acquired during each new round of SCNT.

As TSA and other chemicals with similar inhibitory effects can also dramatically boost the success of SCNT in other species, it is anticipated that serial recloning should prove similarly feasible in larger animals such as cows or pigs. Wakayama also notes that their technique could revive long-lost animal populations. "We plan to use the mouse as a model for resurrecting extinct species," he says. "For example, we have successfully generated clones from frozen mice, and are trying to make clones from taxidermied animals or from the fur of a mouse."

1. Wakayama, S., Kohda, T., Obokata, H., Tokoro, M., Li, C., Terashita, Y., Mizutani, E., Nguyen, V. T., Kishigami, S., Ishino, F. & Wakayama, T. Successful serial recloning in the mouse over multiple generations. *Cell Stem Cell* **12**, 293–297 (2013).

ABOUT THE RESEARCHER



Teruhiko Wakayama received his BSc and MSc from Ibaraki University and was awarded a PhD in reproductive biology from the Department of Veterinary Anatomy at the University of Tokyo in 1996. A Japan Society for the Promotion of Science postdoctoral fellowship enabled Wakayama to spend the following two years in Ryuzo Yanagimachi's laboratory at the John A. Burns School of Medicine at the University of Hawaii, where he succeeded in creating the world's first cloned mouse. He was appointed as assistant professor at the same institution in 1998, and moved to the Rockefeller University as a research assistant professor in 1999. In 2001, he returned to Japan to lead the Laboratory for Genomic Reprogramming at the RIKEN Center for Developmental Biology. Currently, he is also a professor at the University of Yamanashi.

A liquid-crystal force to reckon with

A new chemical strategy forces electrically active molecules to stack into 'superlattice' liquid-crystal films with exceptional charge transport properties

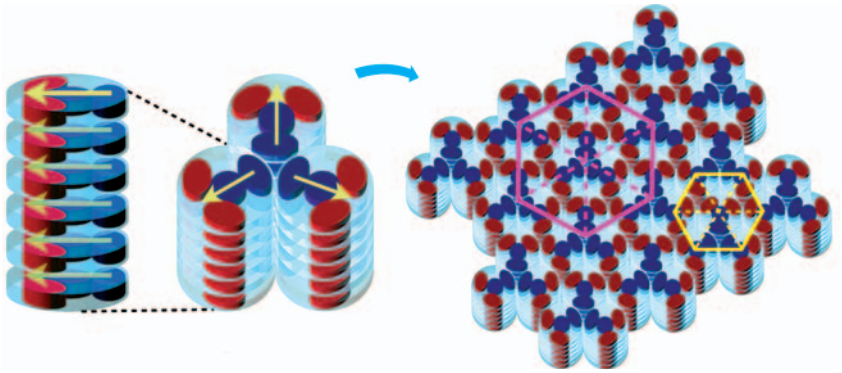


Figure 1: Disrupting the natural packing tendencies of disc-shaped liquid crystals (light blue cylinders) produces stacked columns with significant dipole moments (left). These columns self-assemble into hierarchical triangular (center) and hexagonal films (right) perfectly aligned for organic electronic applications.

A need for fast, solution-based processing of organic electronic devices has sparked increased interest in 'discotic' or disc-shaped liquid crystals. These molecules, which contain a flat aromatic core surrounded by hydrocarbon side chains, can spontaneously pile into column-like structures that could be ideal for one-way charge transport. Research led by Takashi Kajitani and Takanori Fukushima from the RIKEN Advanced Science Institute* has now revealed a way to turn individual discotic columns into liquid-crystal films with unprecedented hierarchical order in two dimensions[1].

Chemists recently discovered that the formation of columns from discotic molecules is enhanced when the molecules contain bond dipoles—electrochemical imbalances that exist between atoms such as carbon and nitrogen. These dipoles increase the polarization of the 'pi'-bonds that stack aromatic rings together, leading to better column alignment and better electronic pathways. However, discotic columns self-assembled in this manner have no net dipole; instead, the discs pack with alternate 'head-to-tail' layering to minimize free energy, which cancels out the columns' electronic activity.

Kajitani, Fukushima and their colleagues tried a different approach in order to produce 'head-to-head' stacked discotic columns with significant dipole moments. Starting with dibenzophenazine, an aromatic ring containing carbon–nitrogen bond dipoles, they attached two types of side chains: one consisting of hydrophobic paraffin chains and another of hydrophilic triethylene glycol. The team anticipated that microscopic phase separation between the different side-chain regions could force the discs into a single orientation during aqueous self-assembly.

Synchrotron x-ray experiments revealed that the modified discotic molecules generated diffraction patterns unlike any seen before—a 'superlattice' of columns packed into triangle shapes that press together into a two-dimensional hexagonal film with extended lateral order. According to the researchers, this is clear evidence that head-to-head discotic stacking was successful. The striking film patterns are a consequence of the self-assembly neutralizing the large column dipoles over an extended space. "Liquid crystals normally occupy a space between states of matter, so finding a stable structure with wide-ranging two-dimensional lattice correlation is remarkable," says Kajitani.

Intriguingly, they also found that the superlattice columns stacked almost perfectly perpendicular to a glass surface, whereas most other discotic techniques generate a mix of horizontal and vertical orientations. Conductivity measurements showed that this spontaneous 'homeotropic' alignment yields directional charge transport properties suitable for high-efficiency organic electronics. Kajitani notes that the unique assemblies created using this technique may be suitable for applications such as organic thin-film solar cells and fuel-cell assemblies.

1. Yeh, M.-C., Su, Y.-L., Tzeng, M.-C., Ong, C. W., Kajitani, T., Enozawa, H., Takata, M., Koizumi, Y., Saeki, A., Seki, S. & Fukushima, T. Amphiphilic design of a discotic liquid-crystalline molecule for dipole manipulation: Hierarchical columnar assemblies with a 2D superlattice structure. *Angewandte Chemie International Edition* **52**, 1031–1034 (2013).

* Reorganized into new centers from April 2013

X-ray surfing

A repetitively strained atomic structure makes it possible to offset the propagation of x-ray light in semiconductor materials

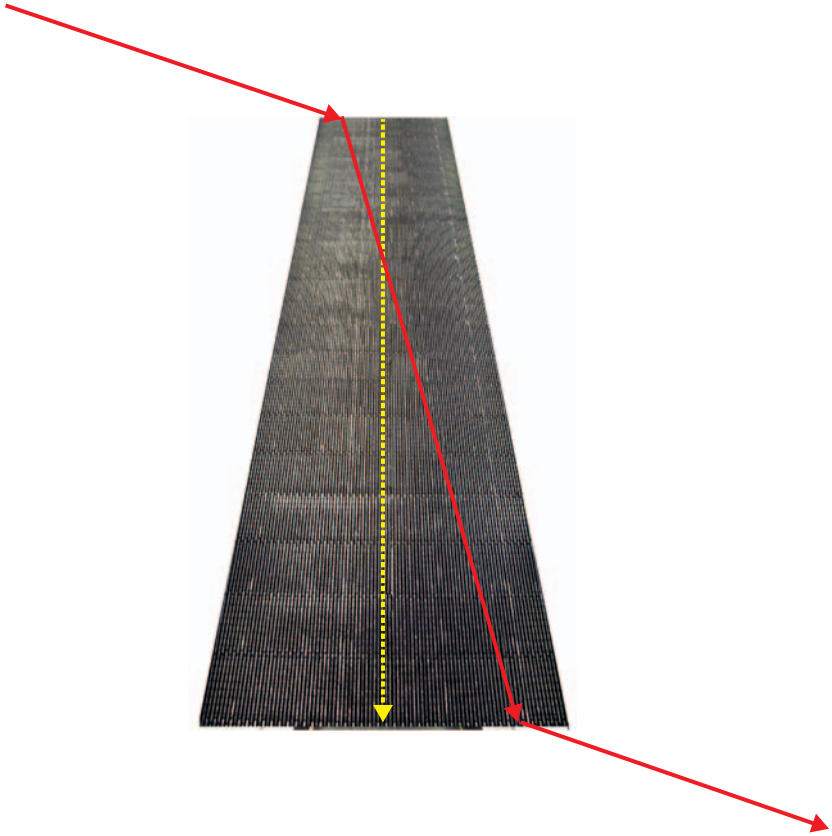


Figure 1: The path of an x-ray beam is offset as it passes through a silicon–germanium crystal with a repetitively strained atomic structure.

X-rays, like visible light, are a form of electromagnetic radiation and have a very short wavelength and a relatively high energy. The utility of x-rays in medical imaging is due to the very weak interaction between these high-energy electromagnetic waves and matter, which makes it possible to see through soft tissue to the bones beneath. However, this property also makes the lenses and mirrors equivalent to those used for visible light much more difficult to develop for x-ray wavelengths. Yoshiki Kohmura and colleagues from the RIKEN SPring-8 Center have now shown that the optical translation of x-rays can be achieved using materials carefully engineered at the atomic scale[1].

The distance separating atoms in many crystals, on the order of a tenth of a nanometer, falls within the range of x-ray wavelengths. This means that the precise atomic arrangement in crystalline materials can have a pronounced influence on the way x-rays propagate through such solids. The phenomenon is in fact exploited for the investigation of unknown crystal structures through analytical methods such as the widely used x-ray diffraction technique. However, Kohmura and his team examined this concept in reverse to find out whether a well-known and tunable atomic structure can be used to control the propagation of x-rays.

The researchers grew a thin film of germanium—just a few atoms thick—on a silicon substrate, and then exposed the film to a beam of x-rays with a wavelength of 0.08 nanometers. By setting the x-ray incidence angle slightly outside of what is known as the Bragg reflection condition, the team found that the x-ray beam, which usually undergoes negligible refraction when passing through crystalline materials, was shifted or 'translated' from the original path by a few hundred micrometers (Fig. 1).

As the atoms in germanium are normally spaced further apart than those in silicon, producing a thin film of germanium on the silicon substrate resulted in the formation of small, self-assembled islands or 'quantum dots' of germanium. The quantum dots also produced strain in the underlying silicon substrate by pulling the silicon atoms further apart than they would normally like. Kohmura believes that this repetitively strained atomic lattice is largely responsible for enhancing the x-ray translation effect, a phenomenon he refers to as 'x-ray surfing'.

"Strain engineering is rapidly developing in the semiconductor industry as a means of enhancing the electronic properties of silicon," explains Kohmura. "By controlling the atomic arrangement to optimize x-ray translation, we will gain a previously unimaginable component for x-ray optics."

1. Kohmura, Y., Sawada, K., Fukatsu, S. & Ishikawa, T. Controlling the propagation of x-ray waves inside a heteroepitaxial crystal containing quantum dots using Berry's phase. *Physical Review Letters* **110**, 057402 (2013).

Fine-tuning plant growth

Finding the missing genes in a hormone-biosynthesis pathway hints at subtle control of growth in rice

Figure 1: Mutant rice plants without the *CYP714B1* and *CYP714B2* genes (right) show enhanced uppermost node lengths, indicating that these genes are negative regulators of growth.

The plant hormones known as gibberellins (GAs) are growth promoters that are involved in a wide range of processes from seed germination to flower development. The details of the biosynthesis of GAs, however, have yet to be fully clarified. Hiroshi Magome and colleagues of the Gene Discovery Research Group at the RIKEN Center for Sustainable Resource Science have now identified genes for two 'cytochrome P450' enzymes involved in GA biosynthesis in rice[1].

Magome's team found that mutant rice plants without the *CYP714B1* and *CYP714B2* genes had lengthened uppermost internodes (Fig. 1), indicating that these two genes are negative regulators of growth. Overexpression of these genes in *Arabidopsis*, on the other hand, resulted in semi-dwarf plants.

Certain carbon atoms in GA molecules may be linked to either a hydrogen (H) or hydroxyl group (OH). At the 13th carbon, the ratio between these forms is determined by GA 13-oxidase enzymes, which hydroxylate the –H group to an –OH. *CYP714B1* and *CYP714B2* are the first GA 13-oxidase genes to be characterized, and represent a key missing link in the GA-biosynthesis pathway.

In rice mutants without the *CYP714B1* and *CYP714B2* genes, levels of 13-OH GAs were reduced and 13-H GAs were increased, indicating that these two genes have a major role in GA 13-hydroxylation in rice. The researchers also found that exogenous application of bioactive GAs upregulates expression of both genes, representing evidence of a homeostatic mechanism regulating GA levels in rice.

Functional analyses applying recombinant CYP714B1 and CYP714B2 proteins to a variety of GA substrates enabled the team to identify the very step in GA synthesis at which the proteins act: hydroxylation of GA_{12} to form GA_{53}. In flowering plants, GA_{12} is a forerunner of GA_4, a highly bioactive GA, while GA_{53} is a precursor of GA_1, which is only weakly active. Growth repression by CYP714B1 and CYP714B2 is therefore caused by hydroxylation of GA_{12} resulting, downstream, in the production of a less-active GA.

In rice, the weakly active GA_1 is the predominant form of GA in most tissues. Magome suggests that this counterintuitive observation might indicate tight regulation of growth through minute modifications in weakly active GAs, in addition to the dramatic changes facilitated by the more active GA_4. This combined approach could allow plants to fine-tune their growth to suit their circumstances. Moderate suppression of plant growth, as provided by CYP714B1 and CYP714B2, is also associated with improved crop yields. "Our findings show promise for the development of new plant-growth control technologies," says Magome.

1. Magome, H., Nomura, T., Hanada, A., Takeda-Kamiya, N., Ohnishi, T., Shinma, Y., Katsumata, T., Kawaide, H., Kamiya, Y. & Yamaguchi, S. *CYP714B1* and *CYP714B2* encode gibberellin 13-oxidases that reduce gibberellin activity in rice. *Proceedings of the National Academy of Sciences USA* **110**, 1947–1952 (2013).

Reassigning cells to fight infection

Unexpected flexibility in immune cell development could help the body rapidly marshal its defenses at sites vulnerable to infection

Just as a uniform helps distinguish a soldier from a police officer, scientists use proteins that immune cells wear on their surfaces to determine their job in the body. T cells, for example, that display the CD8 protein are classified as 'cytotoxic lymphocytes' (Fig. 1), which kill off cancerous or infected cells, whereas those displaying the CD4 protein are identified as 'helper' T cells that coordinate the immune response.

Immunologists have previously viewed these roles as fixed endpoints in development, but research by Ichiro Taniuchi from the RIKEN Center for Integrative Medical Sciences in collaboration with Hilde Cheroutre of the La Jolla Institute for Allergy and Immunology in California and colleagues now reveals that helper T cells retain opportunities for a 'career change'[1].

Cheroutre had noticed that some helper T cells transplanted into immunodeficient recipients unexpectedly began expressing CD8. "This suggested reprogramming of cells from a helper fate into a cytotoxic lineage," says Taniuchi, whose research group studies ThPOK, a 'master' protein that coordinates helper T cell development and suppresses CD8 production.

Teaming up to examine these enigmatic CD4$^+$ cytotoxic lymphocytes (CTLs), the groups of Taniuchi and Cheroutre developed genetically modified mice with fluorescently labeled ThPOK-expressing cells, corresponding primarily to helper T cells. They also isolated unlabeled CD4-expressing cells from the intestine, and closer examination showed that these CD4-positive, ThPOK-negative cells now expressed CD8 and behaved like CTLs.

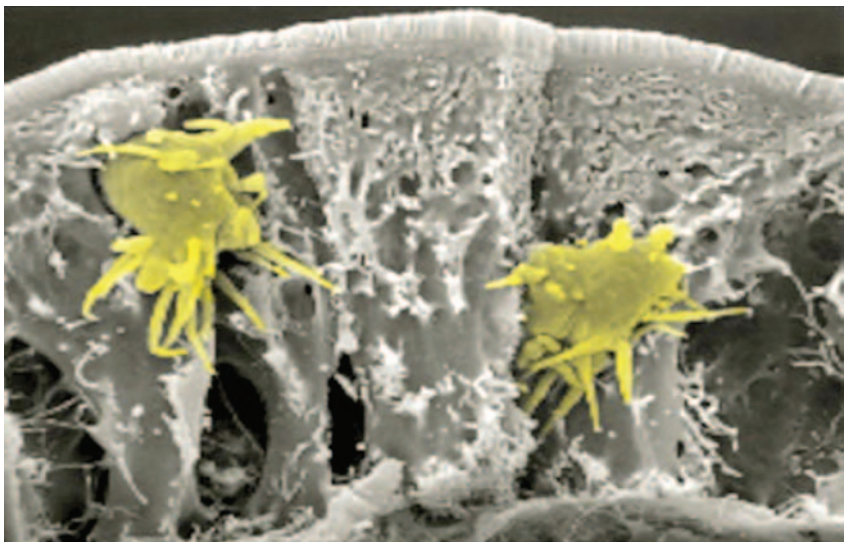


Figure 1: Electron microscope image of T lymphocytes from the intestinal epithelium.

The researchers followed up with a strategy called 'fate mapping', using genetically modified mice whose cells become fluorescently labeled if they express ThPOK at any point—even transiently—in their lifetime. These experiments showed that intestinal CD4$^+$ CTLs begin as ThPOK-expressing helper cells, but switch to a CD8-expressing CTL fate when external factors cause these cells to inactivate ThPOK production via a genetic 'switch' called the 'ThPOK silencer'. "This demonstrates that CD4$^+$ T cells retain unappreciated plasticity for CTL development," says Taniuchi.

This flexibility appears to arise in response to infectious threats, as mice cultivated to be 'germ-free' do not develop CD4$^+$ CTLs. The researchers subsequently determined that helper T cells must be properly exposed both to foreign antigens as well as an immune signaling factor called interleukin-15 to change roles. Although the intestinal wall is a particularly critical point of entry for pathogens, this mechanism may apply more generally as a means of rallying local defenses by retraining helper T cells in a crisis. "I believe other 'barrier sites' in the body may have these cells as well," says Taniuchi.

1. Mucida, D., Husain, M. M., Muroi, S., van Wijk, F., Shinnakasu, R., Naoe, Y., Reis, B. S., Huang, Y., Lambolez, F., Docherty, M. *et al.* Transcriptional reprogramming of mature CD4$^+$ helper T cells generates distinct MHC class II–restricted cytotoxic T lymphocytes. *Nature Immunology* **14**, 281–289 (2013).

Monkey see, monkey do

A new experimental method allows the spontaneous synchronization of arm motions by pairs of Japanese macaques to be observed under controlled conditions

Humans often synchronize their movements when, for example, we cooperate to move a piece of furniture. We also synchronize gestures and facial expressions when we interact. Coordinated actions are in fact surprisingly common in the animal kingdom, as exemplified by the flocking of birds and the schooling of fish. Such behaviors, however, have to date only been observed in the wild. Yasuo Nagasaka and colleagues from the Laboratory for Adaptive Intelligence at the RIKEN Brain Science Institute have now devised the first method for observing coordination under experimental conditions[1].

The researchers individually trained three Japanese macaque monkeys to press two buttons repeatedly and alternately with one hand. They then recorded the monkeys performing this task with a video camera and motion capture device.

Nagasaka and his colleagues later paired the monkeys and had them perform the task again while facing each other (Fig. 1). Initially, each monkey in a pair pressed the buttons at different speeds. However, after a certain amount of time, the two monkeys spontaneously synchronized their button presses by altering the speed of their actions so that their button presses became harmonized with those of their partner.

The speed of repeated button presses differed among the three pairs of monkeys, as did the timing of the synchrony. In one pair, the button presses were synchronized but one monkey was always delayed by 1 millisecond, while in another the delay was 13 milliseconds. In all cases, however, the timing of the actions became closely matched, and the delay seemed to be dependent on exactly which monkeys had been paired together.

The researchers then played back the video recordings of the monkeys performing the task at different speeds while a monkey watched. The monkeys sped up or slowed down their button presses to harmonize their actions with those of the 'virtual' monkey, and they seemed to prefer to slow down their button presses, perhaps to save energy.

In a final set of experiments, the research team allowed the real monkeys to either see or hear the video recordings, and found that visual information is far more important than auditory information for synchronization.

"We believe that this spontaneous synchronization plays an important role in the building of social bonds, and we are now looking for the brain areas responsible," says Nagasaka. "This could be fundamental to understanding the brain itself, and also the social interaction deficits in conditions such as autism."

1. Nagasaka, Y., Chao, Z. C., Hasegawa, N., Notoya, T. & Fujii, N. Spontaneous synchronization of arm motion between Japanese macaques. *Scientific Reports* **3**, 1151 (2013).

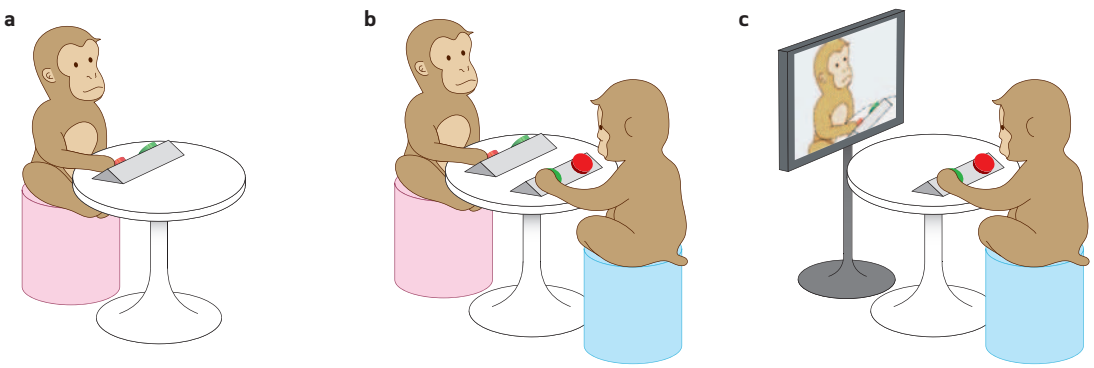


Figure 1: The solo, paired, and video playback conditions of the experiment.

Cell rounding brings development into the fold

The spherical shape that cells take on during cell division promotes the formation of three-dimensional structures in the developing embryo

To form complex, three-dimensional organs during embryonic development, the first flat sheets of cells to develop must undergo a folding procedure known as 'invagination'. Shigeo Hayashi and Takefumi Kondo from the Laboratory for Morphogenetic Signaling at the RIKEN Center for Developmental Biology have now shown that a major driver of this dramatic tissue bending is the spherical shape that cells adopt during cell division[1]. This process, called 'mitotic cell rounding', compresses adjacent cells, forcing layered sheets to cave inwards by a mechanism that is distinct from previously described causes of invagination.

"This mechanism acts by uncovering hidden instability pressure in the tracheal epithelium to trigger sudden morphogenetic change," says Hayashi.

Hayashi and Kondo tracked the formation of the invertebrate trachea—an open respiratory system composed of a series of tubular structures—in developing embryos of the fruit fly *Drosophila melanogaster* using live-cell imaging. They found that invagination in this system takes place in two distinct phases. The first phase occurs slowly over the course of about an hour and relies on signaling via a cell-surface receptor known as epidermal growth factor receptor (EGFR). In this process, EGFR stimulates the contraction of the motor protein myosin, leading to the passive compression of central cells in a thickened region of the epithelium known as the placode.

This slight cellular pinching results in the formation of a shallow pit that gets rapidly pushed inward to form L-shaped tubular structures in the second, faster phase. In this step, the compressed cells enter mitosis and undergo mitotic cell rounding. Using flies with genetic mutations that caused delayed mitotic entry and a drug called colchicine that prevents cell division without blocking cell rounding, Hayashi and Kondo demonstrated that cell rounding, but not cell division, is required to accelerate invagination. The researchers propose that the round shape releases the stored inward pressure associated with the EGFR-triggered myosin contraction, leading to rapid buckling at the cell surface that eventually becomes the inner lining of the tube (Fig. 1).

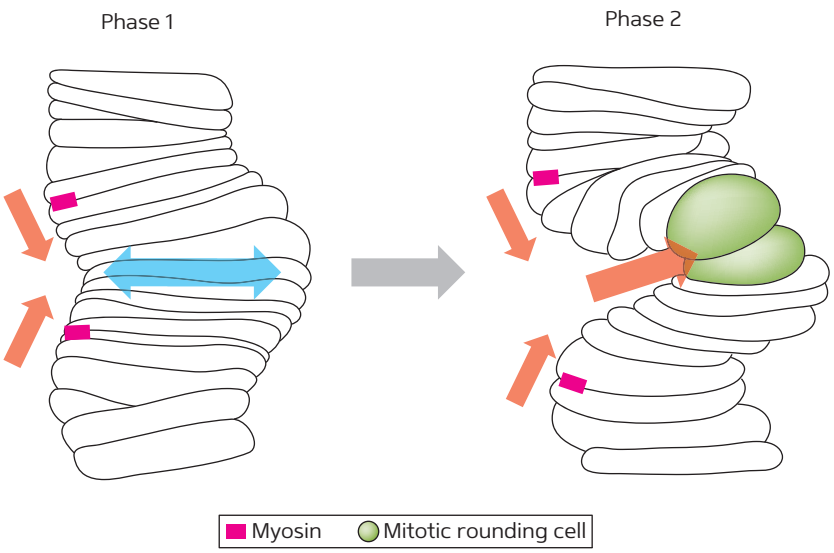


Figure 1: Cell rounding leads to rapid tracheal invagination after the formation of a shallow pit by myosin contraction.

The precise timing and positioning of mitotic cell rounding is crucial to the overall process. If mitosis occurs in cells that are actively undergoing myosin contraction, invagination can be prevented, as several research teams have reported previously. For mitotic cell rounding to achieve proper tissue reorganization, the entire system must be ready with myosin contraction, says Hayashi. "How the mitotic entry is triggered, however, remains a key question to be resolved," he notes.

1. Kondo, T. & Hayashi, S. Mitotic cell rounding accelerates epithelial invagination. *Nature* **494**, 125–129 (2013).

Managing the body's interior architecture

Developing insects regulate growth of respiratory tubules through tightly controlled trafficking of an enzyme that remodels their interior infrastructure

Virtually every multicellular organism requires the means to transport nutrients, water or other materials throughout the body. Tubule networks offer a simple mechanism for achieving this, although careful developmental regulation is required to ensure these tubules are of appropriate width and length to efficiently deliver their cargo.

New research from a team led by Shigeo Hayashi of the Laboratory for Morphogenetic Signaling at the RIKEN Center for Developmental Biology now reveals an important mechanism governing the length of tubules in the tracheal network of flies[1].

Fly respiration depends on proper formation of the system of tubules that comprise the tracheal network. Embryonic tracheal tubules are filled with a matrix of the polymer chitin, which accumulates within the interior, or lumen, of developing tubules (Fig. 1). This matrix is eventually cleared at the end of embryogenesis to make room for air to pass.

It is thought that a putative chitin-modifying enzyme called Serpentine (Serp) acts as a brake to limit tracheal-tube elongation. During the growth process, Serp is taken up from the luminal space into the cells that form the tracheal tubules by a process called endocytosis. However, this is only temporary, and the internalized enzyme is eventually shuttled back to the lumen and reused. Hayashi's team hypothesized that Rab-family proteins might facilitate this 'recycling' process. "These proteins are known to be crucial for membrane trafficking," explains Hayashi, "and we searched for Rab proteins required for tracheal tube morphogenesis."

They learned that embryos lacking Rab9 develop a trachea of excessive length. Examination of Serp localization in these embryos confirmed that this enzyme was being retained by tracheal cells after endocytosis, rather than being shuttled back into the luminal space. Intriguingly, tracheal tubule diameter was not altered in Rab9 mutant embryos, suggesting that diameter and length of tracheal tubules are regulated by separate mechanisms.

Subsequent experiments allowed Hayashi and his colleagues to identify numerous other proteins that collaborate with Rab9 in this process. Importantly, they were also able to observe key steps of the mechanism by which these proteins transfer endocytosed Serp into a 'retrieval' pathway that delivers it back to the lumen.

The team's findings could illuminate similar developmental mechanisms that occur in 'higher' organisms. "Chitin is present only in invertebrates and plants, but in a wider sense, vertebrate tubule organs like blood vessels and the neural tube are all formed through accumulation of luminal matrices," says Hayashi. "It is an open field begging for intense investigation."

1. Dong, B., Kakihara, K., Otani, T., Wada, H. & Hayashi, S. Rab9 and retromer regulate retrograde trafficking of luminal protein required for epithelial tube length control. *Nature Communications* **4**, 1358 (2013).

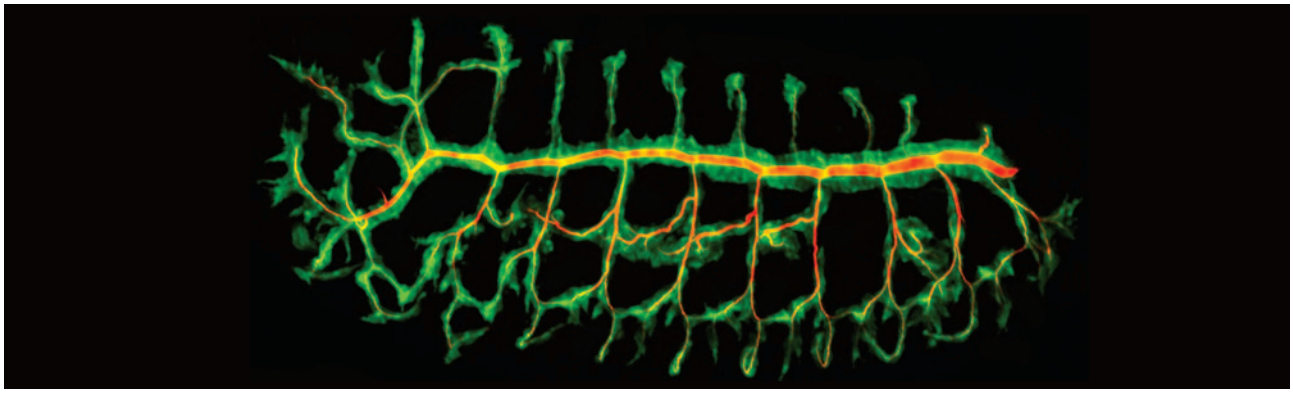


Figure 1: In the fly embryo, chitin polymer (red) accumulates within the luminal spaces of the developing tracheal network (green).

Controlling the cravings

Aberrant frontal circuitry controls smokers' craving for cigarettes

Drug addiction alters the brain's reward system, leading users to place great value in the drugs and seek them out. In ex-addicts, drug-related cues such as the sight of paraphernalia trigger cravings that can lead to relapse. Craving is known to be controlled by the frontal cortex, but exactly how and the areas involved remain unclear. New research led by Takuya Hayashi of the RIKEN Center for Life Science Technologies and Alain Dagher of the Montreal Neurological Institute at McGill University, Canada, has now identified two distinct regions of the frontal cortex that interact to regulate smokers' cravings for cigarettes[1].

The team recruited ten smokers and used functional magnetic resonance imaging (fMRI) to indirectly measure their brain activity while watching film clips of people smoking. On one occasion, the participants were told before the fMRI scan that they could have a cigarette immediately after the scan, and on the other, they were told that they would have to wait four hours before smoking again.

Previously, greater craving-related activity had been shown to occur in the dorsolateral prefrontal cortex (DLPFC) in smokers who were told they could smoke immediately after being scanned. This time, the researchers used a technique called transcranial magnetic stimulation (TMS) to temporarily inactivate the DFPLC of the participants just before their scan.

Craving, as measured by the participants' subjective reports, was stronger when they were told they could smoke immediately. In keeping with the earlier findings, the DLPFC showed greater activity when they were allowed to smoke immediately. However, the researchers also found that craving was represented by activity in another region called the medial orbitofrontal cortex (mOFC), which assigns value to available stimuli and actions.

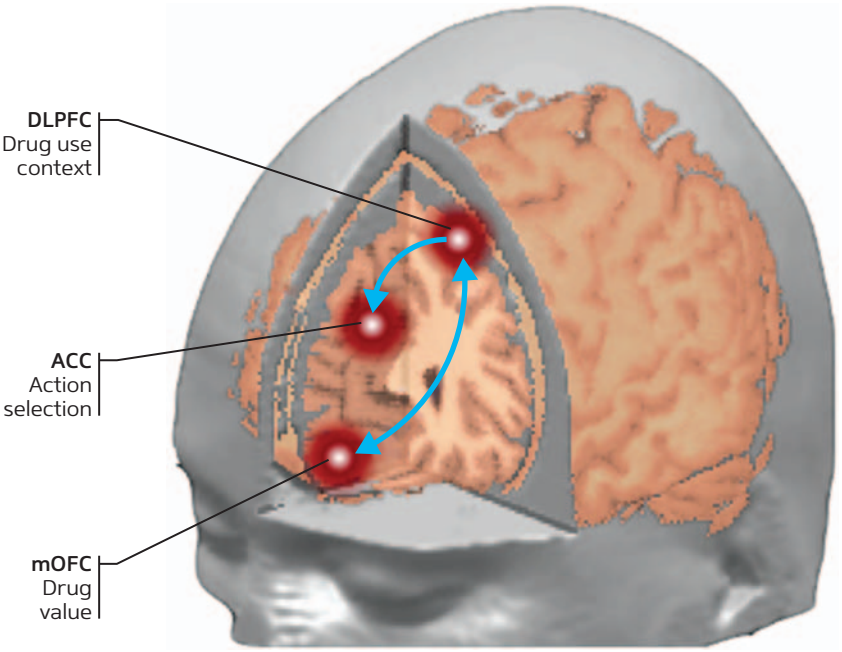


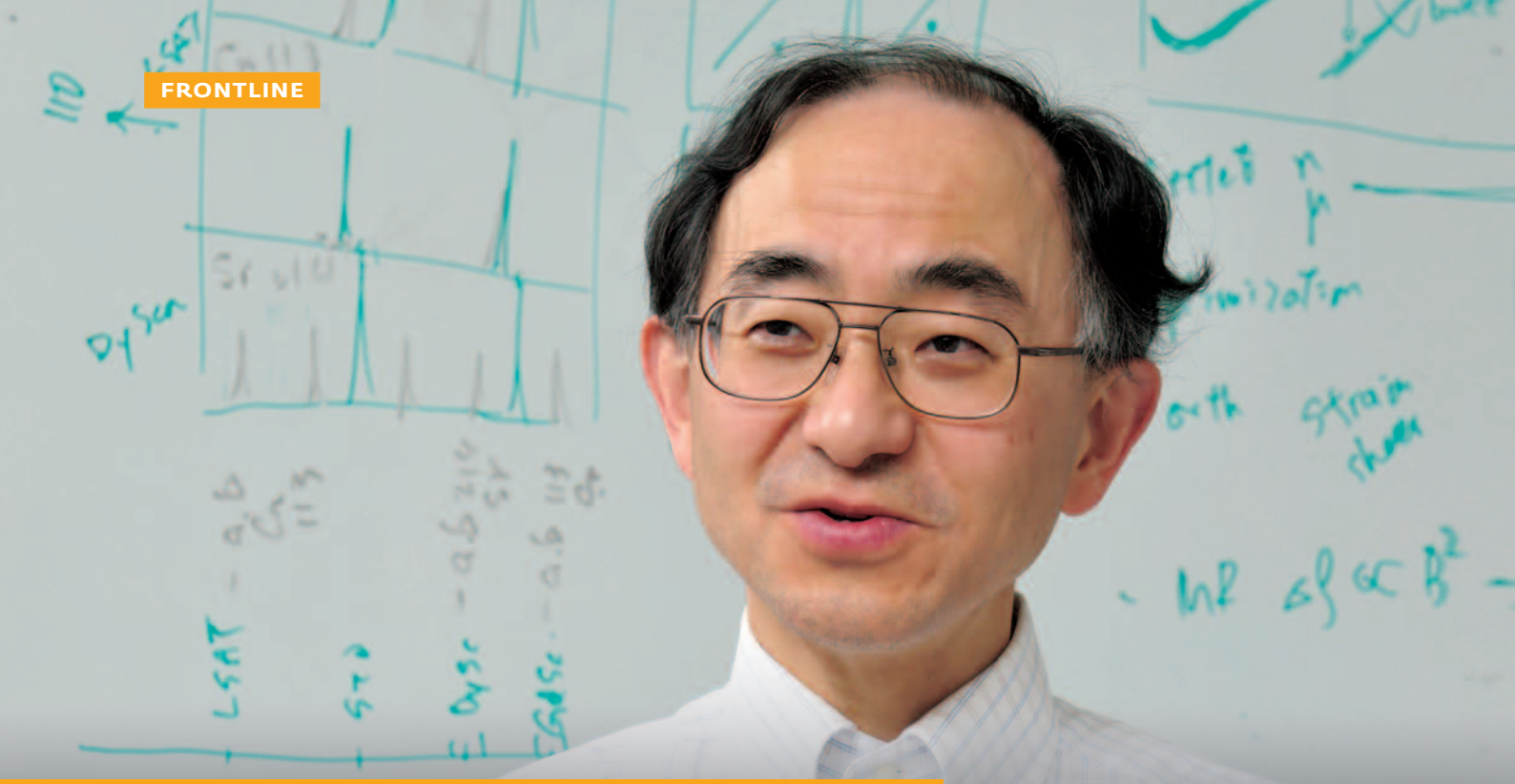
Figure 1: Interactions between the dorsolateral prefrontal cortex (DLPFC) and medial orbitofrontal cortex (mOFC) control cigarette craving.

Inactivation of the DLPFC eliminated the subjective reports of stronger craving when participants had been told they could smoke immediately afterwards, and also attenuated the value-related activity of the mOFC and another region known as the anterior cingulate cortex (ACC)—an area related to transforming value into action (Fig. 1).

The findings suggest that the DLPFC drives decisions about drug seeking based on knowledge of drug availability, and that aberrant frontal circuitry may underlie the pathology of addiction.

"This tells us that addiction is a decision-making problem that may be due to aberrant circuitry in frontal regions," says Hayashi. "Some researchers have been testing the use of TMS of the DLPFC to treat addiction, but the effects are controversial. The study identified the pinpoint frontal location that modulates craving."

1. Hayashi, T., Ko, J. H., Strafella, A. P. & Dagher, A. Dorsolateral prefrontal and orbitofrontal cortex interactions during self-control of cigarette craving. *Proceedings of the National Academy of Sciences USA* **110**, 4422–4427 (2013).



Topological current for energy-saving devices

NAOTO NAGAOSA

Deputy Director
RIKEN Center for Emergent Matter Science

Personal computers soon become hot when in use due to the heat produced when current flows through devices with electrical resistance; its presence indicates that electric power is being wasted. Naoto Nagaosa has discovered that, theoretically, a 'topological current'—a type of electric current that does not generate heat—can flow through normal materials at room temperature. He aims to use topological current as the basis for new devices that require little electricity to work.

Quantum-mechanical phenomena in solids

In 1976, Nagaosa entered the School of Science at the University of Tokyo. "When I joined the university, I studied many subjects including mathematics and chemistry to assess my capability. How one can prove themselves—and in which level of the abstract world—differs from person to person. I felt that pure mathematics was too abstract for me, and that particle physics had also by then become a theoretical world that could hardly be verified by experiment. I therefore decided to focus on the theoretical study of condensed matter physics in which theory is linked to the real world."

In the late twentieth century, condensed matter physics progressed with the help of 'band theory', which has been used to describe the electronic state of a solid. Band theory has assisted the invention of electronic components such as transistors, which are now integrated into a wide range of devices that have revolutionized society and life, including computers.

"In the 1970s, the physical properties of a solid were thought to be fully understood, but in the 1980s two major discoveries were made that could not be explained by conventional theories. One such discovery was the quantum Hall effect," says Nagaosa.

In the quantum Hall effect, when an electron system is subjected to a magnetic field, the transverse conductance of the electrons that move on a two-dimensional surface—such as the interface between a semiconductor and an insulator—is equal to the integer multiple of the value based on a fundamental constant of quantum mechanics.

"I was so surprised to find that a principle of quantum mechanics which describes the microscopic world of atoms and electrons had also been revealed in the macroscopic world of semiconductors, even with their impurities. To explain this, theorists in the field of condensed matter physics introduced topological theory to the Hilbert space." Quantum mechanics dictates that the physical state is represented by a vector in an abstract space known as the Hilbert space. Under a strong magnetic field, for example, the electrons are confined to a part of this space, which is often topologically nontrivial.

Topological theory represents the electronic state of a material as an abstract mathematical representation in the space of a wavefunction—namely the Hilbert space—which is then analyzed by

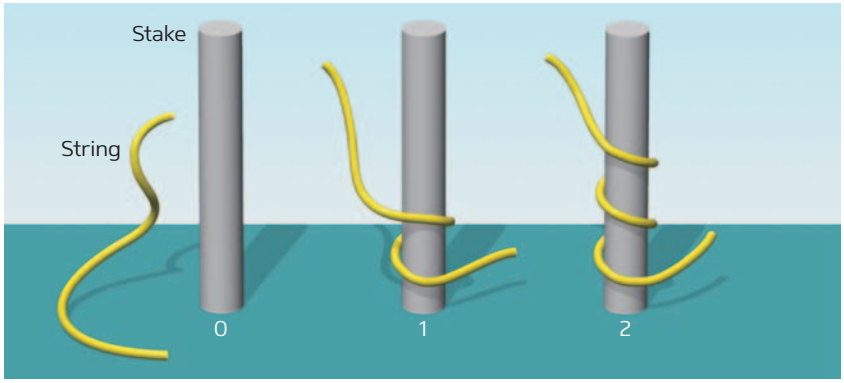


Figure 1: Topological numbers
The number of turns of a string winding around a stake, such as 0, 1, and 2, represents the invariant feature of the figure made by the string. Such numbers are known as topological numbers.

means of topology, a branch of geometry used to examine the invariant features of geometric figures with respect to continuous deformation.

"In the Hilbert space, the electronic state showing the quantum Hall effect corresponds to the geometric figure of a piece of string winding around a stake," explains Nagaosa (Fig. 1). "The number of turns of the string around the stake is equal to an integer such as 0, 1 and 2, not decimal numbers such as 1.1 and 0.9. These integers are called topological numbers. Theorists consider that transverse conductance becomes an integer multiple of the value given by fundamental constants of quantum mechanics because topological numbers are integers." Thus, it was proved for the first time that the very abstract mathematical world of the Hilbert space is actually linked to the real macroscopic world of electrical conductance, says Nagaosa.

"The other major discovery in the 1980s was high-temperature superconductivity," he continues. Conventional superconductivity is a phenomenon whereby a material exhibits no electrical resistance at very low temperatures. But in 1986, copper oxide was found to exhibit superconductivity at a higher temperature than had ever been seen. As superconductivity is a macroscopic manifestation of a quantum-mechanical phenomenon, researchers were puzzled as to how copper oxide—a poor electric conductor—could exhibit this high-temperature superconductivity. Despite its discovery over 25 years ago, the mechanism of superconductivity is still under discussion.

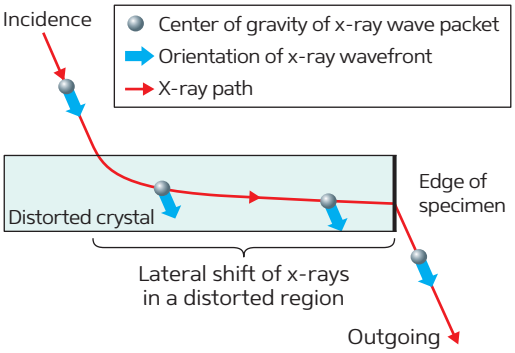
Nagaosa continued theoretical research to understand mechanisms of high-temperature superconductivity in the 1980s and 1990s and found that not only the quantum Hall effect, but also high-temperature superconductivity, could be explained by topology theory in the Hilbert space.

Quantum-mechanical phenomena at room temperature

During the 2000s, Nagaosa made a theoretical discovery which he considers to be the greatest contribution to physics of his research career. "I was probably the first person in the world to notice that quantum-mechanical phenomena can occur not only in special cases such as the quantum Hall effect or high-temperature superconductivity, but also in normal materials at room temperature," he states. "We used to consider that such phenomena could occur only under extreme-heat conditions such as immediately after the Big Bang that created the Universe, or in an extremely low-temperature world. However, thanks to topological theory in the Hilbert space, I discovered that various quantum-mechanical phenomena can occur in materials at room temperature."

This finding led Nagaosa to completely change the direction of his research. "My earlier research had tried to theoretically explain phenomena that were discovered through experiment, such as high-temperature superconductivity. But after my discovery, I changed my research approach and started using topological theory in the Hilbert space to predict phenomena, which could then be tested experimentally by experts." On the basis of topological theory in the Hilbert space, Nagaosa was able to make a number of predictions.

In 2000, Nagaosa and his team suggested that a phenomenon known as the anomalous Hall effect—a Hall effect peculiar to ferromagnets, the mechanism of which had been debated continuously for over a century—can be explained by topological theory in the Hilbert space. They also showed that the principle of the anomalous Hall effect is the same as that of the quantum Hall effect. "In 2003, I extended this concept to spin current and indicated the existence of a topological spin Hall effect for the first time. Then in 2005, I predicted a class of materials called quantum spin Hall insulators."



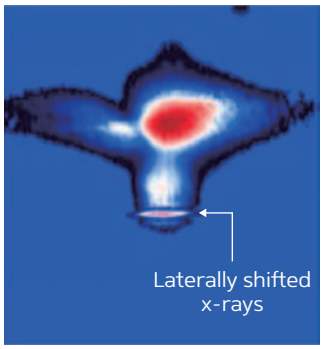


Figure 2: Lateral shift of x-rays in a distorted crystal
A huge lateral shift of an x-ray beam—10^6 times greater than its wavelength—was observed in a distorted crystal through experiments performed at the RIKEN SPring-8 Center. The result verified a prediction made by Nagaosa and his team.

A US theoretician who was working to extend the findings of Nagaosa and his team later predicted the existence of a new type of material known as a 'topological insulator', and that an electron (which has a mass of 1/1836 times that of a proton) flows as a massless particle on the edges or surfaces of the topological insulator—a phenomenon which has now been confirmed by experiment. The discovery of topological insulators attracted much attention as such a material is likely to open up new avenues for research across a range of fields. (See Frontline: "Opening the door to new forms of matter at the Condensed Matter Theory Laboratory," *RIKEN RESEARCH*, December 2010.)

"In the Hilbert space, a topological insulator has the shape of a Mobius strip, which has only one surface. The fact that an electron can flow as a massless particle on the surface of a topological insulator is a phenomenon that can only be explained by topological theory in the Hilbert space," explains Nagaosa.

Interestingly, topological theory is not only applicable to electrons, but also to light. In 2004, Nagaosa and colleagues predicted the occurrence of the Hall effect of light, a lateral shift of light at the time of optical reflection and refraction. Furthermore, in 2006, Nagaosa and the members of his team, together with Kei Sawada, a graduate student in Nagaosa's laboratory at the University of Tokyo, predicted that a beam of x-rays which was expected to travel in a straight line would be subject to a significant lateral shift in a distorted crystal. In 2010, Sawada (who had since joined the RIKEN SPring-8 Center) observed—using the SPring-8 large-scale synchrotron—that a beam of x-rays experienced a lateral shift of as much as 10^6 times greater than the wavelength of the x-ray, thus successfully verifying the team's prediction (Fig. 2).

Developing energy-saving devices

At RIKEN, Nagaosa formed two research groups: the Theoretical Design Team in 2007, and the Strong-Correlation Theoretical Research Team in 2010. "Ninety-nine percent of physical properties are caused by the interaction of a solid with an electromagnetic field such as an electric or magnetic field, visible light or x-rays," notes Nagaosa. "In the past, theorists devised condensed matter theories to describe the interaction between the electron system in a solid and an external electromagnetic field. However, topological theory in the Hilbert space considers that the electron system in a solid produces an 'emergent electromagnetic field,' which interacts with an external electromagnetic field. This is a new view on matter." It is from these principles that Nagaosa aims to develop novel devices by designing an emergent electromagnetic field created by the electron system of a solid.

As part of this goal, Nagaosa and his team are studying Joule heating—also known as Ohmic or resistive heating—a process where heat is produced when an electric current passes through a conductor. "In close collaboration with experimental experts, we aim to reduce power loss occurring as a result of current flowing through devices with electrical resistance—a process that causes electrical devices to become hot—and thereby reduce electric power consumption."

Nagaosa expands upon the process by which Joule heat is generated. "The general current that encounters resistance in accordance with Ohm's Law is called Ohmic current. Quantum mechanics states that an electron has both a particle and wave nature. An electron in Ohmic current strongly displays the properties of a particle, which can be compared to a moving pinball. Like a pinball, an electron moves in a solid and encounters electrical resistance as it collides with impurities, thus producing heat" (Fig. 3, part A).

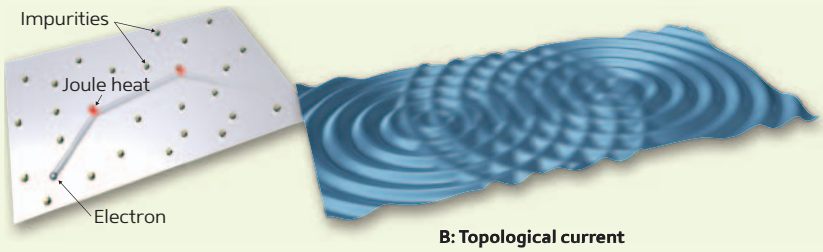
Interestingly, Joule heat is not produced in superconductors because they have no electrical resistance. However, at present, room-temperature superconductors do not exist. Thus, for practical applications, superconductors must still be cooled to ultra-low temperatures, which requires energy.

Nagaosa believes that where current is required to flow, the generation of such heat, or the need to employ cooled superconductors, can be avoided. "Thanks to research into topological theory in the Hilbert space, I am convinced that 'topological current' phenomena occur widely in normal materials at room temperature."

Nagaosa points out that there are three types of electric current that flow in a solid: Ohmic current, superconducting current and topological current. He describes topological current—which essentially does not produce Joule heating—through the following analogy.

"Imagine throwing two stones into a pond. As the two wave patterns expand, the amplitude increases where the amplitudes of the two individual waves are combined, or decreases where the amplitudes of the two waves are cancelled, thus causing interference between the patterns," he says. "Electrons are located in the crest positions where the amplitude of the waves is increased as a result of interference. Electrons are moved when

Figure 3: Schematic representation of Ohmic current and topological current



Illustrated by Nariyuki Toshihara. © 2013 RIKEN

A: Ohmic current
Ohmic current is a flow of electrons that move as particles, rather like pinballs. The electrons collide with impurities in the solid, generating Joule heat.

B: Topological current
Electrons in a topological current are located in the crest positions where the amplitude of waves is increased as a result of interference. Electrons appear to move with a change in interference patterns and travel as waves without generating Joule heat.

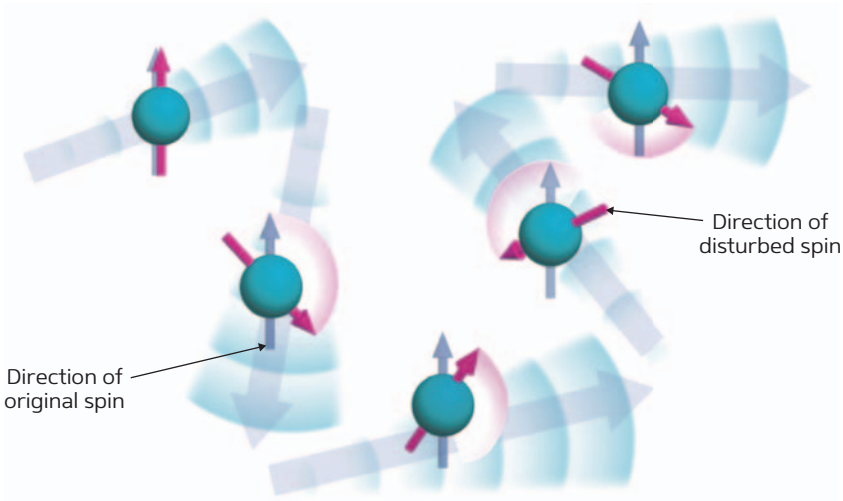


Figure 4: Information on spin direction is preserved in the Hilbert space

When electrons of the same spin direction (blue arrow) are released in a solid, the spin direction of each electron is disturbed (red arrow), resulting in loss of information. Nagaosa and his team discovered that, in theory, information relating to the original spin direction is preserved in the Hilbert space despite appearing to be lost, and can therefore subsequently be retrieved.

the interference pattern changes. That is an image of a topological current (Fig. 3, part B). No Joule heat is produced because electrons move as waves. We can cause a topological current by changing the unique electromagnetic interference pattern produced by the electron system of a solid through exerting a small amount of external force."

Utilizing spin current

Conventional experiments have demonstrated that a topological current can flow through normal materials at room temperature.

"However, the flow of a topological current often involves that of an Ohmic current, producing Joule heat that wastes electric power," explains Nagaosa. "Topological insulators are materials that can minimize the flow of Ohmic current, allowing only the topological current to flow. However, since topological insulators are created from special materials, we plan to exploit a more universal phenomenon of materials such as spin current."

Spin can be explained by looking at electrons, which not only have an electric charge, but also a certain amount of angular momentum—or spin—similar to that of the Earth. There are two spin directions: 'up', which corresponds to the Earth rotating toward the right, and 'down', which corresponds to the Earth rotating to the left.

Conventional electronic engineering focuses on electric charge. For example, information technology is based on the existence, or lack, of an electric current as denoted by the presence or absence of a '1' or '0', respectively. However, in addition to electric charge, researchers are actively studying 'spintronics' where spin direction is used to encode information. High-density memory devices that record information using spin direction, even if power to the device is turned off, have been put to practical use. Such developments are expected to lead to the generation of high-performance, energy-saving devices.

Spin current itself is a difficult concept to explain, but, if electric current is described as the flow of electric charges, then spin current is the flow of spins. And, as for electric current, there are also three types of spin current. Nagaosa's team is planning to apply a spin-current-induced topological current to an electrical insulator, which will then be used to develop new devices.

If spin directions are to be used to store information, they need to be preserved. Fortunately, Nagaosa made a discovery which led to a breakthrough in the development of spintronics. "When an electron moves in a solid, its spin direction is often disturbed, resulting in the loss of information, which is a major problem in spintronics," he explains. "However, we theoretically discovered that information about the original spin direction is preserved in the Hilbert space even though the information appears to be lost—and this information can actually be retrieved" (Fig. 4).

Linking the Hilbert space to the real world

"As a high school student, I was impressed by Einstein and studied his theory of relativity in my own time. I was amazed to learn that the abstract mathematical world in which the theory of relativity is described can be linked to actual physical phenomena," says Nagaosa.

Nagaosa's own work is now fashioning new 'bridges'. As a result of the ideas created by Nagaosa and his team, which—in a similar way to Einstein's theories—are able to link the abstract mathematical world to the real world, new devices are likely to be invented. With their power-saving potential, such devices will undoubtedly contribute to the creation of a sustainable society.

ABOUT THE RESEARCHER

Naoto Nagaosa was born in Hyogo, Japan, in 1958. He graduated from the University of Tokyo in 1980, and obtained his PhD in 1986 from the same institution. Nagaosa then held a postdoctoral position at the Massachusetts Institute of Technology in the United States, before returning to Japan where he was appointed lecturer at the University of Tokyo, and later promoted to professor in 1998. In 2007, he became a team leader at the RIKEN Advanced Science Institute and he is now deputy director of the RIKEN Center for Emergent Matter Science. His main research interests are the theories of strongly correlated electronic systems, spintronics, and the gauge theories of topological aspects of electrons in solids.

Understanding the Universe

Jenny Lee

Researcher
Radioactive Isotope Physics Laboratory
RIKEN Nishina Center for Accelerator-Based Science

What led you join RIKEN?

When I was a graduate student at the National Superconducting Cyclotron Laboratory in the United States, my thesis advisor encouraged me to pursue research at RIKEN, describing its nuclear physics facilities as some of the most advanced in the world. I seized the opportunity to work here and when I arrived, I found that the facilities exceeded my expectations.

Please tell us about the aims of your center.

I am studying nuclear structure using the RIKEN Nishina Center for Accelerator-Based Science's radioactive-isotope beam facility, the Radioactive Isotope Beam Factory. The goal of our research is to try to understand the origins of the Universe—to unlock the secrets of how the Universe, and everything in it, is put together, beginning at the level of the nucleus.

Please tell us more about your work at RIKEN.

We mainly study exotic nuclei using the radioactive-isotope beams. The nucleus is composed of protons and neutrons, and to understand how they are put together, we disturb them by means of nuclear reactions and detect the reaction products using our detectors.

Nuclei can exist in certain configurations, and it turns out that adding just a single neutron or proton, for example, can cause the nucleus to take on a completely different shape with very different properties. By studying the structure of a particular nucleus, we can use the information to investigate nucleosynthesis—how the elements of the Universe were created.

What are your goals while at RIKEN?

As a junior scientist, I am still digesting the bigger picture so that I can create my own theories and research agenda. In the future, I would love to help establish nuclear physics as a field of research in Hong Kong, where I come from. There is no infrastructure there yet, so someday I hope to return to share the knowledge and experience that I have gained at RIKEN. I think it would be challenging, but meaningful, to start something new.

What has surprised you about working at RIKEN?

When I was an undergraduate student in Hong Kong and then studying for my PhD in the United States, people tended to go home right after work. But at the Nishina Center, colleagues often get together after work to socialize and talk about things, and RIKEN holds a number of parties and festivals on its campuses. It is part of an interesting working philosophy.

What advice would you give to other young scientists considering working at RIKEN?

RIKEN has a very open environment, where even young scientists are encouraged to discuss ideas and hypotheses. In the United States, there is a tendency for the number of papers to be very important—everything is about results—whereas here people encourage discussion on 'real physics' and concentrate on how they can perform elegant science. Of course, publishing papers is still important, but there is more of a focus on thinking and more of an emphasis on interaction.

CONTACT INFORMATION

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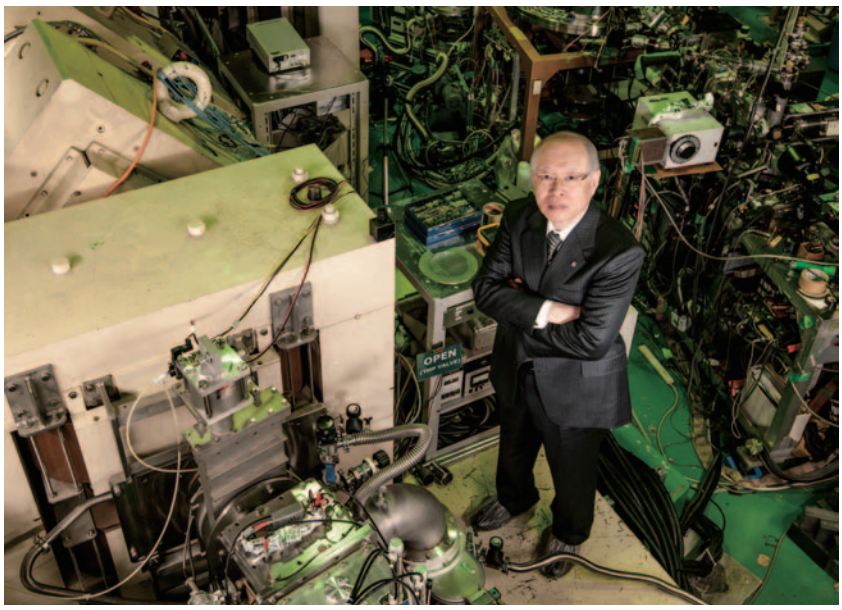
E-mail: pr@riken.jp

RIKEN enters new phase

RIKEN's new five-year term, which began in April of this year, marks the start of a fresh phase for the research institution. Over the next five years, RIKEN's activities will be carried out in accordance with the Japanese government's fourth Science and Technology Basic Plan, which fosters innovative research that expedites reconstruction efforts in the aftermath of 2011's Great East Japan Earthquake. While basic research remains one of RIKEN's primary missions, the focus will shift to uncovering solutions for critical scientific, technical and societal issues, as well as green and life sciences innovation. To meet these newly defined objectives, RIKEN has taken the visionary step of inaugurating five cutting-edge research centers.

Three of the new centers are dedicated to research that will contribute to a more sustainable future. The Center for Emergent Matter Science brings together RIKEN's strengths in materials science to create more energy-efficient materials and devices, while researchers at the Center for Advanced Photonics are developing new technologies based on advances in photonics. The Center for Sustainable Resource Science will concentrate on research into three key areas—carbon, nitrogen and metals—as part of efforts to decrease societal impact on the environment.

Two other centers have been established to contribute to RIKEN's work in the life sciences with a view to strengthening its efforts and advances in health and medicine. The Center for Integrative Medical Sciences is set to focus on medical research for the development of personalized medicine, while the Center for Life Science Technologies aims to integrate RIKEN's life sciences technology research to foster innovation in drug development, medical care and healthcare science. Additionally, the Preventive Medicine & Diagnosis Innovation Program has been created to promote collaborations within RIKEN and also with universities, research institutes and hospitals both in Japan and overseas.



Ryoji Noyori, president of RIKEN, addressed the importance of the recent changes at RIKEN, noting that they would allow the organization to "be an integral part of society."

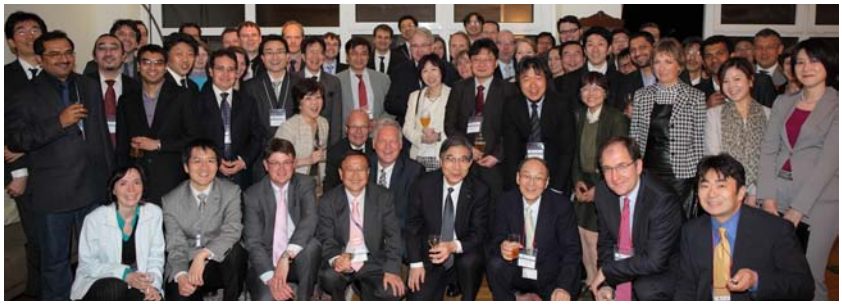
To encourage cooperation between centers under this mission-based structure, new independent laboratories will be created, headed by chief scientists who are encouraged to perform interdisciplinary work and pioneer innovative areas of research. In addition, the Global Research Cluster has been established to strengthen RIKEN's national and international research partnerships. Furthermore, to coincide with the new organizational structure, RIKEN has refreshed its website, www.riken.jp, enabling improved communication of RIKEN's research activities to a global and broad range of audiences.

In a statement to mark the start of RIKEN's third five-year term, Ryoji Noyori, president of RIKEN, aptly summed up the importance of the latest developments: "As Charles Darwin pointed out, it is not the strongest who survive, but rather those who evolve and adapt. I see RIKEN as a living, breathing organism. As such, we must keep up with the changing times to be an integral part of society."

Second RIKEN–Max Planck joint symposium

The second symposium of the RIKEN–Max Planck Joint Research Center was held on 15–17 April 2013 at RIKEN's campus in Wako. The symposium aimed to promote collaborative research in systems chemical biology. RIKEN President Ryoji Noyori and Minister Lutz H. Görgens of the German embassy in Tokyo made the opening remarks. Both leaders reflected on the history of mutual cooperation between the two countries and institutions and expressed their confidence in the creation of even greater partnerships in the future.

On the first evening, RIKEN Executive Directors Maki Kawai and Kenji Oeda were invited, together with Director Kohei Tamao of the RIKEN Global Research Cluster and along with 60 symposium participants, to the official residence of Minister Stefan Herzberg of the German embassy. The group was given an overview of research activities at the RIKEN–Max Planck Joint Research Center and attended a social event.



The second symposium of the RIKEN–Max Planck Joint Research Center brought together scientists from Germany and Japan.

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