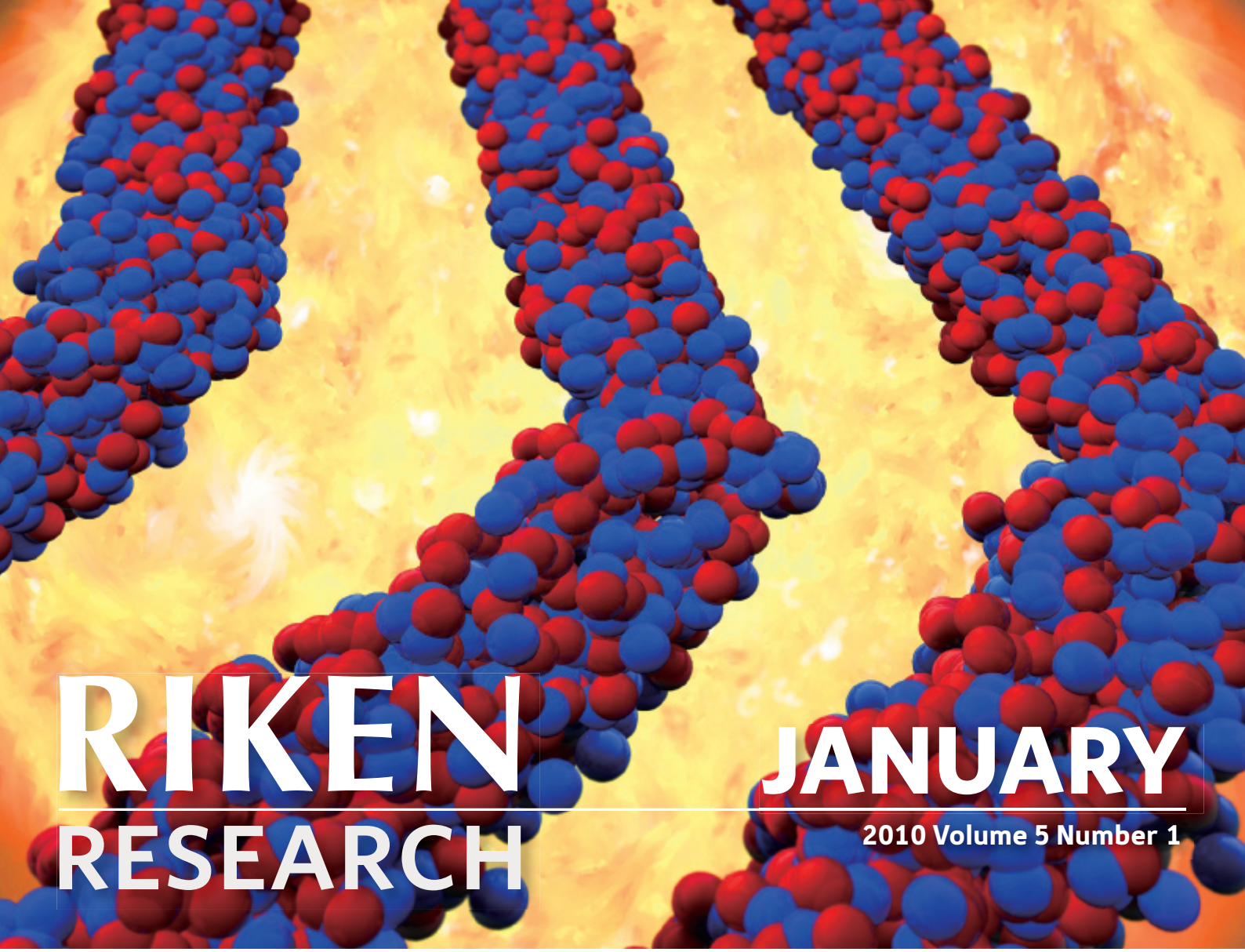

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Physics at the core of supernovae

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Sights set on immunization target

Revelation of key elements of how the digestive system defends the body broadens the scope for oral vaccines

A RIKEN-led research team has unraveled the molecular details of a key mechanism of the immune system in the gut. The work opens the way to new possibilities for developing versatile, inexpensive vaccines that are swallowed, rather than injected.

"The description of this molecular pathway fills a gap in our understanding of the immune response of the digestive system," says team leader Hiroshi Ohno. "And it provides molecular targets for compounds taken orally, and therefore offers the hope of an easy-to-administer, cost-effective weapon against infectious diseases and allergies."

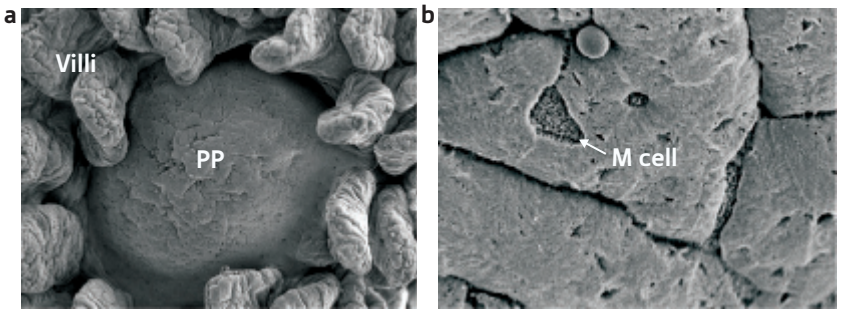

Figure 1: Two scanning electron micrographs of a murine Peyer's patch (PP). In the image of higher magnification (right), an M cell is visible.

Intestinal armory

The mouth is the most significant entry point into the body for pathogens, allergens and poisons. Most of what comes into the body through the mouth moves through the digestive system. As a result, the lining of the gut houses the largest part of the entire immune system. It protects the body from disease organisms and foreign particles by secreting vast amounts of antibodies in the form of Immunoglobulin A.

Antibodies combine with, neutralize and mark out their targets, known as antigens, for future attack by other parts of the immune system. In order to form antibodies targeting specific foreign particles or organisms in the digestive system, the antigens are presented to immature dendritic cells in Peyer's patches—organized bodies of immune system tissue found beneath the epithelial cells that line the gut. It has long been suspected that specialized microfold or M cells (Fig. 1) in the epithelium are involved with the process of moving such antigens from the gut cavity to the immune system cells underneath. Until now, however, the molecular details of the process have remained a mystery.

Investigative artillery

Using the latest techniques of micro-dissection, microarray analysis, staining, microscopy and molecular genetics to investigate, Ohno and colleagues from the RIKEN Research Center for Allergy and Immunology, Yokohama, collaborated with biologists from Yokohama City University, several other Japanese universities and Stanford University in the US.

They uncovered a particular receptor molecule on M cells that stimulates the immune response by binding to a protein on the hair-like projections or pili of bacteria such as *Escherichia coli* and *Salmonella*[1]. The epithelial M cells engulf foreign bodies in the gut cavity, surrounding them with the cell membrane. These membrane-sealed packages or vesicles are then passed through the body of the M cell to waiting immune system cells in a pocket on the underside (Fig. 2).

The researchers speculated that the M cells may have specific molecular receptors to bind to antigen targets. So, working in mice, Ohno and colleagues used the analytical technique known as microarray technology to scan the genome for receptor molecules specific to M cells. Glycoprotein 2 (GP2) was one such molecule and the microarray analysis showed it was highly associated with epithelium close to Peyer's patches. When they stained both GP2 and M cells, they discovered that not only was GP2 restricted to M cells on Peyer's patches in the gut, but that it was also found on M cells associated with immune system tissues in other parts of the body in humans as well as mice. In fact, GP2 is a universal marker for M cells.

Moving in closer, the researchers employed electron microscopy to determine where GP2 was distributed on the M cells. They found it was localized on the membrane facing the gut cavity. By using an antibody that binds only to GP2, they discovered that some was incorporated into vesicular structures in the body of the M cells. This provided further evidence that the receptors were involved in the mechanism for transporting antigens to the immune system cells beneath.

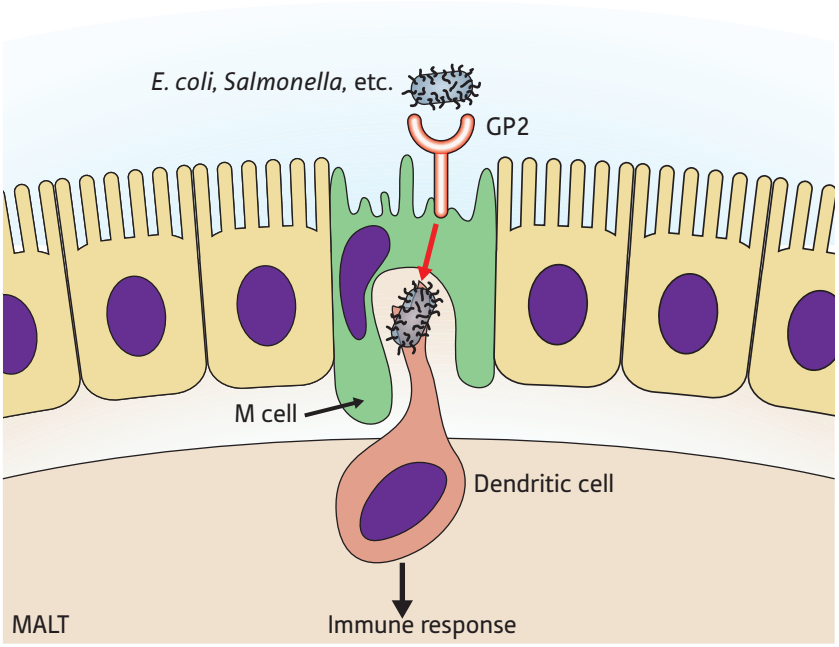

Figure 2: A schematic diagram depicting the role of GP2 in the immune response of the gut.

Profiling glycoprotein 2

The next step was to determine what compound or compounds linked to the GP2 receptor. By assessing the structure of GP2, the researchers discovered that it resembles a protein in the kidney that binds to pathogenic *E. coli* bacteria. Thus, they reasoned that GP2 might also be associated with bacteria in the gut.

Ohno and colleagues then experimented by mixing GP2 with *E. coli,* and found that it bound to the protein FimH on the bacterial pili. In fact, GP2 binds only to bacteria that carry FimH, and not to bacteria that lack this protein, such as *Pseudomonas* and *Listeria.*

Using an intact mouse intestine, the researchers tracked *E. coli* expressing green fluorescent protein. GP2 accumulated around the bacteria, which then could be followed moving inside the M cells. More than 90% of the bacteria transported through the M cells are captured by dendritic cells. They also showed that this process was severely hampered in *E. coli* lacking FimH or mice lacking GP2.

Fortified by FimH

Finally, the research team tested the ability of bacteria with and without FimH to induce the immune response in mice. They used a particular type of *Salmonella* modified to carry a fragment of inactivated toxin used as an antigen in tetanus vaccines. Those bacteria carrying FimH stimulated a much stronger immune response than those without, which the team showed was not the result of any inherent deficiency in the mouse immune system.

"It is reasonable to assume there are other molecules on the M-cell surface responsible for binding and uptake of bacteria lacking FimH," Ohno says. "This is one of the projects we are now working on. Another is to screen for small compounds that bind tightly to GP2. We can then use these molecules to target to GP2 on M cells in an effort to develop efficient oral vaccines." ■

1. Hase, K., Kawano, K., Nochi, T., Pontes, G.S., Fukuda, S., Ebisawa, M., Kadokura, K., Tobe, T., Fujimura, Y., Kawano, S., *et al.* Uptake through glycoprotein 2 of FimH$^+$ bacteria by M cells initiates mucosal immune response. *Nature* **462,** 226–232 (2009).

About the researcher

Hiroshi Ohno was born in Tokyo, Japan, in 1958. He graduated from the School of Medicine, Chiba University, in 1983, and obtained his PhD in 1991 from the same university. He then became an assistant professor of the School of Medicine, and was promoted to associate professor in 1997. He spent three years from 1994 to 1997 as a visiting scientist at the National Institute of Child Health and Human Development, National Institutes of Health in the USA. In 1999, he became a full professor at the Cancer Research Institute of Kanazawa University. He joined the RIKEN Research Center for Allergy and Immunology as team leader in 2002, where his research focuses on mucosal immunology, and in particular the role of epithelial cells in the development of the mucosal immune system, mucosal antigen uptake and initiation of mucosal immune responses.

Emulation for understanding

Controllable quantum systems that allow us to better understand complex physical processes are now within reach

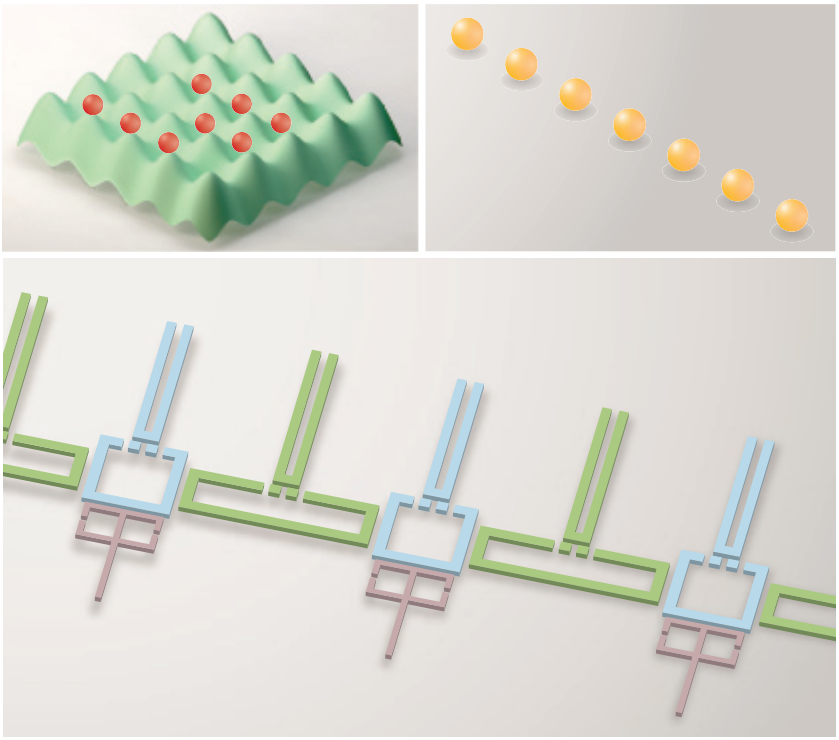

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Figure 1: Schematic diagrams of three types of quantum simulators: atoms (red) held in place by an optical field (green; top left); ions (yellow) aligned using an electromagnetic field (top right); and superconducting circuits (bottom).

Physical processes affect almost every aspect of our lives, yet physicists still grapple with understanding and modeling the behavior of many such processes—particularly complex quantum physical processes, including certain superconducting effects. To circumvent the limitations of conventional computers in tackling these problems, physicists have proposed using well-understood quantum systems called 'quantum simulators' (or 'quantum emulators') to emulate similar, but otherwise poorly understood, quantum systems. In a review of the different approaches taken in developing these simulators, Iulia Buluta and Franco Nori from the RIKEN Advanced Science Institute, Wako (and the University of Michigan, USA), have concluded that the first practical applications may soon be a reality[1].

"Quantum emulators could be employed in fields such as atomic physics or condensed-matter physics," explains Nori. However, he says, the detailed study of known physical processes is just one advantage: these controllable quantum emulators would also allow the exploration of novel physical processes that are typically hard to study.

Among the various physical systems that could be used to build a quantum simulator, one possibility is the use of regular arrays of atoms or ions that are held in place by laser fields. According to Buluta and Nori, the interactions between these atoms provide a good model for emulating the interaction between other particles in complex systems. To model electrical conductivity, for example, this type of quantum simulator can be used to study the transition from the insulating state to the conducting state, where the atoms switch from being fixed to being free to move.

Buluta and Nori also point out that electronic devices fabricated on a computer chip could be used as a controllable quantum system. In this system, small circuits made from superconducting wires possess quantum physical properties that could be used to model atomic physics problems.

These quantum systems have been demonstrated experimentally (Fig. 1); however, challenges remain until more advanced and versatile quantum simulators can be built. Synchronizing the operation of a large number of components, for example, has not yet been achieved, Buluta notes. From a theoretical viewpoint, she says that much also needs to be learned about meaningfully programming quantum simulators.

Nevertheless, Nori believes that, in contrast to the situation 25 years ago when Richard Feynman first proposed quantum simulators, the experimental demonstrations of the basic components for quantum computers completed to date suggest an optimistic outlook. "The necessary level of control of quantum systems is now within reach," he says. ■

1. Buluta, I. & Nori. F. Quantum simulators. *Science* **326,** 108–111 (2009).

Supernovae host a pasta dinner

The nuclei in the core of a collapsing supernova can form a range of unusual 'pasta-like' structures

The dense matter at the interior of a collapsing star—or 'supernova'—is unlike anything that can be replicated in a laboratory. Scientists therefore rely on simulations to predict the behavior of electrons, protons and neutrons in these stellar explosions.

Now, scientists at the RIKEN Nishina Center for Accelerator-Based Science in Wako, and several other institutions in Japan, have shown that the proton- and neutron-containing nuclei at the core of supernovae are likely to form a range of unusual shapes[1]. These structures, called 'pasta phases' because of their similarity to strands of spaghetti or flat slabs of lasagna, are different from the mostly spherical nuclei found at the center of atoms and are likely to affect the dynamics of supernova explosions.

Predictions that pasta phases form in supernovae are not new, but the earlier work was based on models that assumed the nuclear structures did not change through time. In an actual collapsing supernova, however, the core density is not static. Gentaro Watanabe, a lead author of the paper, and his colleagues therefore performed new simulations to look for pasta phases in a supernova. They used a method called 'quantum molecular dynamics' that takes into account the realistic time evolution of nuclei at the supernova core.

The researchers started by assuming that large spherical nuclei are distributed periodically in a lattice such that the total density is about 15% that of normal nuclear matter. They then simulated what happens to the nuclei as the lattice is compressed. They found that the spherical nuclei merge into zigzag shapes and ultimately into columns (Fig. 1), confirming that pasta phases should exist in supernovae.

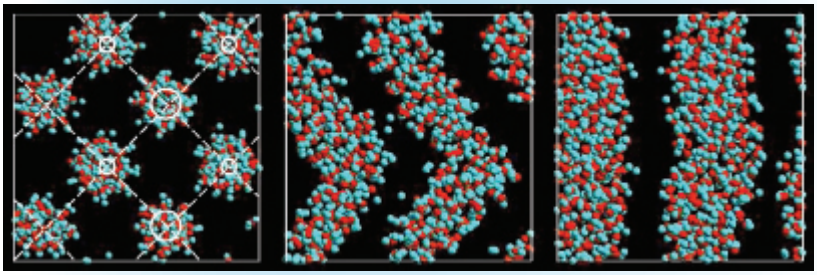


Figure 1: Simulations showing the transformation of spherical nuclei in a lattice (left) into zigzag shapes (center) and columns (right) when pressure is applied to the lattice. Such shape transitions may occur at the core of a supernova (protons, red; neutrons, blue).

Scientists had long believed that what caused spherical nuclei to deform into longer rod-like shapes was a so-called fission instability that forced the nuclei to break apart. "The actual formation process of the pasta phases is very different from this generally accepted scenario," says Watanabe. He and his team have shown that the attraction between neighboring nuclei is what drives the shape changes.

In addition to advancing the understanding of nuclear structures, the results will also be important for astrophysics. "Supernova explosions are very complicated phenomena, and we do not know exactly how pasta phases change the dynamics of supernova explosions," explains Watanabe. "In the future, we would like to simulate the collapse of the supernova core, taking into account the effect of the pasta phases." ■

1. Watanabe, G., Sonoda, H., Maruyama, T., Sato, K., Yasuoka, K. & Ebisuzaki, T. Formation of nuclear "pasta" in supernovae. *Physical Review Letters* **103,** 121101 (2009).

Sensitive hybrid

Combining design concepts produces a high-sensitivity detector that could enable greater exploitation of terahertz radiation

Terahertz (THz) radiation has unique characteristics with the potential to provide the basis of new imaging techniques for detecting and diagnosing cancer and other medical conditions. Moreover, because the energy of the photons that carry THz radiation is around 100,000 times less than that of x-rays, it is expected to be much safer.

Unfortunately, the efficiency of devices built to detect THz radiation has been poor. This is because the frequency of THz radiation is too high to be handled by microwave electronic circuits, and its energy is too low for conventional optoelectronic devices. By combining several advanced device concepts, Yukio Kawano and colleagues of the RIKEN Advanced Science Institute, Wako, have succeeded in building a highly sensitive THz detector (Fig. 1) that is capable of sensing just a handful of THz photons at a time[1].

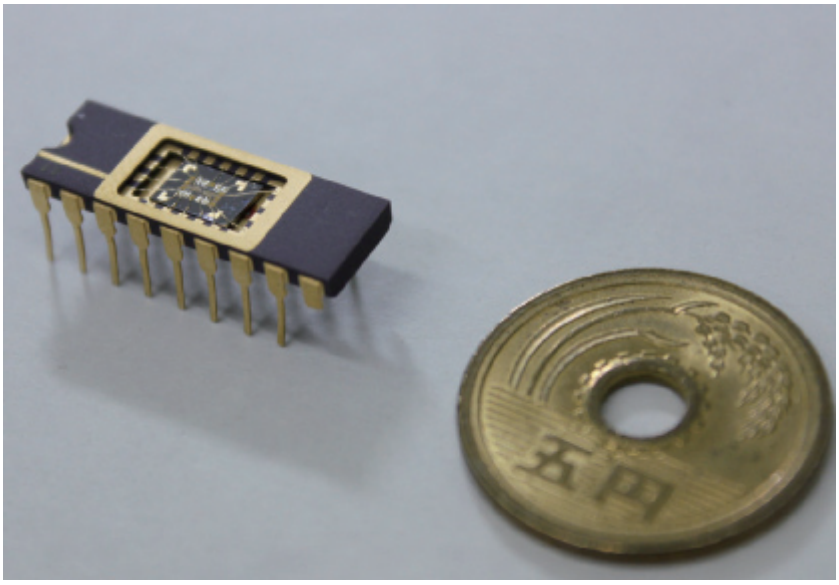


Figure 1: A photograph of the chip in which the high-sensitivity detector is mounted.

Practical sensing devices must perform two key tasks: absorb the radiation they are trying to detect, and then react to this absorption in some measurable way. These tasks are usually performed by a single component of a device. Kawano and colleagues' device, however, performs them with two different components.

One component, a structure known as a two-dimensional electron gas (2DEG), absorbs THz radiation. A 2DEG is a thin layer of electrons that are confined between two different semiconducting materials—in this case, GaAs and AlGaAs—but are free to move within the layer. A 2DEG is highly conducting and therefore highly absorbing of THz radiation.

On top of the 2DEG, the researchers built a carbon nanotube single-electron transistor—a device that behaves like a switch with electrical characteristics controlled by the presence or absence of individual electrons in its channel. They found that when the 2DEG beneath this transistor absorbed a burst of THz radiation, it shifted the voltage at which the transistor switched. At the lowest levels of radiation, they also observed intermittent switching behavior known as telegraph noise, suggesting that only a few photons were needed to induce switching.

As well as achieving unprecedented detection sensitivity, the device operates at temperatures much higher than most previously demonstrated detectors. This means it needs much simpler refrigeration techniques, making it cheaper and easier to use in practical applications.

"Our detector, having the ultimate sensitivity, could be used as a powerful tool in fields such as nanomaterials, bioscience and astronomy where important information is expected to be concealed in the THz region," says Kawano. ■

1. Kawano, Y., Uchida, T. & Ishibashi, K. Terahertz sensing with a carbon nanotube/two-dimensional electron gas hybrid transistor. *Applied Physics Letters* **95,** 083123 (2009).

Seeing the sense in it all

Structural details of an environment-sensing protein complex could guide development of new drugs to direct plant growth or combat bacterial infection

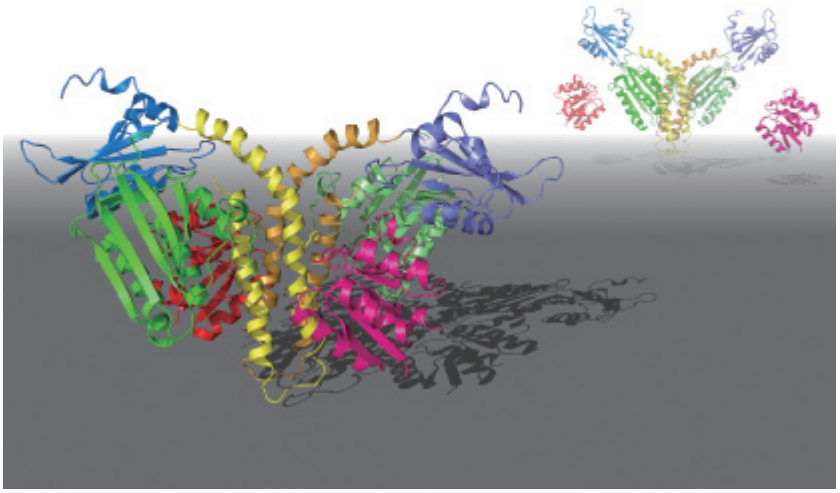


Figure 1: Model of the ThkA/TrrA TCS complex. The structure in the foreground may represent the 'off' state for this system, where the catalytic domain is physically blocked from interaction with the receptor phosphorylation site. In the background is a proposed structure of the system in its 'on' configuration.

Plant, fungal and bacterial species all rely on cellular sensory processes known as 'two component systems' (TCS) to monitor external conditions and deliver instructions on how to respond to fluctuations. "TCS can sense and respond to a variety of environmental changes related to osmotic pressure, oxygen, amino acids, metal ions, nutrients, light, hormones, and so on," explains Yoshitsugu Shiro of the RIKEN SPring-8 Center in Harima.

Each TCS is composed of two proteins: a histidine kinase (HK), which is the outward-facing receptor, and a response regulator (RR), which transmits HK signals within the cell. Following activation by an external stimulus, HK picks up a phosphate group from an adenosine triphosphate (ATP) molecule, which it subsequently transfers to the RR in order to activate it.

Many mysteries remain about TCS signaling mechanisms, partly because the proteins involved are complicated and contain floppy, mobile regions that make structural analysis arduous. Shiro and his colleagues recently achieved a breakthrough on this front, however, by assembling a high-resolution reconstruction of the ThkA/TrrA TCS complex from *Thermotoga maritima*[1]. This bacterium is normally found within geothermal vents, and its proteins exhibit greatly enhanced stability at working temperatures, making structural analysis more feasible.

The HK component, ThkA, assembles in pairs through interactions at a 'dimerization domain', which also contains the histidine amino acid that receives—and eventually transfers—the phosphate group. Accordingly, Shiro's team found that the TrrA 'phosphoacceptor' aligns closely with this residue, with its orientation governed primarily by three points of interaction between the two proteins. However, they also noted that ThkA's sensor domain appears to interact with the catalytic domain, blocking access to the phosphate-receiving histidine and thereby preventing activation. Since ThkA/TrrA is similar to another bacterial signaling TCS, FixL/FixJ, which undergoes physical rearrangements in the presence of oxygen, they hypothesize that this blocked configuration might represent the 'off state' of a switch that enables selective activation (Fig. 1). "The mode of interaction between the sensor and catalytic domains of HK reveals the signal-transduction pathway at an atomic level," says Shiro.

Encouraged by these and other mechanistic insights, Shiro's team is now moving on to study the TCS governing plant response to the growth hormone ethylene, but he sees other potential benefits from this work. "Structural information from TCS could help the development of anti-bacterial drugs without undesirable side effects," says Shiro, "because TCS is essential for the bacterial life cycle, but not present in humans." ■

1. Yamada, S., Sugimoto, H., Kobayashi, M., Ohno, A., Nakamura, H. & Shiro, Y. Structure of PAS-linked histidine kinase and the response regulator complex. *Structure* **17**, 1333–1344 (2009).

Shaping and sharpening movements

A new recording method shows how microcircuitry in the motor cortex of the brain controls voluntary movements

Using a novel technique to record the electrical activity of multiple nerve cells in moving animals, RIKEN researchers have clarified how the brain controls self-initiated voluntary movements. Their findings, published in *Nature Neuroscience*[1], reveal the dynamic properties of small networks of neurons, and show that movements are controlled by the co-ordinated activity of distinct groups of at least two different types of cell.

Voluntary movements are controlled by the primary motor cortex, which is subdivided into five or six layers, each containing distinct populations of pyramidal cells that are presumably activated at different times during the preparation, initiation and execution phases of a movement. The dynamics of these groups of cells is, however, poorly understood. Each layer also contains diverse populations of interneurons, the most abundant being fast-spiking (FS) interneurons, which are thought to regulate the output of the motor cortex by inhibiting the activity of pyramidal cells.

Yoshikazu Isomura of the RIKEN Brain Science Institute and his colleagues trained rats to spontaneously pull a lever with their forelimbs. While the animals performed the movement, the researchers were able to identify the type and layer of the active cells using their new recording method. They also used electrodes to simultaneously record the activity of multiple pyramidal cells and interneurons within the motor cortex to examine how they are connected to one another.

The researchers found that the pyramidal neurons in all cortical layers fire during every phase of voluntary movement. They also identified several distinct patterns of pyramidal cell activity, which occur in sequence and at different times, corresponding to each phase of movement. By contrast, FS interneurons were found to be activated during the execution of movements, but not during the preparation phase (Fig. 1).

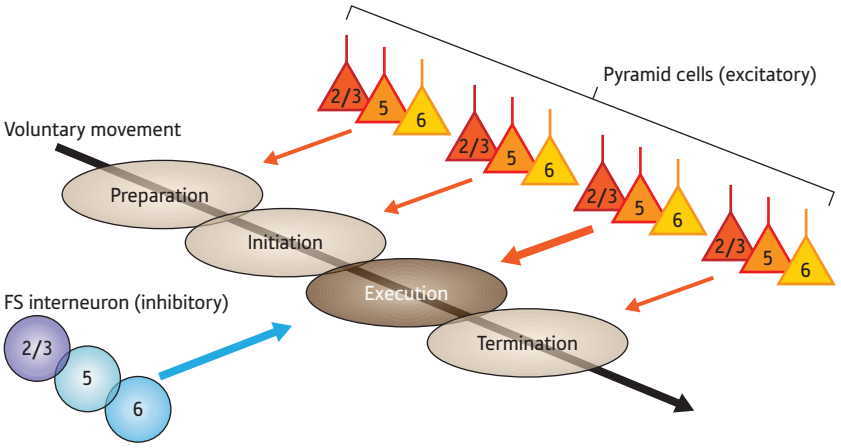


Figure 1: Schematic diagram showing that pyramidal cells in layers 2/3, 5 and 6 of the motor cortex are involved in preparation, initiation, execution and termination of voluntary movements, whereas fast-spiking interneurons are involved only in the execution phase.

FS interneurons therefore appear to modulate the output of pyramidal neurons, rather than inhibit their activity until onset of a movement as previously thought. They shape the motor commands sent from the motor cortex to the spinal cord and other brain regions involved in controlling movement, and make them sharper by limiting pyramidal neuron activity to shorter time windows, according to Isomura. The interneurons may also suppress irrelevant motor commands during the execution of movements.

"Our findings will eventually lead to more efficient rehabilitation for people with brain damage," says Isomura. "They will also enable easier detection of motor control signals, so that brain-machine interfaces for such patients, which consist of implantable electrode arrays, will become less invasive." ■

1. Isomura, Y., Harukuni, R., Takekawa, T., Aizawa, H. & Fukai, T. Microcircuitry coordination of cortical information in self-initiation of voluntary movements. *Nature Neuroscience* **12**, 1586–1593 (2009).

Derailing inner ear development

A molecule that regulates expression of two growth factors is critical for inner ear development

During mammalian neural development, cells extend projections to target tissues from which they derive growth factors needed for survival. Initially, neurons of the inner ear require brain-derived neurotrophic factor (Bdnf) and neurotrophin 3 (Ntf3) from the otic placode, the sensory epithelium from which the cells are derived. Later, the cells express these growth factors themselves, which support the neurons that project to them.

Although this process is well known, the underlying molecular mechanisms have been unclear. Slitrk6, a transmembrane protein with structural similarities to the Slit family of axon guidance molecules and to the Ntrk neurotrophic factor receptors, is known to be expressed in the otic placode, and is therefore likely to play a role in inner ear development.

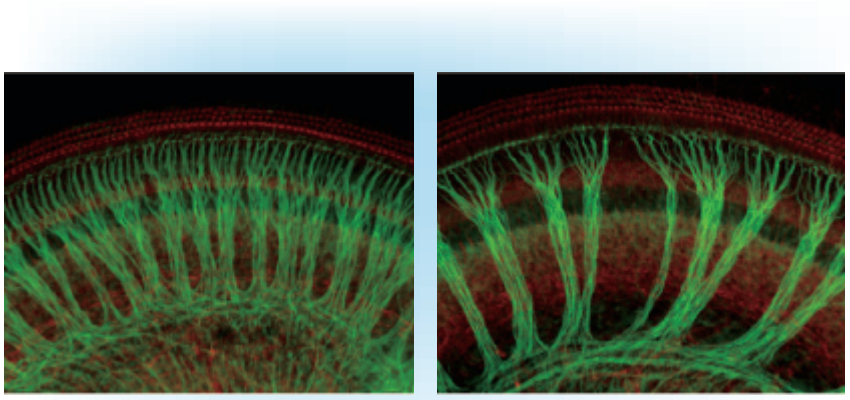


Figure 1: Newborn mice lacking the *Slitrk6* gene (right) have severe reductions in the numbers of nerve fiber bundles innervating the inner ear compared to wild-type animals (left).

To investigate this, Kei-ichi Katayama of the RIKEN Brain Science Institute and his colleagues generated mice lacking the *Slitrk6* gene. They found that the gross structure of the inner appeared normal[1]. However, they observed a significant reduction in the number of nerve fiber bundles projecting to the cochlea (Fig. 1). These defects were evident during late embryonic stages, and persisted throughout postnatal development. In the vestibular region, the defects were more severe, with nerve fibers bundles completely absent in this region in most mutant animals. In others, they were significantly reduced or had abnormal trajectories.

Next the researchers examined the spiral and vestibular ganglia, which contain neurons that project to the cochlea and vestibular region, respectively. Cell death was significantly higher in the mutants than in wild-type animals, so that the structures were up to 75% smaller than normal by the time of birth.

They also found that cultured sensory neurons from the spiral ganglion of mutant mice could grow projections towards sensory epithelial tissue from normal, but not mutant, mice. Further experiments revealed a mild but significant reduction in levels of Bdnf and Ntf-3 in the developing inner ear of the mutants. The phenotype of the mutant mice is therefore not due to axon guidance defects. Instead, these results suggest that Slitrk6 is part of a signaling pathway that increases expression of Bdnf and Ntf3 in the sensory epithelia.

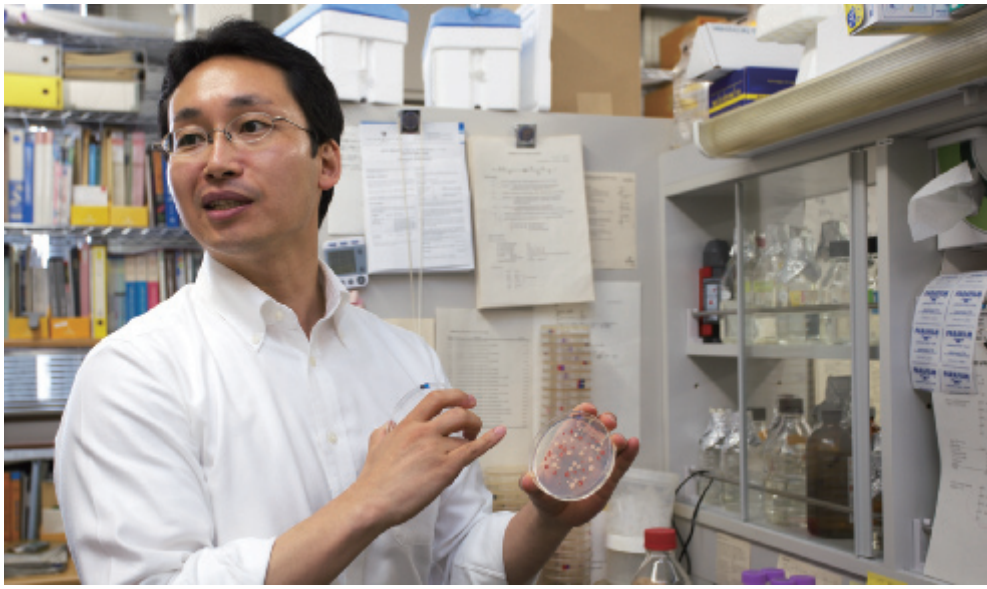
"Behavioral tests show that the *Slitrk6* knockout mice exhibit hearing loss," says senior author Jun Aruga, "and we are now investigating whether they also have balance deficits. We believe our mutant mice are a good animal model of sensorineural deafness, which occurs because of improper cochlear development, following over-exposure to loud noises, and as a result of aging." ■

1. Katayama, K., Zine, A., Ota, M., Matsumoto, Y., Inoue, T., Fritsch, B. & Aruga, J. Disorganized innervation and neuronal loss in the inner ear of *Slitrk6*-deficient mice. *PLoS One* **4**, e7786 (2009).

The role of chromatin structure in the regulation of gene switching

Jun-ichi Nakayama

Team Leader
Laboratory for Chromatin Dynamics
RIKEN Center for Developmental Biology



The human body consists of about 60 trillion cells, each of which contains all the genes necessary to define a 'human'. However, not all genes are active, and excellent genes may lie dormant and inactive without exhibiting their functions. The cells of individual organs, such as the skin, muscles and nerves, have certain genes switched on while other genes are suppressed. Recent research has revealed that the mechanism for regulating the switching of genetic expression is present in the chromatin structure formed by DNA winding around proteins.

Chromatin structure and gene switching

Our personalities and abilities are not solely dependent on the genome inherited from our parents—instead they can be varied by a process known as 'gene switching', which causes some genes to function and others to be deactivated. "It was around the mid-1990s when the mechanism for regulating the switching on and off of genes started to become clear," says Jun-ichi Nakayama, team leader of the Laboratory for Chromatin Dynamics at the RIKEN Center for Developmental Biology, Kobe.

In 1996, when Nakayama was engaged in research into proteins related to aging and cancer and making the best use of the biochemical techniques available at graduate school, he stumbled upon a paper that would later lead him to dramatically change his research theme. "Dr David Allis, currently at the Rockefeller University, discovered an enzyme that catalyzes the attachment of an acetyl group to a particular site in a histone, and demonstrated its association with switching on genes."

A gene is encoded by its base sequence, or the arrangement of the four bases in the DNA: adenine (A), thymine (T), guanine (G) and cytosine (C) (Fig. 1). DNA comprises two strands that are complementarily bound with each other between A and T and between G and C, forming its double helical structure. In this structure, a portion of the DNA contains genes bearing information for protein production. When one of these genes is switched on, the base sequence of the gene region of the DNA is read as RNA (transcription), and the unwanted portions are cut off. This process produces messenger RNA (mRNA), which subsequently becomes a protein.

What does it mean when a gene is switched 'on'? The DNA contained in a single human cell measures about 1.8 m in length when drawn out. The DNA winds around the histone proteins, forming a structure known as chromatin. This chromatin is condensed and housed in the cell nucleus. Highly condensed chromatin is called heterochromatin (Fig. 2).

"For a gene to get switched on, the condensed chromatin structure must loosen to detach or shift the histones and unwind the DNA's double helix. As the helix is unwound, the base sequence of the gene becomes available in a readable state as RNA. It was known that histones undergo modification by the attachment of various chemical entities such as acetyl groups and methyl groups. However, their role was elusive and did not attract significant attention. Dr Allis demonstrated that when an acetyl group attaches to a particular site in a histone, the chromatin structure loosens to allow the gene to be switched on."

In 1999, Nakayama joined Cold Spring Harbor Laboratories as a postdoctoral researcher. "Dr Allis successfully linked the results of a biochemical study of tetrahymena,

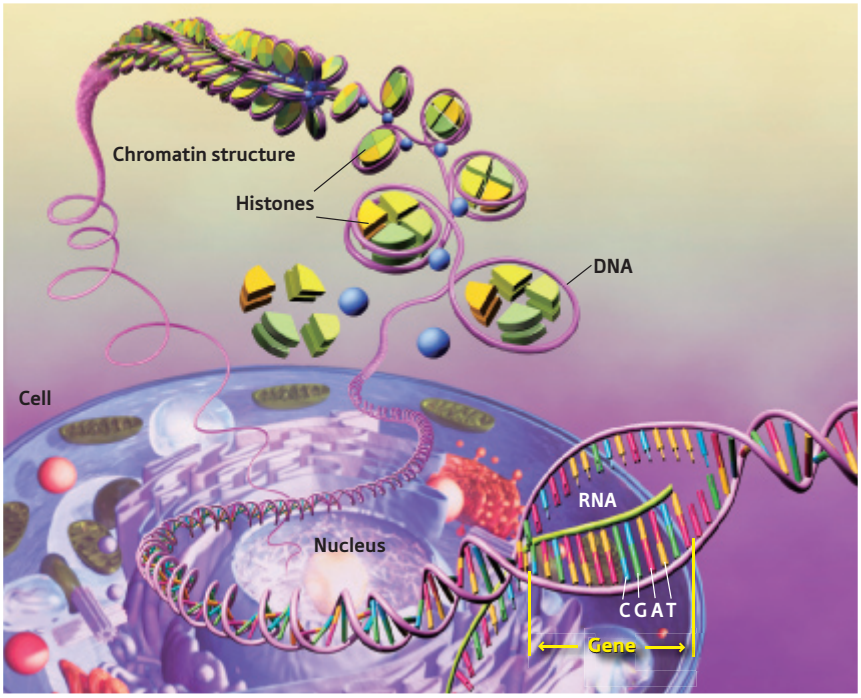


Figure 1: The structure of a gene.
DNA winds around histones to form the chromatin structure. A gene cannot be switched on unless the chromatin structure loosens to detach or shift the histones and unwind the DNA's double helix, allowing the base sequence to be 'read' as RNA.

a model organism, to the genetics of yeast, thus clarifying the role of histone acetylation. I was deeply impressed by his approach, and it made me want to link my own biochemical skills to genetic investigations, and also to research that may lead to resolving the question of chromatin."

In 2001, as a result of his study of fission yeast, Nakayama made the discovery that a methyl group is attached to a site in the heterochromatin histone H3K9 (Fig. 3). "Upon methylation of H3K9 by the action of methylase Clr4, a protein called HP1 that recognizes it assembles to condense the chromatin and form heterochromatin. Thus, the gene of interest remains off. Surprisingly, this mechanism was found to work the same way in humans."

RNA interference and heterochromatin formation

In 2002, Nakayama established his own research unit, the Laboratory for Chromatin Dynamics, at the RIKEN Center for Developmental Biology. "One of the questions to be resolved concerns how the histones surrounding a particular gene are methylated to form heterochromatin in order to switch off the gene. A US study group pointed out that RNA interference is involved in the mechanism. Hence, we began studying the association in detail."

RNA interference, a phenomenon that was observed for the first time in nematodes in 1998, marked a major breakthrough in life science, and its discovery was awarded the 2006 Nobel Prize in Physiology or Medicine. "Double-stranded RNA in a cell is cut into shorter fragments, which in turn bind to protein to form a complex. This complex complementarily binds to a particular mRNA to degrade it. This is RNA interference. Hence, RNA interference may be described as another mechanism for gene suppression."

Several research groups around the world have engaged in experimental studies using fission yeast and have shown that methylation of a particular histone involves utilization of part of the RNA interference mechanism. The methylation is generally assumed to proceed as follows. First, the base sequence is read from a region of the DNA where the gene is otherwise suppressed, resulting in the formation of double-stranded RNA. The double-stranded RNA is then cut into shorter fragments through the same mechanism as RNA interference, thus forming a complex with the protein. Subsequently, the complex returns to the region where double-stranded RNA produced and complementarily binds to the RNA being transcribed. Triggered by this process, the complex attracts methylase Clr4 for attachment of a methyl group to the histone, and also the protein HP1, which recognizes the methyl group and condenses chromatin to form heterochromatin (Fig. 4). "We discovered an important protein that serves in this

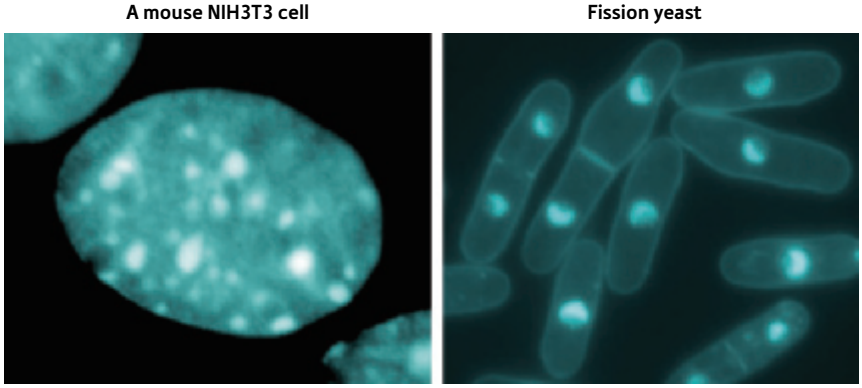


Figure 2: Heterochromatin.
When mammalian cell DNA is stained with fluorescent dye, heterochromatin, which is a densely condensed assembly of chromatin, becomes visible under bright light (left). The basic structure of heterochromatin is also found in fission yeast (right).

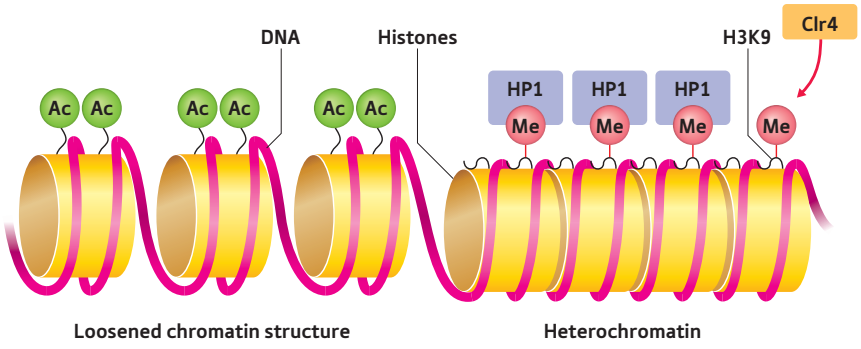


Figure 3: Gene switching and chromatin structural change.
When an acetyl group (Ac) attaches to a particular site in a histone, the chromatin structure loosens to allow the gene to be switched on (left). Meanwhile, when methylase Clr4 acts to attach a methyl group (Me) to a site in histone H3K9, the HP1 protein gathers there with the methyl group as a marker, resulting in chromatin condensation and heterochromatin formation; the gene thus remains off (right).

complex mechanism, and have been working to elucidate its functions."

Traditionally, it had been thought that only as little as 2% of all DNA is read into RNA, the remainder being deemed 'junk' DNA. In 2005, however, Yoshihide Hayashizaki, director of the RIKEN Omics Science Center, Yokohama, and his colleagues upset this common belief. They found that more than 70% of DNA is read into RNA. More surprisingly, they revealed that much RNA lacks information for protein production. "It was found that some of this RNA is involved in the dynamic structural change of chromatin to regulate the switching on and off of genes. Hence, the 'junk' DNA proved to have a function," says Nakayama.

Mechanism for flexible changes in the chromatin structure

In chromatin structure research, it is necessary to comprehensively clarify not only the mechanism for attaching a methyl group to a particular site in a histone, but also the process for recognizing the methyl group and forming heterochromatin. In 2008, Nakayama and his colleagues discovered that a complex mechanism is at work in this process. "There are two types of HP1 protein in fission yeast, which are distinguished by their slightly different shapes. One promotes heterochromatin formation, and the other suppresses heterochromatin formation. The promoting type of HP1 alone cannot form and maintain heterochromatin. We discovered that heterochromatin cannot be formed and maintained unless the two functionally opposite types of HP1 assemble in a certain balance." Why is this complex mechanism required? "Probably to allow the chromatin structure to change flexibly according to varied circumstances."

Three mechanisms involved in cell differentiation

In higher organisms like humans, a mechanism is also available in which genes are strongly suppressed by direct methylation of the DNA, not the histones. DNA methylation has also been found to be closely related to histone modification. "DNA methylation and RNA interference are thought to have originally emerged as a defensive system by which the DNA and RNA of cell-invading viruses are recognized and suppressed. Assuming that the defensive system has evolved to cause chromatin structural change by histone modification, it is easy to understand why these mechanisms are closely related to each other. Furthermore, this defensive system can be assumed to have changed in such a way that it is available in the process of differentiation, which produces a wide variety of cells in multicellular organisms."

In the genesis of multicellular organisms, one fertilized egg proliferates and differentiates into a wide variety of cells, including muscle, skin and nerves, to give rise to an individual. Each cell has all the genes required for the entire organism. However, a fertilized egg cannot differentiate into skin cells, for example, unless only the genes required for the skin cells are switched on and the other genes are suppressed so that they do not work. This suppression is thought to be mediated by three closely associated mechanisms: DNA methylation, RNA interference and chromatin structural change by histone modification.

iPS cells and chromatin structure

Research into the regulatory mechanism for gene switching is also important in regenerative medicine. Induced pluripotent stem cells (iPS cells) are attracting attention as a key factor for major advances in regenerative medicine. When several genes are introduced into a somatic cell after differentiating into skin cells and the like, the somatic cell restores its ability to differentiate into all types of cell, like the cells in the early developmental stages. As such, iPS cells are versatile. Because iPS cells can be created from somatic cells, they offer great possibilities for application to regenerative medicine with minimal ethical concerns. "In the cells of the skin and muscles, for example, unnecessary genes are suppressed by the chromatin structure. In iPS cells, the system for gene suppression seems to be 'reprogrammed' to loosen the chromatin structure and allow all of the genes to be switched. However, the mechanism behind this change in the chromatin structure, which is induced merely by introducing several genes, remains unknown."

The efficiency of iPS cell production remains low. "Studies on the chromatin structure are expected to contribute to research into the mechanism behind iPS cell genesis, leading to increases in the production efficiency and differentiation of iPS cells into selected cell types."

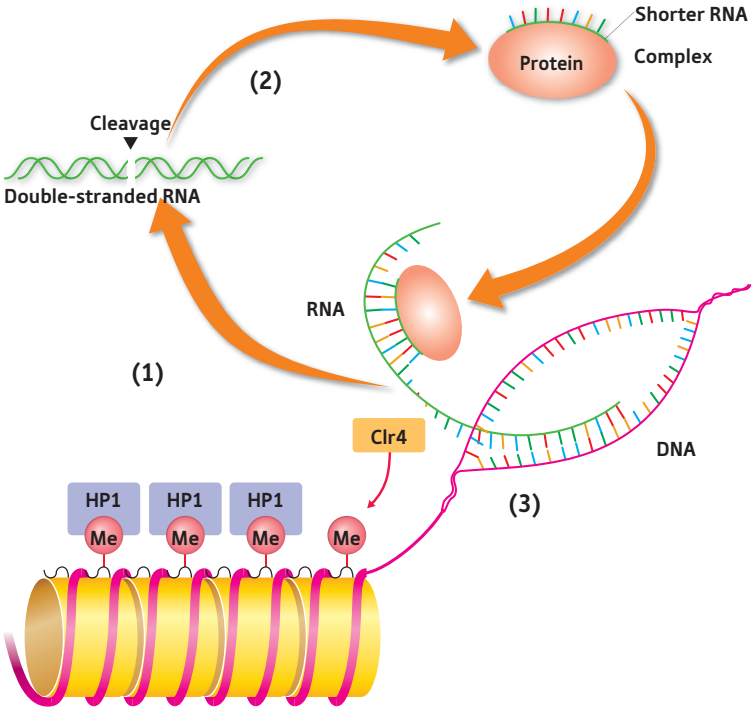


Figure 4: RNA interference and heterochromatin formation.
(1) The DNA base sequence is read, and double-stranded RNA is produced. (2) The double-stranded RNA is cleaved to produce shorter RNA, which in turn binds to protein to form a complex. (3) The complex returns to the region where double-stranded RNA is produced, and complementarily binds to the RNA being transcribed. The complex attracts methylase Clr4 and HP1 protein to form heterochromatin.

Epigenetics, a new keyword in life science

There is another type of behavioral change in genes that cannot be explained solely by the DNA base sequence: epigenetics. "Epigenetics gives rise to variations such as personality differences between monozygotic twins. Although they share exactly the same genome, monozygotic twins have different characters and abilities. In some cases, the elder twin remains healthy, whereas the younger contracts lifestyle-related diseases or cancer. It is thought that environmental factors such as diet, exercise, learning and stress have differential effects and cause slightly different on-off states for various genes, and that the accumulation of these differences gives rise to the personality differences between monozygotic twins." Are there excellent genes in our body that remain inactive and do not exhibit their functions? "Maybe so. I think a good living environment may lead to the accumulation of better on-off states in the genes. Conversely, a bad lifestyle and stress may lead to the accumulation of on-off states that herald the onset of disease."

Epigenetic research is important. "Since the base sequences of the genomes of a wide variety of organisms have been decoded, a major challenge ahead is to elucidate the epigenetics." This could lead to a better understanding of life phenomena, allow the causes of disease to be clarified and therapies to be developed, and ultimately form the basis for regenerative medicine. Epigenetic research is currently being most energetically conducted in Europe and the US. The Japanese Society for Epigenetics was formed in 2007. "Epigenetic research has been advanced through basic studies using various model organisms, including yeast, red bread mould, plants, nematodes and Drosophila. These seemingly modest investigations have come into the limelight. A strong point of research in Europe and the US is that there is a tradition to place importance on basic research."

The EU and US are about to launch a major project called the Human Epigenome Project with the aim of exhaustively investigating all regions of the genome for DNA methylation and histone modification in a wide variety of cells. Histone modification includes not only acetylation and methylation, but also ubiquitination and phosphorylation, and even the same modification produces different modes in the regulation of gene switching depending on the portion of the histone that is modified. There is thus much to investigate. "The Epigenome Project is certainly important. However, I want to emphasize that the system for regulating the switching of genes cannot be clarified merely by examining their modifications. How do the modifications take place, and how are the modifications read to regulate switching? These are critical. I want to continue to work to elucidate the mechanism. To understand the regulatory system for the gene as a whole, basic research using a wide variety of model organisms must continue. We are determined to continue to work on elucidating this complex mechanism using fission yeast, and to conduct investigations to clarify how the mechanism found in that organism works in humans. We want our work to contribute to elucidating the mechanisms for genesis and to the development of regenerative medicine."

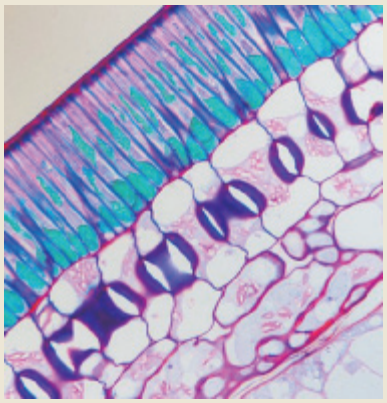
About the researcher

Jun-ichi Nakayama was born in Tokyo, Japan, in 1971. He graduated from the Department of Biosciences of the Tokyo Institute of Technology in 1994, and obtained his PhD in 1999 from the same institute. After two years postdoctoral training at Cold Spring Harbor Laboratories in the US, he returned to Japan as a PRESTO researcher of the Japan Science and Technology Agency, where he started his career in chromatin dynamics. He was appointed team leader of the Laboratory for Chromatin Dynamics at the RIKEN Center for Developmental Biology in 2002. Since then, he has led his own research team. His research interests lie in elucidating the molecular mechanisms underlying chromatin dynamics and epigenetic gene regulation.

RIKEN Researchers win Honorable Mention in International Digital Imaging Competition

A photograph taken by researchers at the RIKEN Plant Science Center (PSC) has been awarded an Honorable Mention in the prestigious Olympus BioScapes International Digital Imaging Competition. The photograph, taken by technician Mayumi Wakazaki and researcher Kiminori Toyooka as part of joint research with scientists at the University of Hyogo, depicts epidermal layer cells of dry *Lotus japonicus* seed, dyed so as to highlight seed segmentation.

Now in its sixth year, the Olympus BioScapes International Digital Imaging Competition highlights the best microscopic life science photography of the year from around the world. In 2009, the competition received nearly 2,000 entries from 62 countries. The photo by Wakazaki and Toyooka was one of 65 honorable mentions awarded by Olympus in addition to ten winning entries. An awards reception for winners of the 2009 prizes was held on December 6 in San Diego, in conjunction with the annual meeting of the American Society for Cell Biology.



Epidermal layer cells of dry *Lotus japonicus* seed.

Cheiron School 2009 — The third AOFSRR workshop

The Asia-Oceania Forum for Synchrotron Radiation Research (AOFSRR) held its third school from November 2 to 11 at SPring-8, the world-largest synchrotron radiation facility located at the RIKEN Harima Institute. The students at the school were 55 young scientists and engineers from nine countries: Australia, China, India, South Korea, New Zealand, Singapore, Taiwan, Thailand and Japan. The school promotes better understanding of synchrotron radiation science as well as building a network among students.

The event was cosponsored by RIKEN, the Japan Synchrotron Radiation Research Institute (JASRI), which operates SPring-8, the High Energy Accelerator Research Organization (KEK) and AOFSRR. The curriculum included lectures on synchrotron radiation science and technology, covering a wide-range of topics from accelerator science to X-ray physics as well as applications to materials science and biology. Through the lectures, students learned new scientific directions and potentials in synchrotron radiation science.

One of the most popular lectures, given by Tsumoru Shintake, a chief scientist of RIKEN, was on the 'X-ray Free Electron Laser'. X-ray free-electron lasers are currently in the global spotlight as an ultimate source of intense pulsed coherent radiation at wavelengths as short as 0.06 nm.

A two-day beamline practical course was particularly attractive for students, allowing them to experience the measurement process using beamlines. The 'Meet the Expert' course—round-table discussions between students and SR science specialists—characterizes the concept of the 'Cheiron' school. Those experts gave students useful advice on challenges they are facing.

One of the students expressed their appreciation by saying, "I have learned a lot during the ten days I spent here. It is very well organized and created a necessary network I would certainly use in my future research." Other students mentioned that, "by attending this school, I surely expand my vision. I also made new friends from other synchrotron facilities in the Asia-Oceania area," and, "everything here is fantastic and excellent including the lectures, lecturers, staff, secretaries, guest house and cafeteria. I miss the nice staff at the Cheiron School. If I have chance, I want to work at SPring-8."

This school's name, Cheiron, is taken from ancient Greek mythology. The immortal god as well as a teacher, Cheiron, would impart his knowledge only to those mortals most worthy of it. The concept of Cheiron aligns well with the purpose of the school, which seeks to train the best and brightest young minds from the Asia-Oceania region. Tetsuya Ishikawa, director of the RIKEN SPring-8 Center, says, "We believe they will become next-generation leaders in synchrotron radiation science for the Asia-Oceania region."

Nobel laureates deliver message to Prime Minister Hatoyama

Eight Nobel laureates, including RIKEN President Ryoji Noyori and Brain Science Institute Director Susumu Tonegawa, delivered a message to Japanese Prime Minister Yukio Hatoyama on November 26 rejecting recently proposed cuts to basic science budgets. The cuts have been recommended by working groups of the newly created Government Revitalization Unit in an effort to trim next year's national budget by three trillion yen. In their current form, the cuts would drastically reduce funding for facilities such as RIKEN's SPring-8 synchrotron facility in Harima and the Next-Generation Supercomputer in Kobe, while also significantly reducing financial grants for individual scientists and research groups.

In a hastily convened symposium at the University of Tokyo on November 26, the eight Nobel laureates publicly decried the proposed cutbacks, warning that, "Weakening science and technology will lead to the decline of our resource-poor country." RIKEN President Noyori, in a separate message, describes science and technology as Japan's "lifeline". "It takes time," he explained, "before scientific results bear fruit and begin to spur innovation. Instead of demanding results right away, I ask that you take a long-term view and consider supporting science and technology as an investment in the future."

With the recommendations currently working their way through government, the future of many of RIKEN's research initiatives, and of Japan's commitment to basic science, hangs in the balance. "I really have to wonder whether the people who insist on carelessly terminating and freezing operations will be prepared, in the future, to stand behind these decisions in the courtroom of history," says Noyori. "Besides being the only means for our country to make it through this century of global competition, science and technology is also the pillar of international cooperative efforts to solve the many problems facing the continued existence of humankind."

Dr. Toshikazu Ebisuzaki
Computational Astrophysics Laboratory
RIKEN Advanced Science Institute
2-1 Hirosawa, Wako, Saitama
351-0198 Japan

Dear Ebisuzaki-sensei,

As I write this letter I remember well my three and a half years at RIKEN as a JSPS fellow, first in Shimizu-sensei's laboratory and then as a contract researcher in your laboratory. At the end of this month, it will be exactly three years since I left your lab to return to Italy, where I now have a permanent position at Torino University. My current position is one of the great outcomes of my stay at RIKEN, which was really an important cornerstone of my scientific curriculum vitae.

As you know, since I left your laboratory, I have visited RIKEN more than ten times, and you have visited Torino University twice. Together we have organized workshops in Torino and in Tokyo. All of this shows how we have built a strong collaboration since my departure, and I consider all of these things the second most important outcome of my research activity at RIKEN.

RIKEN for me is a synonym for organization, efficiency, top-level research, synergy and cooperation among quite different disciplines. As an example, in your laboratory, I met colleagues working on cosmic rays like me, but at the same time, some of them were interested in developing electronic circuits or advanced computers, while others were dealing with the formation and evolution of galaxies, or molecular dynamics. I have experienced a similar situation in my previous research at the former Image Information Unit, and I clearly understood that one of the characteristics of laboratories at RIKEN is that they are headed by eclectic team leaders with several fields of interest. And I believe this is one of the main reasons that research at RIKEN is at the top level in the world and has produced several Nobel laureates in the past. In fact, we need open-minded researchers with many fields of interest in order to generate great ideas.

From my stay in Japan, I learned that RIKEN bets on new ideas, revolutionary projects and young researchers, giving very good salaries and ample funding to develop research. This is the dream of every scientist. Unfortunately, it is one of the aspects I have missed most since my return to Italy.

At RIKEN, I also learned that secretaries and assistants are as important as researchers. I'm deeply indebted to Suzuki-san and Ohata-san who helped me to solve all of the obstacles that a foreign person encounters while living in Japan, and in doing so allowed me to concentrate on my work.

I also have to thank all of my Japanese colleagues who taught me quite a lot about Japanese life, culture and language. They spoke with me mostly in Japanese, as I was the only foreigner working in the two labs. It was tough at the beginning, but thanks to the Japanese language classes taken at JALS of RIKEN, I managed to overcome the initial difficulties.

Finally, I envy RIKEN for its excellent facilities: the nice soccer field, the comfortable international house and its kind personnel, as well as the friendly staff of the ICO room. I miss the variety of food of the two *shokudo* (cafeteria), the 'blood orange juice' of Tully's, and last but not least, the pleasant *nomikai* (get-together) we had with you and our colleagues from time to time, as well, of course, as the business trips and holidays all over Japan!

I am looking forward to cooperating with you even more than before on our JEM-EUSO project. See you soon at the next collaboration meeting in Japan!

With my best regards,

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RIKEN

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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.

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