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Breathing new life

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The roots of respiration

The structure of a greenhouse gas-producing bacterial enzyme may yield insights into the evolution of our earliest oxygen-breathing ancestors

Every year, nitrogen-metabolizing bacteria in the soil and seas churn out more than ten billion kilograms of nitrous oxide (N_2O) gas as they respire in these oxygen-deficient environments.

Nitric oxide reductase (NOR) enzymes are the powerhouse underlying production of this gas, taking pairs of nitric oxide (NO) molecules and transforming them into N_2O and water via a chemical reaction known as 'reduction'. These enzymes also help pathogenic bacteria to evade destruction by the immune system, as some T cells use NO as a chemical weapon against infectious agents.

More generally, scientists are interested in the potential to employ these enzymes as a tool for synthesizing useful, customized molecules for a variety of applications. "The nitrogen-oxygen bond cleavage and nitrogen–nitrogen bond formation reactions executed by these enzymes are the essence of chemistry," says Yoshitsugu Shiro of the RIKEN SPring-8 Center in Harima.

Although a great deal is known about the biochemical properties of these proteins, scientists have found it challenging to determine the structure of bacterial NOR in fine detail. Now, after seven years of hard work, Shiro and colleagues have finally obtained the first such structure for NOR from *Pseudomonas aeruginosa*[1], a pathogenic bacterium associated with opportunistic infections in immune-compromised patients (Fig. 1).

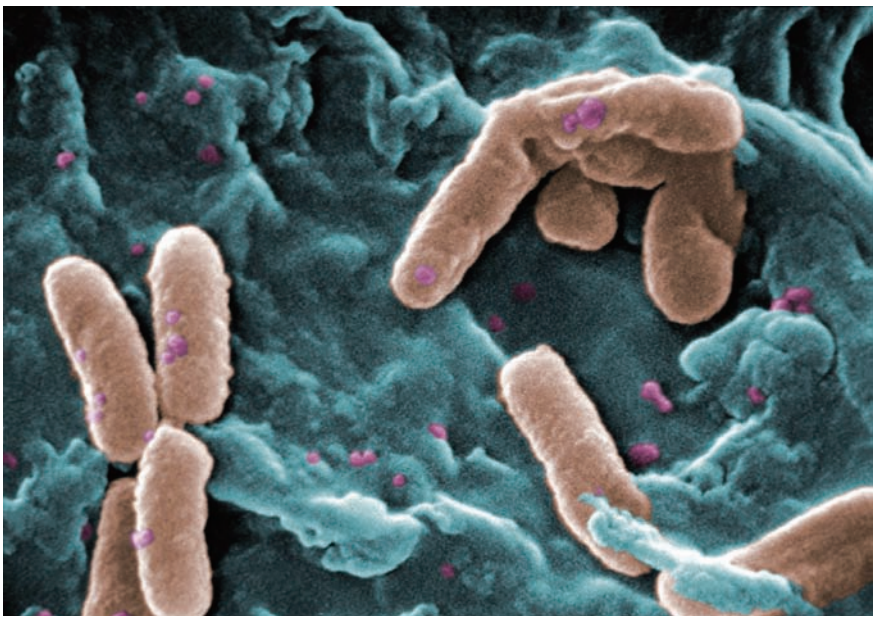


Image courtesy of US Centers for Disease Control/Janice Haney Carr

Figure 1: Scanning electron micrograph of *P. aeruginosa* bacteria.

Not-so-distant relations

Although all NOR enzymes execute essentially the same chemical reaction to produce N_2O, they can be subdivided into three major classes: cNOR, qNOR and qCuNOR. The enzyme crystallized by Shiro and colleagues (Fig. 2) belongs to the cNOR family, and contains a subunit known as cytochrome c that also enables bacteria to engage in aerobic (oxygen-driven) respiration. This ability to switch from oxygen-based to nitrogen-based respiration is highly beneficial for survival in the oxygen-poor conditions deep in the soil or beneath the waves.

Accordingly, cNORs are thought to be closely related to the cytochrome oxidases (COX), enzymes that play a central role in aerobic respiration, and these new findings have revealed a number of structural parallels between the two. "There is a long history of research into these respiratory enzymes, COX and NOR, and a lot of knowledge on NOR has been accumulated by biochemical, chemical, molecular biology and microbiological studies," says Shiro. "From these points of view, our NOR structure is not surprising, but seeing is believing!"

The reduction process is dependent on the directional transport of electrons and protons, and COX and cNOR appear to closely resemble one another in terms of the structure of their electron-transfer networks. Both enzymes depend on precisely positioned metal ions to enable electron transport, and the four iron atoms contained within cNOR are arranged in a configuration that closely resembles COX, maintained via interactions between these positively charged iron atoms and a set of evolutionarily conserved, negatively charged histidine and glutamate amino acids.

On the other hand, the researchers observed some notable differences with regard to the movement of protons. Both COX and cNOR are bound within membranes, but COX contains channels

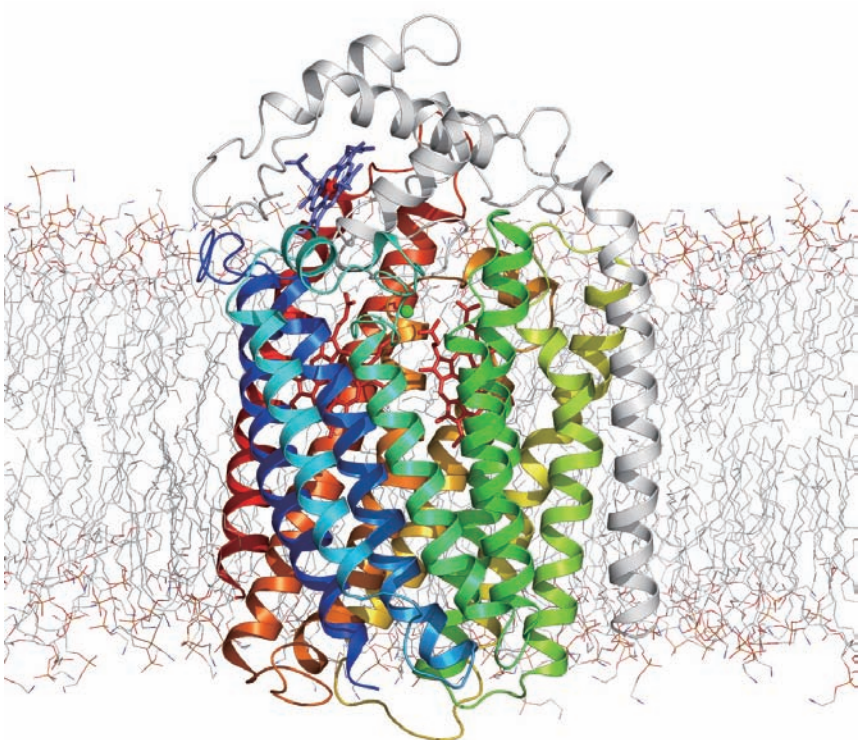


Figure 2: Structure of cNOR from *P. aeruginosa* within the context of the cellular membrane.

that are believed to direct the flow of protons across the membrane from the interior of the cell. This flow helps generate electrical potential that subsequently powers a variety of cellular motors. However, cNOR lacks such membrane-spanning channels, and protons entering the enzyme from the exterior of the cell only make it as far as the membrane interior, where the reductase catalytic site is located.

Back to the beginning

Even with a structure in hand for this well-studied enzyme, a number of mysteries remain to be addressed. For example, these data are insufficient to resolve an ongoing debate over the fine details of the N_2O production mechanism. Shiro and colleagues were readily able to identify the two iron atoms involved in catalysis, but their structure reveals insufficient space at this 'active site' to accommodate the two molecules of NO believed to be required for this reaction.

"The NOR active site is tightly packed and very crowded," says Shiro. "This observation suggests that some conformational change [is] needed to achieve catalytic turnover, but no one knows of any such conformational change so far." Resolving this issue will require the acquisition of additional, high-resolution structures that might offer clear snapshots of the enzyme at intermediate stages in the catalytic process.

This structure offers tentative support for the hypothesis that the COX aerobic respiratory machinery originally evolved from NOR enzymes, although additional work will clearly be required to confirm this. Unlike NOR, which exclusively employs iron ions, COX makes use of both copper and iron for catalysis, and the researchers have tentatively identified a few amino acid changes that might have enabled this transition to take place. In addition, although NOR lacks the 'K-channel' that allows COX to deliver protons from the cytoplasm, Shiro's team has identified some structural elements that could potentially represent early evolutionary precursors in the formation of this channel.

In future studies, Shiro plans to develop experimental tests for some of these still-speculative models. "We want to follow the molecular evolution of the respiratory enzymes from anaerobic to aerobic conditions on Earth, from NOR to COX," he says. "Using mutagenesis, based on our structural comparisons, we are hoping to convert NO-reducing NOR into oxygen-reducing COX." For the present, though, he is optimistic that this structure will give a boost to researchers seeking to understand and manipulate this enzymatic process. "Scientists worldwide who are interested in NO reduction can enter a new stage of NOR research with this structure," he says. ■

1. Hino, T., Matsumoto, Y., Nagano, S., Sugimoto, H., Fukumori, Y., Murata, T., Iwata, S. & Shiro, Y. Structural basis of biological N_2O generation by bacterial nitric oxide reductase. *Science* **330**, 1666–1670 (2010).

About the researcher

Yoshitsugu Shiro was born in Nagoya, Japan, in 1956. He graduated from the Faculty of Engineering, Kyoto University, in 1980, and obtained his PhD in 1985 from the same university. After two years of postdoctoral training with the Japan Society for the Promotion of Science at Kyoto University, he moved to RIKEN as a research scientist. In 1990, he was a visiting scholar for one year at the Department of Chemistry, Stanford University. Since then, he has been very interested in the chemistry of metal-containing proteins and enzymes, based on their molecular structures that can be examined using x-ray techniques including crystallography, small angle scattering and spectroscopy. In 1997, when SPring-8 was opened to the public, he moved to the Harima Institute from the Wako campus, and was promoted to chief scientist in 2000. Since then, he has been director of his own research group. In addition to metal-containing proteins, his current research interest focuses on the structures/functions of proteins related to the dynamics of metal elements in biology, e.g. sensing, transport, storage and utilization.

A novel vanishing act

Microwave photons can nullify the conductivity of electrons confined to the surface of liquid helium

Trapping electrons in a flat plane prevents them from moving freely in the third dimension and opens the door to a whole range of unusual physics. These effects are harnessed, for example, in modern ultrafast transistors, which confine electrons to thin layers of high-quality semiconductor crystals such as gallium arsenide. But scattering from impurities in semiconductors can mask the deeper underlying physics of these so-called two-dimensional electron gases (2DEGs). Liquid helium may provide an alternative to semiconductors since it is largely impurity free. Using this approach, Denis Konstantinov and Kimitoshi Kono from the RIKEN Advanced Science Institute have demonstrated a novel effect where light totally switches off the conductivity of 2DEGs[1].

Two-dimensional electron gases form naturally at the surface of helium because an intrinsic energy barrier prevents electrons from penetrating any deeper into the liquid. These gases vary markedly from their three-dimensional counterparts because the electron motion in one direction becomes quantized—that is, their velocity in this direction is governed by quantum mechanics and is restricted to a range of discrete values.

Konstantinov and Kono cooled liquid helium-3 to 0.3 kelvin. They supplied electrons from a nearby hot filament, and applied voltage to a plate below the helium to control the number of electrons per unit area. Then, they fired microwave radiation at the 2DEG (Fig. 1) and measured the longitudinal conductivity—the current induced by an electric field applied along one direction—as a function of external magnetic field. They saw that the conductivity periodically fell to zero as they increased the magnetic field. When they switched off the source of microwave photons, however, this effect ceased.

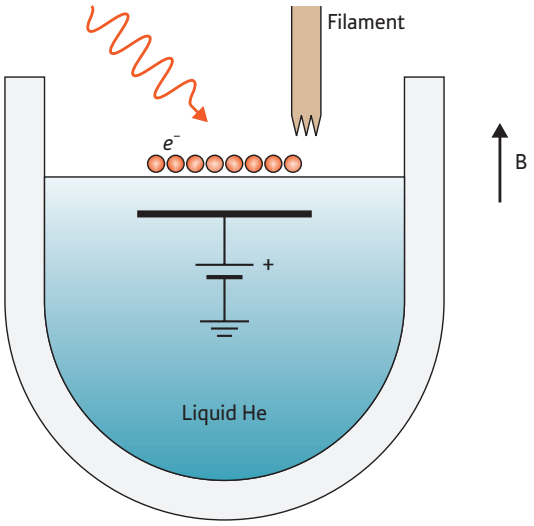


Figure 1: At specific values of external magnetic field B, and under microwave irradiation (red arrow), the conductance of a two-dimensional electron (e^-, generated by a nearby filament) gas on the surface of liquid helium falls to zero.

This previously unidentified nullifying effect of microwave photons on conductivity is a consequence of energy-conserved scattering of the liquid helium's electrons between different energy states—specifically, the first excited and ground sub-bands. "When the electrons stay in the ground sub-band, the effects are rather dull," says Kono. "In our experiment, absorption of microwave photons transfers electrons to a higher energy sub-band," Konstantinov adds. "As we change the magnetic field, the energies of states in two subbands cross, and scattering redistributes electrons between the sub-bands."

Kono and Konstantinov believe that the result will lead to the observation of more novel phenomena in these two-dimensional systems when they are shifted out of their equilibrium state. "The study of nonequilibrium transport in the extremely clean helium system will complement studies of electron transport in semiconductors," explains Konstantinov. ■

1. Konstantinov, D. & Kono, K. Photon-induced vanishing of magnetoconductance in 2D electrons on liquid helium. *Physical Review Letters* **105**, 226801 (2010).

Finding solid ground

Experimental evidence adds to the likelihood of the existence of supersolids, an exotic phase of matter

Figure 1: A custom-developed rotating cryostat capable of 15 millikelvin temperatures is helping physicists find evidence for the existence of supersolids.

Supersolids and superfluids rank among the most exotic of quantum mechanical phenomena. Superfluids can flow without any viscosity, and experience no friction as they flow along the walls of a container, because their atoms 'condense' into a highly coherent state of matter. Supersolids are also characterized by coherent effects, but between vacancies in a crystal lattice rather than between the solid's atoms themselves.

The reduction in the rotational inertia of a bar of solid helium-4 as it was cooled to very low temperatures provided the first experimental evidence for supersolids. Physicists interpreted the reduction to mean that some amount of supersolid helium had formed and decoupled from the remainder of the bar, affecting its rotational inertia and frequency. Others argued that the reduction in inertia resulted from a change in the helium's viscosity and elasticity with temperature, rather than from the onset of supersolidity.

Kimitoshi Kono from the RIKEN Advanced Science Institute in Wako, Eunseong Kim from KAIST in Korea, and their colleagues from these institutes have now disproved the alternative interpretation by simultaneously measuring the shear modulus (a measure of viscosity and elasticity) and the rotational inertia of a solid helium-4 cell as its temperature dropped from 1 kelvin to 15 thousandths of a kelvin[1]. The cell was made to rotate clockwise and then counterclockwise periodically, as well as to rotate clockwise or counterclockwise continuously (Fig. 1). The continuous rotation affected the inertial mass of the helium but its shear modulus, allowing these quantities to be monitored independently.

Under continuous rotation, the degree of change in the rotational inertia had a clear dependence on rotation velocity, while the shear modulus did not. In addition, the energy dissipated by the rotation increased at high speeds. Both of these observations contrast to what would be expected if viscoelastic effects were at play, rather than supersolidity. The researchers also found that periodic rotation and continuous rotation affected the rotation differently, raising new questions about the experimental system.

The data support the interpretation that changes in the rotational inertia of helium-4 at low temperature result from supersolidity. This is important because of the novel and surprising nature of the phenomenon itself, says Kono. "Superfluidity in a solid is a very radical concept which, if proven, is certainly a good candidate for the Nobel Prize" he adds. "Therefore the first priority is to determine whether it can be proven in a fashion that will convince the low-temperature physics community." ■

1. Choi, H., Takahashi, D., Kono, K. & Kim, E. Evidence of supersolidity in rotating solid helium. *Science* **330**, 1512–1515 (2010).

Carrying calcium

Molecular simulations explain how enzymatic pumps transport calcium ions within muscle cells

The transport of ions is essential for the routine maintenance of the body. For this reason cells contain specialized enzymes that act as pumps that help ions to move around and pass through boundaries.

Now, Yuji Sugita at the RIKEN Advanced Science Institute in Wako and colleagues have used computer simulations to explain how one particularly important pump enzyme, Ca^{2+}-ATPase, transports calcium ions in muscle cells[1].

"The calcium ion pump is an essential membrane protein that can transport Ca^{2+} ions across biological membranes against a large concentration gradient," explains Sugita. "It is believed to be related to some human heart diseases because there are large concentrations of the pump in the cell membranes of the heart, and the calcium ions work as messengers in muscle cells."

Specifically, each molecule of Ca^{2+}-ATPase transports two calcium ions at a time from a cell's cytoplasm into the sarcoplasmic reticulum (SR), a subcellular structure devoted to regulating calcium levels. By this method the SR releases calcium ions when muscles are contracted, then reabsorbs them when muscles are relaxed.

Previous research has shown that Ca^{2+}-ATPase on the SR membrane functions by switching between two states. First the binding sites face outwards and have high affinity for calcium in order to 'gather' ions from the cytoplasm. Then they face inwards and lose their affinity, thus releasing the ions into the SR.

To investigate exactly how this switching mechanism works, Sugita and colleagues focused on the role of a glutamate called Glu771, which is often found near the calcium binding sites. Studies have shown that when Glu771 is removed by mutation the calcium pump loses all its calcium-binding ability.

The researchers ran molecular dynamics simulations for both normal and mutant versions of the calcium pump. Their results revealed that the presence of Glu771 prevents water molecules from interfering at the binding site (Fig. 1). In the Glu771 mutant, this system breaks down and the binding sites get flooded with water.

This is the first time that research has indicated a relationship between calcium binding and the shielding of water, the team notes. Sugita is hopeful that with the help of more powerful computers, he and his colleagues could soon simulate a full enzymatic cycle of the calcium pump.

"We are developing new algorithms that combine all-atom and coarse-grained models," he says. "Using such multi-scale simulation methods, we hope to elucidate further molecular mechanisms of ion uptake by the calcium pump or other ion pumps." ■

Figure 1: Just as a breakwater can protect a shore, Glu771 can protect binding sites from water molecules.

1. Sugita, Y., Ikeguchi, M. & Toyoshima, C. Relationship between Ca^{2+}-affinity and shielding of bulk water in the Ca^{2+}-pump from molecular dynamics simulations. *Proceedings of the National Academy of Sciences USA* **107**, 21465–21469 (2010).

Secretions of the mind

Insights into a specific secretion mechanism in the brain could lead to a better understanding of anxiety in unfamiliar or stressful environments

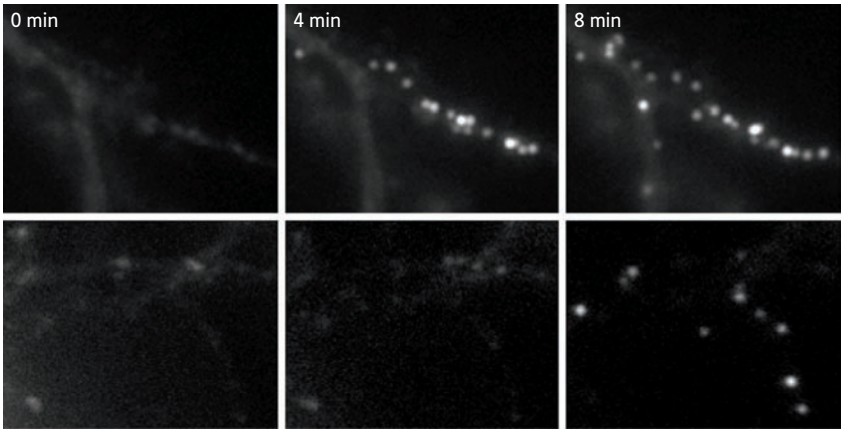
A molecule called calcium-dependent activator protein for secretion 2 (CAPS2) promotes the secretion of a neurotrophic factor that is critical for the proper development and survival of networks of interneurons in the brain's hippocampus, researchers in Japan have shown[1].

Teiichi Furuichi of the RIKEN Brain Science Institute in Wako, and his colleagues showed previously that CAPS2 is involved in secretion of brain-derived neurotrophic factor (BDNF) from cerebellar granule cells and neurons in the cerebral cortex, but its exact role in secretion was unclear.

Yo Shinoda, a researcher of the Furuichi's group used antibody staining to examine the distribution of CAPS2 in cultured hippocampal neurons of mice. He saw that most CAPS2 localized along the axons, but found some on secretory vesicles that contain and release BDNF.

To investigate the role of CAPS2 in BDNF secretion, the researchers visualized BDNF secretion in cells from mutant mice lacking the *CAPS2* gene. They found that these cells secreted significantly less BDNF than normal cells, but the level returned to normal or became enhanced when they transfected the cells with CAPS2 (Fig. 1).

The researchers then examined hippocampal interneurons in the mutant mice and compared them with those in normal animals. These interneurons synthesize and secrete γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain. The mutants had reduced numbers of these cells in hippocampus of the brain. Furthermore, analysis of inhibitory synapses under the electron microscope revealed that the mutants had fewer synaptic vesicles than the normal animals. The researchers also revealed that the vesicles were distributed over a smaller area within presynaptic boutons, the specialized area where loaded vesicles dock to release their contents.



Enhanced BDNF secretion by overexpression of CAPS2 (top), at four-minute intervals, in hippocampal neurons from mice lacking the *CAPS2* gene (bottom: without CAPS2 expression).

Finally, the researchers used microelectrodes to examine the electrical activity of the cells from the mutants and discovered that there was a significant reduction in both the number and size of spontaneous inhibitory postsynaptic currents. Consequently, the mutant mice displayed anxiety-like behaviors that would be expected with a GABA signaling impairment.

The findings show that CAPS2 promotes BDNF secretion by affecting the kinetics of its release from dense-core vesicles, and that BDNF is essential for proper development and function of the networks of inhibitory interneurons in the hippocampus, the researchers conclude. "We are interested in the molecular mechanism underlying the enhanced BDNF secretion, and would like to analyze the kinetics of secretion using state-of-the art cell imaging technology," Furuichi explains. "We also want to study relation of CAPS2-BDNF-GABA pathways in anxiety and depressive behavior." ■

1. Shinoda, Y., Sadakata, T. Nakao, K., Katoh-Semba, R., Kinameri, E., Furuya, A., Yanagawa, Y., Hirase, H. & Furuichi, T. Calcium-dependent activator protein for secretion 2 (CAPS2) promotes BDNF secretion and is critical for the development of GABAergic interneuron network. *Proceedings of the National Academy of Sciences USA* **108**, 373–378 (2011).

Unearthing a pathway to brain damage

Particular types of neuronal loss and brain damage are caused by a molecular mechanism associated with calcium signaling

Neuroscientists have long suspected that abnormal calcium signaling and accumulation of misfolded proteins cause an intracellular membrane-bound organelle called the endoplasmic reticulum (ER) to trigger the abnormal death of cells implicated in many neurodegenerative diseases. However, the underlying mechanisms have proved elusive.

The ER is crucial for synthesizing proteins and maintaining their quality, and also acts as a reservoir of calcium ions essential for numerous cellular events. However, it is sensitive to alterations of surrounding environments, causing a process called ER stress.

Katsuhiko Mikoshiba and Takayasu Higo at the RIKEN Brain Science Institute, Wako, and their colleagues now report that a calcium channel called IP_3 receptor type1 (IP_3R1), which mediates the release of calcium ions from ER, is destroyed by ER stress and that this induces neuronal cell death and brain damage[1] (Fig. 1).

Using a calcium imaging technique, the researchers revealed that IP_3R1 released less calcium in cultured neurons treated with an ER stress inducer than in controls. To investigate the significance of this dysfunction, they bred mice lacking the gene for IP_3R1, which caused brain damage under ER stress conditions.

In an exploration into how ER stress impairs IP_3R1 and induces neuronal cell death. Mikoshiba and colleagues identified GRP78, a molecular 'chaperone' that normally regulates the cellular response to misfolded proteins, as an interacting partner of IP_3R1. RNA interference experiments revealed that GRP78 positively regulates the assembly of IP_3R1, which consists of four subunits. They also found that this interaction was inhibited under ER stress conditions.

In a further set of experiments, the researchers then examined the involvement of the interaction in neurodegenerative diseases using a mouse model of Huntington's disease (HD). They found that both the protein interaction and IP_3R1 channel activity were significantly impaired in parts of the brain most affected in HD.

The findings demonstrate a novel mechanism by which ER stress impairs the regulation of IP_3R1 by GRP78. Mikoshiba and Higo propose that IP_3R1 functions to protect the brain against stress and that the link between ER stress, IP_3/calcium signaling, and neuronal cell death is associated with neurodegenerative disease.

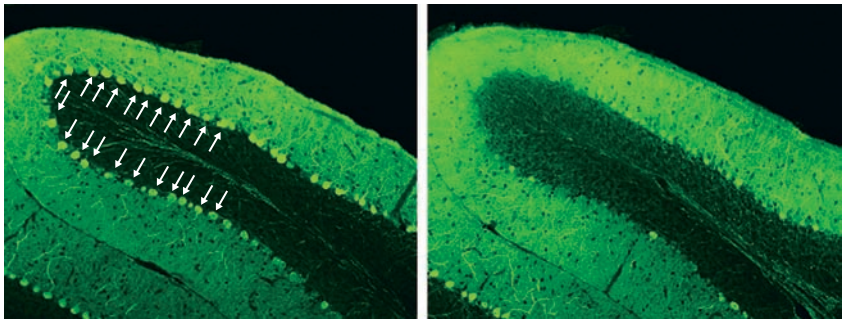


Figure 1: Cross-sections of the brain's cerebellum showing normal neurons (left) and ones lacking IP_3R1 (right), which results in abnormal cell death and brain damage under ER stress conditions.

"It has been suggested that neurodegenerative conditions including Huntington's disease are associated with deranged calcium signaling and ER stress," says Mikoshiba. "We hypothesize that IP_3R1 functions to protect the brain from ER stress, so development of a method to restore or enhance IP_3R1 could prevent disease progression or alleviate the symptoms. Our findings might be applied to other neurodegenerative diseases such as Alzheimer's disease." ■

1. Higo, T., Hamada, K., Hisatsune, C., Nukina, N., Hashikawa, T., Hattori, M., Nakamura, T. & Mikoshiba, K. Mechanism of ER stress-induced brain damage by IP_3 receptor. *Neuron* **68**, 865–878 (2010).

Fighting their way to the middle

Immune cells get switched off by the accumulation of dense clusters of inhibitory proteins

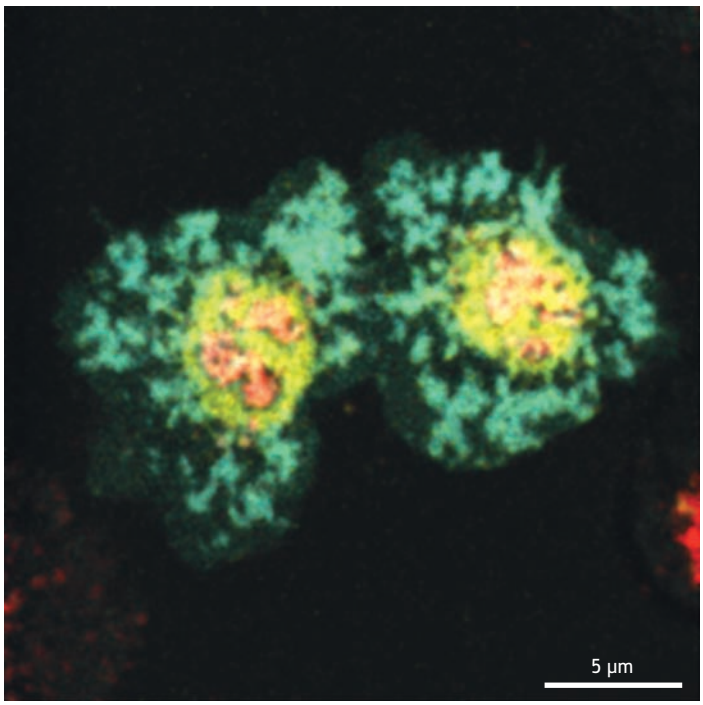


Figure 1: Over time, clusters of CTLA-4 molecules (yellow) gather alongside TCRs (red) within the cSMAC; in doing so, they displace CD28 (green) and suppress TCR signaling.

Foreign entities within the body get chopped into pieces by antigen-presenting cells (APCs), which display the resulting chunks on their surface. These antigens can subsequently be recognized and bound by T cell receptors (TCRs), and the interaction between a T cell and an antigen-bearing APC eventually triggers the onset of an immune response against the antigen.

Inappropriate responses by this system, however, can give rise to disastrous medical consequences, and there is keen interest in developing more sophisticated ways to modulate how T cells react to perceived threats. "Ultimately, we would like to regulate T cell function and activation in order to overcome autoimmune diseases, allergy, or infectious diseases," explains Takashi Saito, of the RIKEN Center for Allergy and Immunology (RCAI) in Yokohama.

New findings from a team led by Saito and RCAI colleague Tadashi Yokosuka could ultimately prove valuable for such efforts, by revealing insights into the mode of action of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), an inhibitor of TCR signaling[1]. When T cells associate with APCs, they form what is known as an 'immunological synapse', a juncture where numerous proteins assemble into elaborate complexes, such as the central supramolecular activation cluster (cSMAC). By establishing experimental conditions that simulate this cellular interaction, the researchers were able to monitor the dynamic rearrangements that take place at the cSMAC and its environs.

CD28, a T cell protein that promotes TCR signaling, typically assembles at a narrowly defined region of the cSMAC, where it interacts with CD80, a protein expressed on the surface of APCs. Saito, Yokosuka and colleagues determined that CTLA-4 gradually gathers at the immunological synapse and subsequently forms into clusters within the exact same area of the cSMAC as CD28, where it directly competes to bind CD80 (Fig. 1). "Positive regulation by CD28 and negative regulation by CTLA-4 are induced at the same place in the cell," says Saito. "Without accumulating at the cSMAC, CTLA-4 cannot inhibit T cell activation."

In addition to illuminating a mechanism by which T cell responses get fine-tuned, these findings could ultimately yield benefits for patients suffering from a variety of conditions. "Anti-CTLA-4 antibody therapy has been utilized for cancer patients, enhancing tumor immunity by inhibiting regulatory T cells, and CTLA-4-based fusion proteins have been used to block autoimmune diseases such as arthritis," says Saito. "Our findings will enable us to explore new therapeutic concepts based on the inhibition of the dynamic movement of regulatory molecules such as CTLA-4." ■

1. Yokosuka, T., Kobayashi, W., Takamatsu, M., Sakata-Sogawa, K., Zeng, H., Hashimoto-Tane, A., Yagita, H., Tokunaga, M. & Saito, T. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T Cell activation. *Immunity* **33**, 326–339 (2010).

Maintaining independence

A set of neighboring immune-system genes each receive separate activation instructions despite being controlled by a common factor

As part of the immune response to foreign antigens, naïve T cells mature into different types of helper T cells. T_H1 cells and T_H17 cells, for example, secrete a subset of signaling factors known as cytokines that promote inflammatory responses to viral infections, while T_H2 cells secrete cytokines that promote antibody secretion by B cells and drive allergic reactions.

The GATA-3 protein is known as a 'master switch' for T_H2 differentiation, stimulating production of cytokines such as interleukin (IL)-4 and IL-13, but new findings from a team led by Masato Kubo at the RIKEN Center for Allergy and Immunology in Yokohama have revealed an unexpected degree of complexity in this activation process[1].

"The idea that genes encoding T_H2 cytokines are coordinately regulated … has been widely accepted," says Kubo. Many of these genes are situated in the same chromosomal neighborhood, and some scientists believe that the chromosome physically loops so that DNA-bound GATA can regulate multiple sites simultaneously. However, Kubo and colleagues found that GATA appears to independently bind multiple, distinct sites that each confer regulatory control over individual T_H2-associated genes.

One of these sites, HS2, specifically governs IL-4 expression, and GATA binding at this site induces chemical modification of the DNA segment containing the *Il4* gene, leading to increased cytokine production. Naïve T cells from mice lacking this chromosomal region give rise to T_H2 cells that generate normal levels of most cytokines, but fail to produce IL-4; these animals also show fundamental defects in their allergic response.

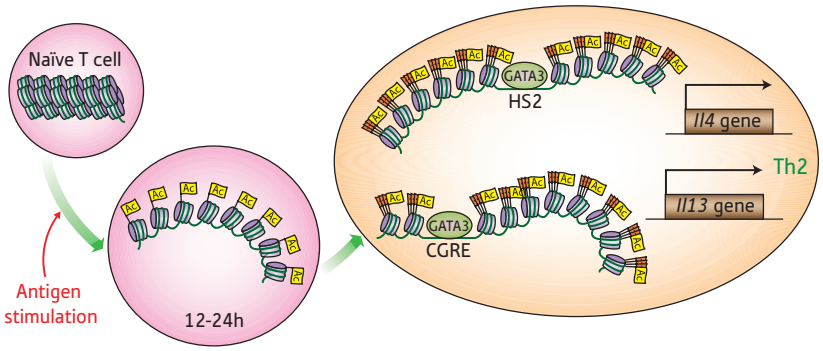


Figure 1: Naïve T cells (left) begin to mature into T_H2 cells within 12–24 hours of being stimulated by the presence of a foreign antigen (middle). As a component of this process, a 'master switch' protein drives the production of T_H2 cytokines IL-4 and IL-13 by binding to and promoting chemical modification in the vicinity of the HS2 or CGRE sites, respectively (right).

In parallel, the researchers identified a second GATA-binding site, CGRE, which specifically regulates production of IL-13. Like HS2, GATA interaction with this site is associated with targeted chemical modification of a nearby stretch of DNA containing the *Il13* gene, and disruption of CGRE essentially eliminates production of this cytokine while leaving IL-4 production unaffected (Fig. 1). "These results came as a surprise," says Kubo. "They indicate that the independent recruitment of GATA-3 to locus-specific regulatory elements controls the expression status of individual genes encoding T_H2 cytokines." These findings also parallel previous data suggesting that GATA coordinates expression of IL-5, another T_H2 cytokine, independently of IL-13.

Other types of immune cells also secrete T_H2 cytokines, and Kubo and colleagues now hope to determine whether their findings represent a broadly used mechanism for regulating production of these cytokines. "Our next priority will be exploring the relative contribution of these discrete elements to transcriptional regulation of IL-4 and IL-13 among these cell types," he says. ■

1. Tanaka, S., Motomura, Y., Suzuki, Y., Yagi, R., Inoue, H., Miyatake, S. & Kubo, M. The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells. *Nature Immunology* **12**, 77–85 (2011).

Caught in the act

An analysis of the interactions of a gene-reading enzyme with an inhibitor protein provides surprising insights

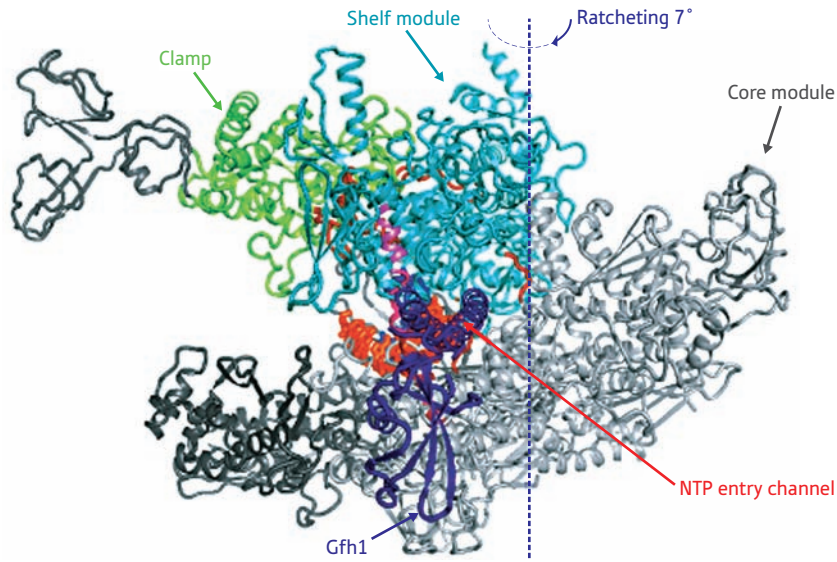


Figure 1: In the RNAP-Gfh1 protein complex the transcription factor (purple) inserts itself in the channel where nucleotides (NTPs) enter (red). This can only happen when the channel has expanded by a ratcheting motion that alters the relative position of the core module (gray) and shelf and clamp modules (light blue and green).

Within the cells, the RNA polymerase (RNAP) protein complex clutches DNA like a crab claw, scanning across gene-coding regions and transcribing these sequences into the messenger RNA molecules that will ultimately provide the blueprint for protein production.

This process can be impaired or assisted through interactions with proteins known as transcription factors, but understanding how these factors influence RNAP function can pose a serious challenge for structural biologists. "It is very difficult to crystallize RNAP, which is an unusually large enzyme," says Shigeyuki Yokoyama, director of the RIKEN Systems and Structural Biology Center in Yokohama. "In particular, no crystal structures of bacterial RNAP-transcription factor complexes have ever been reported." Recently, however, Yokoyama and colleagues successfully obtained a crystal structure that captures RNAP in the midst of transcription while bound to Gre factor homologue 1 (Gfh1), a transcription factor from the bacterium *Thermus thermophilus*[1].

RNAP consists of several discrete modules connected by flexible linker regions, with most of the enzymatic machinery residing in the 'shelf' and 'core' modules that serve as the main body of the RNAP 'claw'. In their structure, the researchers uncovered a never-before-seen arrangement of the RNAP modules, where some sort of 'ratcheting' action has created notable displacement between the shelf and core relative to its normal structure.

In fact, the binding of Gfh1 appears to lock RNAP into this configuration. This transcription factor—a known inhibitor—inserts itself into a channel on the complex that normally accepts nucleotides for addition onto newly synthesized RNA molecules (Fig. 1). However, such insertion would be impossible with the normal RNAP complex, where the channel is too narrow. This suggests that RNAP executes this unexpected ratcheting motion as part of its normal behavior, which in turn leaves it vulnerable to Gfh1 inhibition. "This conformational change was most surprising," says Yokoyama. "It was simply impossible to predict this before the structure of RNAP-Gfh1 was solved."

In subsequent biochemical experiments, he and his colleagues managed to essentially catch RNAP in the act of ratcheting, providing further evidence that this behavior occurs spontaneously in nature and is likely to contribute directly to the enzyme's transcriptional activity. "We hypothesize that RNAP uses this ratcheted state to slide along DNA chains as an intermediate step in the course of normal transcription," says Yokoyama. "This state may also be used an intermediate for transcriptional termination, in which the [RNA] dissociates from the RNAP." He adds that validating these and other hypotheses will be top priorities for future experimental efforts. ■

1. Tagami, S., Sekine, S., Kumarevel, T., Hino, N., Murayama, Y., Kamegamori, S., Yamamoto, M., Sakamoto, K. & Yokoyama, S. Crystal structure of bacterial RNA polymerase bound with a transcription inhibitor protein. *Nature* **468**, 978–982 (2010).

Gearing-up for spring

Rapid activation of specific genes readies the mammalian body for seasonal change

The genes that regulate the process called photoperiodism—the seasonal responses induced in organisms by changing day length—have been found by researchers from the RIKEN Center for Developmental Biology, Kobe, and Kinki University, Osaka. Led by Kohhei Masumoto and Hiroki R. Ueda from RIKEN, the researchers also discovered how these genes can be activated within a single day[1]. The work bears relevance to seasonal human disorders, such as winter depression, and symptoms associated with conditions such as bipolar disease.

Organisms need to alter body functions and behavior to accommodate seasonal changes in their environment (Fig. 1). The measurement of day length is one obvious way of determining the time of year. To this end, the body uses its internal circadian clock, and against this background measures the extent and timing of light and dark.

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Figure 1: Changes in the timing of light and dark in a mammal's environment trigger functional and behavioral changes.

The team noted that an increase in day length induces activity in the gene for thyroid stimulating hormone beta (*TSH*β) in the pars tuberalis (PT) region of the pituitary gland. *TSH*β plays a key role in the pathway that regulates photoperiodism in vertebrate animals. However, the detailed mechanism that links information about day length with induction of the production of *TSH*β is unknown.

Masumoto, Ueda and colleagues found the genes that stimulate the activity of the *TSH*β gene in mammals by observing the activity of genes in the PT of photoperiod-responsive mice under chronic 'short-day' (eight hours of light) and 'long-day' (16 hours) conditions. They identified 57 genes stimulated by short days and 246, including *TSH*β, by long days.

Then, the researchers placed chronic short-day mice into a long-day regime—they switched off the lights eight hours later—and observed that it took five days for *TSH*β to become fully active. They could, however, stimulate full activity of *TSH*β within a single 24-hour period if they subjected the mice to a short burst of light during a sensitive 'photo-inducible' period late at night. Thirty-four other long-day genes responded in the same way, including the transcription factor, *Eya3*, which seemed a likely candidate for regulating *TSH*β activity. In laboratory studies, the researchers determined that *Eya3* and its partner binding factor *Six1* do indeed act together to activate *TSH*β. And this activity is enhanced by two other genes, *Tef* and *Hlf*.

"We are next planning to identify the upstream gene of *Eya3*," Ueda says. "And we are also hoping to elucidate why the photo-inducible phase is late at night." ■

1. Masumoto, KH., Ukai-Tadenuma, M., Kasukawa, T., Nagano, M., Uno, K.D., Tsujino, K., Horikawa, K., Shigeyoshi, Y. & Ueda, H.R. Acute induction of *Eya3* by late-night light stimulation triggers *TSH* β expression in photoperiodism. *Current Biology* **20**, 2199–2206 (2010).

Simpler fabrication of nanogaps

A template-based coating techniques allows the production of gapped nanostructures over large areas

Plasmons, which are density waves of electrons, are of great interest to pure and applied scientists because of their novel properties, and because of their application to sensing and photonic technologies. These applications are possible because plasmons are sensitive to surface properties, and allow for the concentration of electric fields into small volumes. Fabricating the intricate nanostructures necessary to support plasmons, however, has proved a challenge. Now a straightforward fabrication technique, capable of generating plasmon-supporting nanogap structures over large areas, has been demonstrated by Wakana Kubo and Shigenori Fujikawa from the RIKEN Innovation Center, Wako, and the Japan Science and Technology Agency[1].

The researchers fabricated many copies of a structure consisting of two nested vertical gold cylinders, with the cylinders spaced apart by tens of nanometers. This structure, called a 'double nanopillar', was designed to support a highly concentrated electric field in the gap between the cylinders, in response to illumination with light. When the gap was filled with a liquid or gas, the optical properties of the double nanopillar changed, making it a useful sensor.

Typically, closely gapped structures such as the double nanopillar are fabricated individually by carving a polymer resist with an electron beam, but this process is slow and can pattern only small areas. Fujikawa and colleagues used a template-based coating process instead. They etched a silicon wafer to make a mold of periodically spaced holes, and applied the mold to a soft polymer film, resulting in an array of polymer pillars. They then coated these pillars with a gold layer, followed by a spacer, and a second gold layer. Finally, they removed the polymer film and spacer layers, leaving a double nanopillar array (Fig. 1). Using this process, the researchers could make a patterned area as large as the original template, and adapt it to include different spacer materials with finely controlled thicknesses.

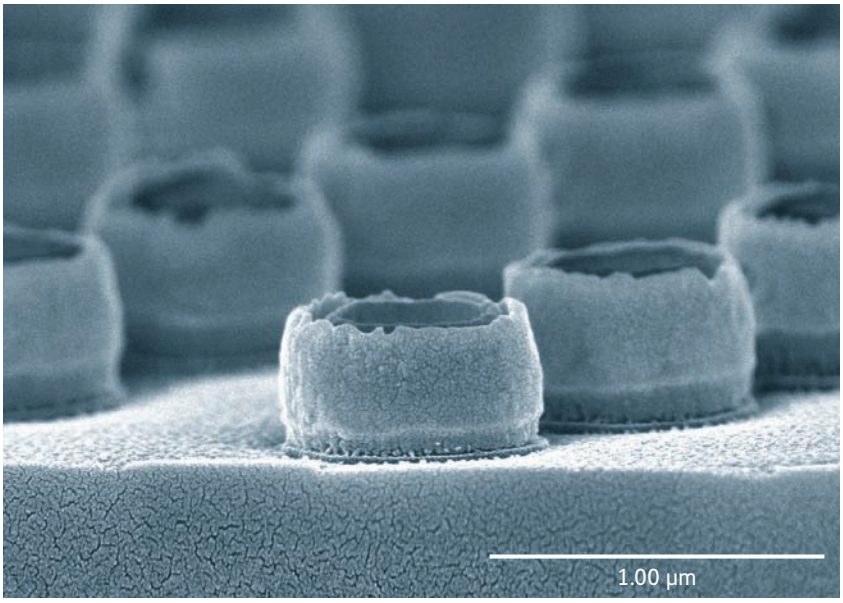


Figure 1: A micrograph of a completed gold double-nanopillar array.

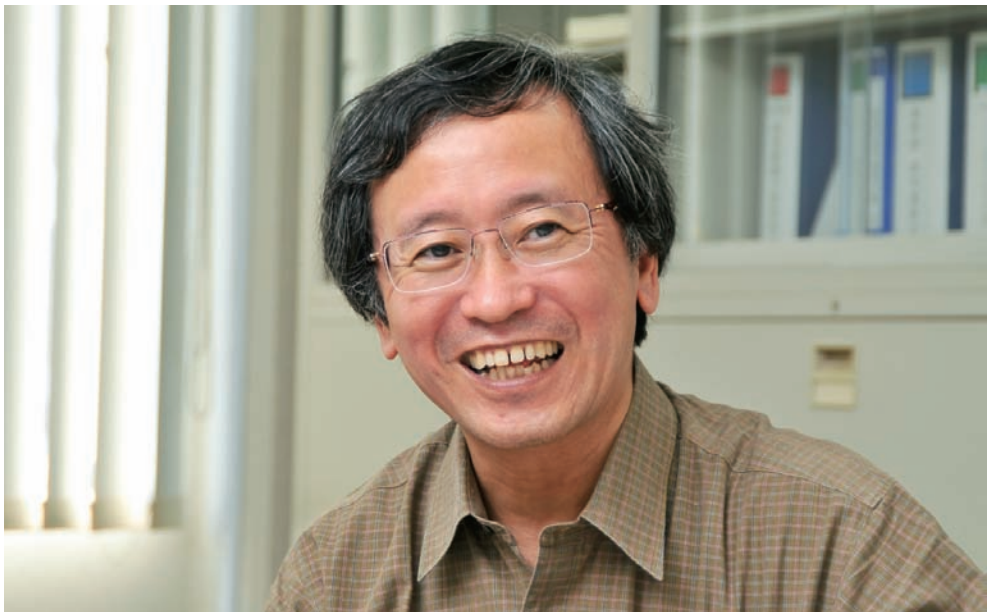
Kubo and Fujikawa tested the double nanopillars as sensors of refractive index, which showed sensitivities that were greater than sensors that had equivalent metal surface areas, but which did not have a nanoscale gap. This comparison demonstrated that the electric field in the double nanopillars was indeed highly concentrated. The new fabrication process marks just the beginning of an extended research program, says Fujikawa. "We do not fully understand the optical behavior of these nanostructures," he explains. "We will seek out collaborations with other researchers to investigate them further, and will try including magnetic, electric and organic materials into our process."

1. Kubo, W. & Fujikawa, S. Au double nanopillars with nanogap for plasmonic sensor. *Nano Letters* **11**, 8–15 (2011).

The physics of a sustainable society revolution

Yoshinori Tokura

Group Director
Cross-Correlated Materials Research Group
Correlated Electron Research Group
RIKEN Advanced Science Institute



Faced with global issues concerning the environment and energy and the need to build a sustainable society, we must develop new technologies for generating and using energy efficiently. Yoshinori Tokura, group director of the Correlated Electron Research Group and the Cross-correlated Materials Research Group at RIKEN's Advanced Science Institute, and his colleagues are working to develop electronic technologies based on new principles to allow information processing to be performed with minimal power consumption and more efficient conversion of light and heat to electrical power.

Innovative physics will lead the way

"I decided to become a scientist when I was a second-grade student at elementary school. Reading biographies of Nobel laureates, I admired scientists for contributing to society through their work. When I was young, the most famous scientists in Japan were the physicists Hideki Yukawa and Shinichiro Tomonaga, who inspired me to become a physicist," says Tokura. "Physics has led to major revolutions in human society," he points out. "A good example is electromagnetic induction discovered by the British physicist Michael Faraday in the nineteenth century."

Electromagnetic induction is the phenomenon by which an electric current flows through a coil when a magnet is inserted into the coil and pulled out again. Its discovery led to the development of power generators, thus laying the foundation for our electricity-powered modern society. Modern civilization is critically reliant on ubiquitous supply of electrical power, all of which has been built on the discovery of electromagnetic induction. "The recent spread of information technology devices, including mobile phones, personal computers and the Internet, has dramatically changed society and economies, and even our lifestyles. This major revolution began with the emergence of semiconductor electronics, with the development of the transistor about 60 years ago. Such breakthroughs are based on physics."

Tokura has a vision for another revolution, which he calls 'Innovation 4'. He believes that four key technological breakthroughs could once again change society as we know it: an increase in solar cell conversion efficiency to 40% or more, an increase in the thermoelectric conversion figure of merit to 4 or more, an increase in the critical temperature of superconductivity to 400 K or well above room temperature, and an in increase in battery energy density to 400 watt-hours per kilogram or more. "These numerical targets represent a tripling of existing performance indexes. Another goal is to achieve electronic information processing with minimal power consumption to conserve energy. If realized, 'Innovation 4' will lead to a sustainable society revolution, but it is difficult to achieve

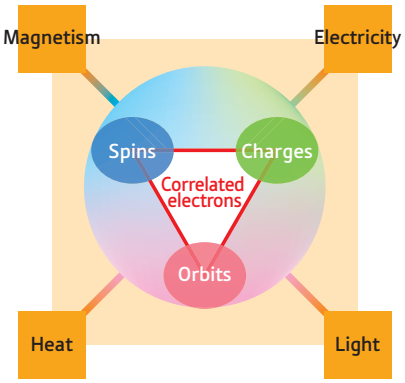


Figure 1: The principle of cross-correlation. By linking electron spins, charges and orbitals using a strongly correlated electron system, it is possible to achieve cross-correlation, an unusual form of responses of electricity, magnetism, light and heat.

these breakthroughs merely by improving existing technologies. We need to develop electronic technologies based on new principles."

Tokura and his colleagues have been researching electronic technologies based on principles that are totally different from the mainstream semiconductor electronics of today. "It is assumed that electrons are sparse in conventional semiconductor devices, so the entanglement of electrons is weak. A group of many densely packed electrons, however, interact strongly with each other in what is known as 'a strongly correlated electron system'. In such a system, non-charge properties that are not important in semiconductors, such as electron spin and orbital, also play important roles. We are seeking to create new functions that are not possible using independent electrons alone by utilizing the features of strongly correlated electron systems. High-temperature superconductivity is another phenomenon that occurs in strongly correlated electron systems. Electronic engineering still has infinite potential.

"The electron state in strongly correlated electron systems can be described as a solid produced by electrons. The electron state is like a dilute gas in semiconductors or a liquid in metals. Just as a liquid flows when the container is inclined, electricity flows when a voltage is applied to a metal. In a strongly correlated electron system, where the electron state is 'solid', electrons are unable to move because of mutual electrical repellence due to their dense packing. Even when a voltage is applied, no electricity flows. Hence, a strongly correlated electron system is an insulator, or specifically, a Mott insulator. When a minor stimulus such as heat, light or an electric field is applied from outside, a phase change from solid to liquid occurs instantaneously, allowing the electrons to move. In strongly correlated electron systems, this state can be changed at ultra-high speed on a nanometer scale."

A bridge across electron functions

"In a strongly correlated electron system, cross-correlation is possible," continues Tokura. "When a voltage is applied, an electric current flows. When a magnetic field is applied, the system becomes magnetized. These are the usual responses. By bridging different functions of the electron, unusual responses are induced. We call this phenomenon 'cross-correlation'.

A typical example of cross-correlation (Fig. 1) is the 'colossal' magnetoresistance effect, which was achieved with manganese oxide by Tokura in the 1990s. In this phenomenon, electric resistance decreases dramatically by a factor of one-thousand when a magnetic field is applied. This unusual response—a change in electrical resistance when a magnetic field is applied—is an example of cross-correlation. By utilizing a strongly correlated electron system, it is possible to produce a state in which an insulator lacking magnetization and a metal having magnetization compete with each other (Fig. 2). Cross-correlation allows two different functions of the electron to compete in a pair-like manner. When a magnetic field is applied to an insulator, it becomes magnetized and metallic, resulting in a dramatic drop in electric resistance.

Low-energy information processing

In 2007, Tokura established his own research group, the Cross-Correlated Materials Research Group, at RIKEN, and has since been conducting research on Innovation 4. "We aim to develop electronic technologies based on new principles for processing and recording information without conducting electrons."

Existing semiconductor devices process information by conducting electrons. However, this involves the use of electrical power, and energy is wasted in the form of waste heat generated due to electric resistance. The same applies to information recording. In hard disks, for example, an electric current is passed through a coil to generate a magnetic field to reverse the orientation of magnetization in a storage bit during information recording. This also requires

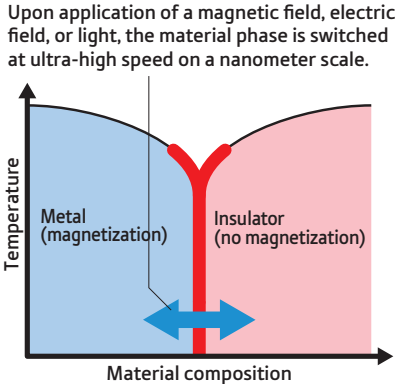


Figure 2: The principle of 'colossal' magnetoresistance. A state is created in which an insulator lacking magnetization and a metal with magnetization compete in a pair-like manner. When a magnetic field is applied to the insulator, it becomes magnetized and turns metallic, resulting in a dramatic decrease in electrical resistance. This rapid phase change can also be achieved by exposure to light or application of an electric field.

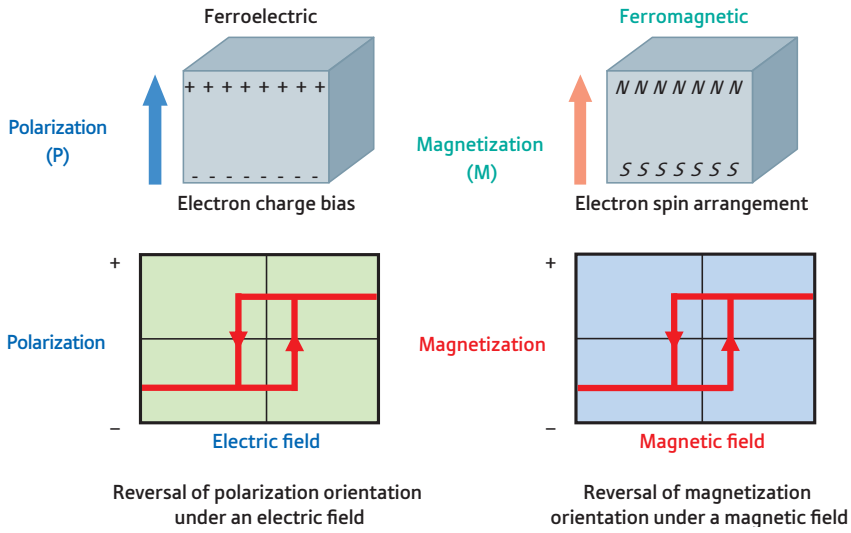


Figure 3: Ferroelectrics and ferromagnetics.
Ferroelectrics and ferromagnetics permit the orientations of electrical polarization and magnetization to be reversed by applying an electric field and magnetic field, respectively.

a electrical power, generating waste heat and wasting energy, and in large computers, the waste heat generated must be cooled using air-conditioners, consuming additional electrical power.

"If cross correlation, that is, the unusual inversion of magnetization using an electric field, rather than the inversion of magnetization using a magnetic field, could be achieved, it will be possible to record information without wasting energy and with minimal power consumption. We are working on using multiferroics to achieve this."

Multiferroics exhibit both ferroelectricity and ferromagnetism. A ferroelectric (Fig. 3) exhibits polarization, with one end positively charged and the other end negatively charged, even in the absence of an external electric field. When an electric field is applied to a ferroelectric, the two poles (+ and –) reverse themselves, allowing information to be rewritten. This phenomenon is used in some prepaid 'e-money' card systems for transport and shopping. A ferromagnetic, on the other hand, exhibits magnetization in the absence of a magnetic field, and the orientation of magnetization can be reversed by applying a magnetic field. Ferromagnetics are utilized in hard disks and other data recording devices. "In multiferroics, it is possible to realize the unusual response of reversing magnetization and simultaneously reversing the electrical polarization using an electric field by linking the orientations of electrical polarization and magnetization."

Polarization is caused by a bias in the distribution of electrons in a material, whereas magnetization occurs when electron spins line up, which otherwise can have either upward or downward orientations, become aligned in a given orientation. Electron spin thus serves as the origin of magnetization (Fig. 4).

"The orientation of polarization can be reversed by deforming the orbital in which the electron is accommodated. By utilizing a strongly correlated electron system of multiferroics, the orientation of electron spins can be reversed by deforming the orbital or electron cloud, which would make it possible to link the polarization and magnetization."

In 2009, Tokura and his colleagues succeeded in experimentally changing the orientation of magnetization at temperatures below –271 °C using an electric field. "If we can improve on this and simultaneously reverse the orientations of magnetization and polarization at room temperature using an electric field, then we will be able to create large-capacity memory that consumes almost no electrical power."

More recently in June 2010, Tokura's research group became the first in the world to directly observe skyrmion crystallization, the phenomenon by which electron spin vortices are regularly arranged like a crystal. The result attracted worldwide attention (Fig. 5). "It is thought that these electron spin vortices can be moved with a small amount of electric current. Hence, by merely changing the orientation of electron spins one after another, it is possible to move the electron spin vertexes to achieve information processing. This has potential

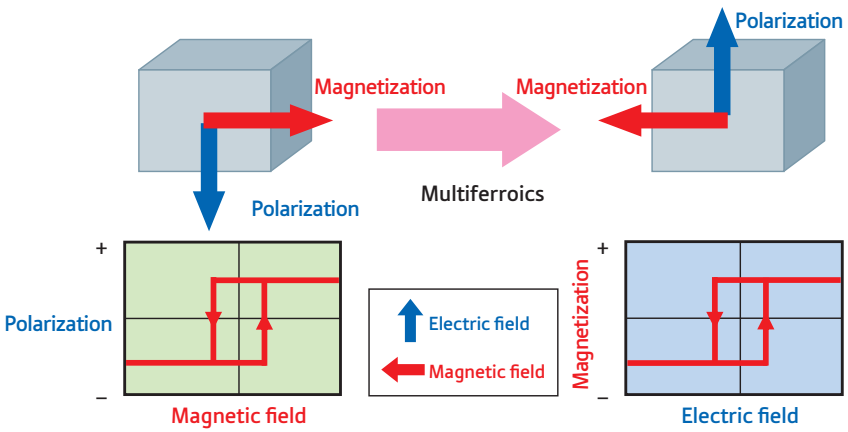


Figure 4: Reversal of magnetization in multiferroics using an electric field.
By linking the electrical polarization and magnetization using multiferroics exhibiting both ferroelectric and ferromagnetic properties, it is possible to reverse the orientations of electrical polarization and magnetization using a magnetic field and electric field, respectively.

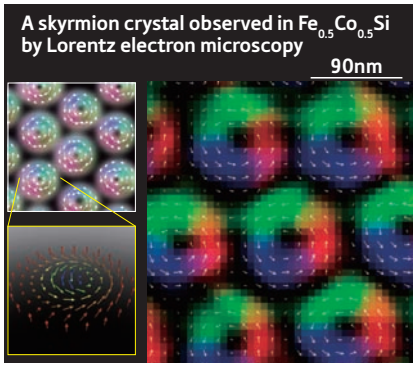


Figure 5: Skyrmion crystal.
Simulated spin structure of skyrmion crystal (left). A skyrmion crystal observed in $Fe_{0.5}Co_{0.5}Si$ by Lorentz electron microscopy (right). The skyrmion state, in which electron spins are arranged in vertexes, is estimated to be movable at currents much lower than conventional levels. Skyrmions are expected to be useful for information processing with low power consumption.

for information processing with minimal power consumption."

New principles for highly efficient solar cells

"We also have an idea for dramatically improving the power efficiency of solar cells," says Tokura. In conventional solar cells, a medium such as a semiconductor absorbs photons and generates a free pair of negative and positive charges. By separating the negative electron and positive 'hole' and transporting them to opposite electrodes, a voltage can be produced. The light-to-electricity conversion efficiencies of modern solar cells is just over 10%, but it should be possible to improve on this efficiency. "Solar radiation contains a broad range of wavelengths. Semiconductor solar cells actually achieve nearly 100% conversion efficiency at particular wavelengths of light, because electron–hole pair can be produced from a single photon at a particular wavelength in each semiconductor with nearly 100% probability. However, an electron–hole pair is also produced when a photon with a shorter wavelength and higher energy level is absorbed, in which case the excess energy is wasted as heat. This accounts for the low power efficiency. If we use a strongly correlated electron system, the wasted energy could be used to create a metallic state and produce a large number of electrons and holes by another mechanism, which could dramatically improve conversion efficiency (Fig. 6). Strongly correlated electron systems are being actively studied worldwide, but Tokura and his colleagues are the only group researching their use in highly efficient solar cells.

Basic science will build a bright future

"Physics will continue to yield major revolutions in human society. In recent years, however, it has become increasingly difficult for a single scientist to make a breakthrough alone."

The Japanese government this year established the Quantum Science on Strong Correlation project with the support of the Funding Program for World-Leading Innovative R&D on Science and Technology. RIKEN is responsible for providing research support, and Tokura serves as key investigator. For this project, he has established a dedicated research group, the Correlated Electron Research Group. "This project aims to set groundbreaking principles for realizing Innovation 4 through integrated joint research among outstanding researchers in a broad range of fields, including physics theory, thin-film growth, structural analysis and instrumentation measurement technology. However, if someone ordered me to produce results that could be used in practical applications within several years, I would struggle. Faraday, when asked about why his discovery of electromagnetic induction was so important, answered, "Who can predict what a newborn baby will become?" The usefulness of the results of basic research like ours is sometimes unpredictable. In the long term, 50 or 100 years, however, they have the potential to produce major revolutions and contribute greatly to future society."

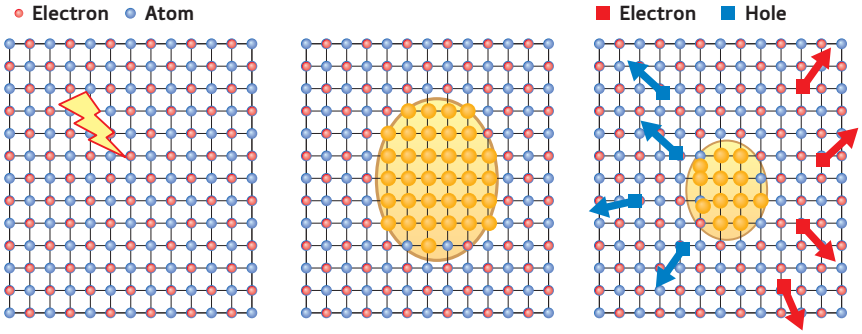


Figure 6: Principle of high-efficiency solar cells using a strongly correlated electron system.
When absorbed by a strongly correlated electron system (a 'solid' electron state), high-energy light 'melts' the solid to generate free electrons and holes, which could be separated and transported to opposite electrical to produce an electrical current.

Yoshinori Tokura

Yoshinori Tokura was born in Hyogo, Japan, in 1954. He obtained his DEng degree from The University of Tokyo's Graduate School of Engineering in 1981, and was appointed professor of the Department of Applied Physics in 1994. He has served as director of the Correlated Electron Research Center at the National Institute of Advanced Industrial Science and Technology, and as department head and group director at the RIKEN Advanced Science Institute since 2007. His research focuses on the exploration of materials and physics of correlated electrons, including metal–insulator transitions, multiferroics, high-temperature superconductors, colossal magnetoresistance, photo-induced phase transitions and organic ferroelectrics.

The Brain Science Institute holds twin symposiums on the mind

The RIKEN Brain Science Institute (BSI) recently held two symposiums as part of efforts to forge collaborative ties among widely disparate disciplines of academic inquiry.

The first of these events, a BSI symposium entitled 'Moral and Brain', was held on 27 January 2011 at the Suzuki Umetaro Hall on the RIKEN Wako campus. Supported with funding from the President's discretionary fund, this symposium is part of an ongoing program to explore connections between the humanities and neuroscience.

The event was launched with a keynote lecture by Paul Bloom, a Yale professor and well-known authority on developmental psychology. That lecture was followed by presentations from Atsushi Iriki, head of the BSI Laboratory for Symbolic Cognitive Development; Kazuo Okanoya, head of the BSI Laboratory for Biolinguistics; Toshio Yamagishi, a social psychology professor at the Hokkaido University Graduate School of Letters; and Yoshimichi Saito, professor of philosophy at Keio University. The event was modulated by Reiko Mazuka, head of the BSI Laboratory for Language Development. The latter half of the event was given over to lively discussion among the speakers and members of the audience.

Initially, there was concern that an event of this kind, bringing together the two completely different disciplines of humanities and science, would not attract many people. This proved not to be the case, however, with a strong showing of more than 200 participants from both RIKEN and other institutions indicating the high degree of interest in these topics.

The symposium focused on human and animal 'morality'. While the speakers had different views on how the concept should be defined, by the end of the event everyone was using the same terminology to communicate their various points of view. The resulting discussion was lively and engaging for all, a sure sign that important progress is being made toward the evolution of a new kind of 'whole human' science.

Invited Speakers at the 'Biology of Mind' symposium, including Hideyuki Okano (front center), Atsushi Iriki (back right), Keiji Tanaka (third from back right) and Shigeyoshi Itohara (third from back left).

The other symposium, on the 'Biology of the Mind—A Human Evolutionary Perspective', was held on 3 February 2011 at the Marunouchi Building Hall in downtown Tokyo. Jointly organized by the BSI and the Central Institute for Experimental Animals, the symposium was also part of the Strategic Exploitation of Neuro-Genetics for Emergence of Mind program headed by Hideyuki Okano, a professor at the Keio University School of Medicine and a Representative Researcher from the FIRST program (Funding Program for World-Leading Innovative R&D on Science and Technology). The event attracted a large audience of 360.

Okano is the lead researcher for a cutting-edge brain science research project focusing on mechanisms underlying the evolution of the mind and emotions. The project has attracted significant attention among the 30 FIRST projects currently underway as it goes well beyond basic and applied science, exploring areas traditionally reserved for the humanities and social sciences.

The symposium included lectures and discussions by 14 distinguished researchers in neuroscience as well as law and religion, and proved a fitting event for the launching of the Okano project with its far-reaching objectives.

Both of these symposiums focused from an academic perspective on what it is that makes us human, an area of inquiry that we are only just beginning to explore. By continuing to pursue this kind of collaboration with the humanities and social sciences, the BSI hopes to open up new avenues of thought and stimulate new discussions. ■

Tahei Tahara
Molecular Spectroscopy Laboratory
RIKEN Advanced Science Institute
Wako, Saitama, Japan

Dear Tahara-san,
Two months ago I returned to India after successfully completing two years of postdoctoral research work as a JSPS fellow in your laboratory at RIKEN. I am now working as an assistant professor in chemistry at Burdwan Raj College.

During my stay in Japan I fully enjoyed the top-class research work I had the opportunity to take part in as a member of your group. I am very fortunate to be engaged in advanced research topics like heterodyne-detected electronic sum frequency generation at the air/water interface, and to have used the research instrument at RIKEN, which is the only one of its kind in the world. Because of your excellent guidance and advice, I learned a lot not only about scientific matters but also many other things that have helped me in my personal life. The group meeting every week was particularly valuable, where I learned how to present a complicated topic in way that can be well understood by everybody. In just two years, my knowledge level and confidence increased tremendously, which I regard as the most valuable gift from being a member of your group. Among our research group I would especially like to thank Dr Yamaguchi, from whom I learned many things. He is not only a good scientist but also an excellent teacher.

Some of my warmest memories of my time at RIKEN are of the entertaining lab parties and barbeques. It was a pleasure to spend time with all my exceptionally helpful and friendly colleagues in your group. I am very grateful for all the help I received from them, and from the RIKEN staff at the ICO Room, who made my stay in Japan easy and enjoyable. The natural beauty of the campus is also something I remember fondly, especially in spring when the cherry blossoms are in bloom.

While studying in your laboratory I also had the opportunity to get to know the impressive research activities being conducted by other groups at RIKEN. The instrumental facilities and intense scientific environment of RIKEN is really impressive. RIKEN's Open House day is also one of the most unforgettable events of my time there. On that day, I was very happy to see the strong interest of Japanese people toward scientific research. The humanity, politeness, honesty, sincerity and culture of Japanese people are the things I liked most about Japan.

I would like to thank you, Prof. Tahara, for making my time at RIKEN so rewarding. I wish to always keep in touch, and hopefully I will see you again someday.

With best regards,

Sudip Kumar Mondal
Burdwan Raj College
Burdwan-713 104, WB, India

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