RIKEN RESEARCH

NOVEMBER
2008 Volume 3 Number 11

Break down for tissue development

www.rikenresearch.riken.jp

Brain waves

Electrical oscillations in one part of the brain suggest that it may interact with another to guide body movements

A seemingly simple action, such as picking up a pencil, actually involves complex communication between many parts of the central nervous system. Information about the pencil and its location enters the body through the eye, and eventually reaches a part of the brain called the somatosensory cortex. There, this information seems to be encoded as two types of brain waves: gamma waves, which oscillate 30–80 times per second, and very fast oscillations (VFOs), which oscillate 80–160 times per second. These brain rhythms may then be conveyed to other parts of the brain to initiate and control the action of reaching out an arm to pick up the pencil.

If other parts of the brain also produce gamma waves and VFOs, it is possible that these brain regions could receive these signals from the somatosensory cortex, and communicate with this or other portions of the cerebral cortex to control movements. In fact, recent work measuring brain waves from the cerebellum, the part of the brain responsible for motor learning, indicates that the cerebellum may communicate with the cerebral cortex to regulate movement. A team of researchers, including Steven Middleton and Thomas Knöpfel from the RIKEN Brain Science Institute (BSI), Wako, Miles Whittington from Newcastle University, United Kingdom, and Roger Traub, now at IBM in New York, report these findings in the journal *Neuron*[1].

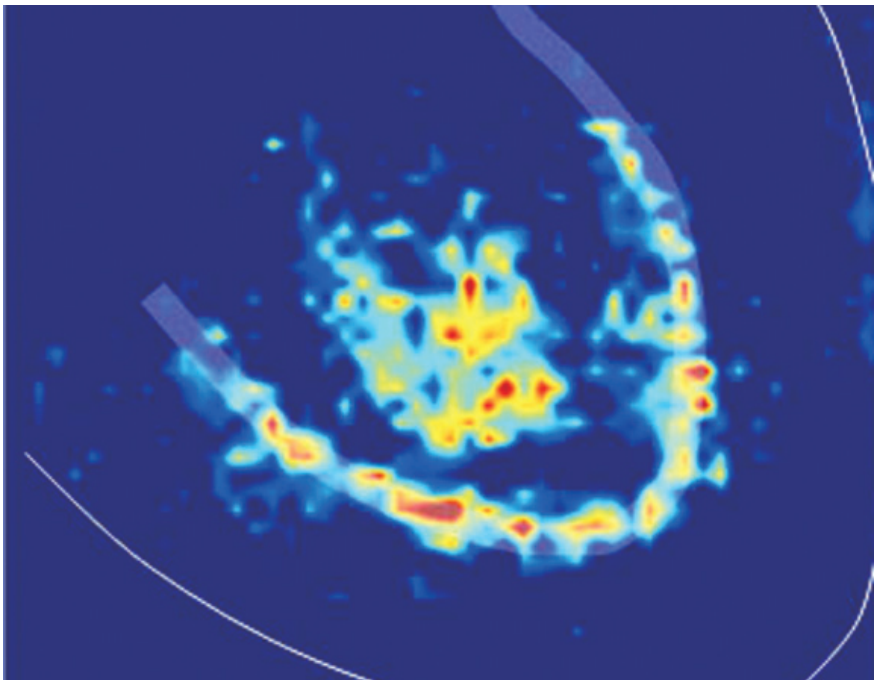


Figure 1: Optical imaging of brain waves using a voltage sensitive dye. Localization of the brain waves around cerebellar neuron cell bodies (thick white line) and their axons (the area enclosed by this line).

Tapping into brain waves

In slices from the mouse cerebellum that they had treated with nicotine, the researchers measured the frequency of oscillations using two methods: electrode recordings, and visualization of a voltage-sensitive dye (Fig. 1). By both methods, they found that the cerebellar oscillations were a mixture of gamma waves and VFOs. These waves were almost identical in frequency to oscillations others had measured in the cerebral cortex during the same experimental conditions. This frequency match suggests that the cerebellum and cerebral cortex may exchange signals to control movement.

The cerebral cortex contains many types of neurons that are both excitatory and inhibitory. The excitatory neurons, which use glutamate as their chemical neurotransmitter, play an important role in regulating the oscillations of the cerebral cortical neuronal network. The cerebellum also contains some excitatory (granule) cells, while the rest consists of inhibitory neurons, which use GABA (γ-aminobutyric acid) as their neurotransmitter. The researchers demonstrated that the granule cells were not involved in generating the brain waves, so it was surprising that they observed these oscillations at all, since they had to have been generated by inhibitory neuronal populations only. The findings therefore indicate that brain areas with vastly different neuronal compositions can still produce similar rhythms.

Middleton, Knöpfel and colleagues also found another important difference between the cerebellum and the cerebral cortex. Oscillations in both brain regions can be triggered by activation of receptors for the neurotransmitter acetylcholine; however, the receptors in the cortex are so-called muscarinic receptors, which are not activated by nicotine, whereas the receptors in the cerebellum are triggered

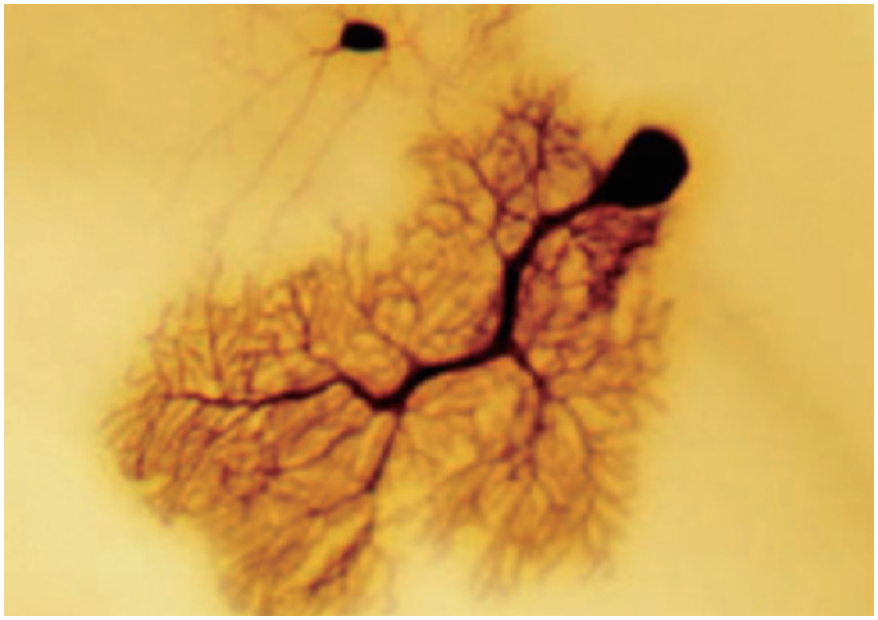


Figure 2: Dye-coupling between a cerebellar output neuron (bottom) and an interneuron (top).

by nicotine. Furthermore, the cerebellar nicotine receptor that is acting to induce the brain waves seemed to be a 'nonclassical' nicotine receptor.

Unraveling neuronal communication

The network oscillations in the cerebral cortex occur due, in part, to gap junctions between cortical neurons, in which electrical activity in one cell can spread through channels that connect that neuron directly to its partner. The researchers also found many pieces of evidence that suggest that electrical connections also exist between cerebellar neurons.

First, they showed that a dye injected into a cerebellar output neuron, called the Purkinje cell, could diffuse to its neighboring local cerebellar interneuron, called a basket cell or a stellate cell (Fig. 2). Then, they blocked all chemical communication that occurs in the spaces between neurons, called 'synaptic neurotransmission', by removing calcium ions from the solution bathing the cerebellar slices, and still observed VFOs. Finally, they blocked gap junctions with a drug, and this manipulation was sufficient to block both the gamma waves and the VFOs. Their results suggest that direct electrical connections between cerebellar neurons may be one mechanism by which network oscillations are regulated.

Visualizing the source of brain waves

Middleton, Knöpfel and colleagues then used electrical and optical recordings to pinpoint the area of the cerebellum which was responsible for generating the gamma waves and the VFOs. "Optical voltage imaging is a technique for which the RIKEN BSI Laboratory for Neuronal Circuit Dynamics attains world-wide recognition," says Knöpfel. "We are expecting that the use of optical voltage imaging in this research field will increase over the coming years."

The researchers also confirmed their findings in slices from the human cerebellum, suggesting that the data could also be relevant to motor function in humans. Because the oscillations were stimulated by nicotine, the findings imply that nicotine from cigarette smoking may have effects on motion—such as tremor—owing to effects on network oscillations in the cerebellum.

This research provides insight into how the cerebellum and cerebral cortex may communicate with each other to create, organize, and control movements. The researchers believe that their work establishes a new approach to the understanding of how the cerebellum handles information, suggesting that, as in cerebral cortex, oscillations are used for temporal coding of information.

"Startup of this exciting new research was made possible through a generous one-year grant from the directors' fund of former BSI director Shunichi Amari," explains Knöpfel. "While we have established the mechanisms underlying cerebellar oscillation generation, we now aim to study the behavioral correlates of these rhythms," say Middleton and Knöpfel.

1. Middleton, S.J., Racca, C., Cunningham, M.O., Traub, R.D., Monyer, H., Knöpfel, T., Schofield, I.S., Jenkins, A. & Whittington, M.A. High-frequency network oscillations in cerebellar cortex. *Neuron* **58,** 763–774 (2008).

About the researchers

Steven Middleton was born in Blackpool, UK, in 1981. After graduating in biochemistry from Lancaster University, UK, he obtained his PhD in 2005 at Leeds University, UK. After two years postdoctoral training at Newcastle University, UK, during which time he visited RIKEN Brain Science Institute (BSI) under a collaboration between Newcastle University and RIKEN BSI. In 2007 he joined the laboratory for Neuronal Circuit Dynamics at RIKEN BSI as a research scientist, where his research addresses the complex neuronal network dynamics seen in EEGs (electroencephalographs) that underlie cognitive processes.

Thomas Knöpfel was born in 1959 in Germany. He earned his MD in 1984 and his Master of Science in 1985, both from the University of Ulm. He then obtained his doctorate in physiology in 1985 and privatdozent (PD) in 1992, both from the University of Zurich, Switzerland. He was assistant (1985) and assistant professor (1989) at the Brain Science Institute, University of Zurich. In 1992, he became a team leader and project leader at Ciba-Geigy Pharmaceuticals (now Novartis). After working as a guest scientist at University College London in 1996, he joined the RIKEN Brain Science Institute as head of the Laboratory for Neuronal Circuit Dynamics in 1998.

New view on an archetypical oxide

New experiments and calculations clarify the origin of the insulating behavior of nickel oxide

Owing to its simplicity in chemical composition and crystal structure, nickel oxide (NiO) is a model system for the understanding of a whole family of oxide compounds. However, even for such a basic two-atom compound the physics governing its physical properties can be rather complex. Indeed, a detailed fundamental understanding as to why this basic oxide is insulating rather than conductive has eluded physicists since the 1930s. Now researchers from the RIKEN SPring-8 Center in Harima have uncovered the fundamentals of the material's insulating behavior.

Based on existing knowledge in the 1930s, physicists initially assumed that all the bound electrons in NiO were situated at the oxygen atoms (light blue stripe in Fig. 1), while the free electrons at the top of the so-called valence band would occupy electronic states of nickel (dark blue stripe). From the 1950s to 1970s, the insulating nature of NiO was thought to result from strong interactions between the nickel electrons (Mott insulation). Then, in the 1980s, oxygen states were considered to be the ones at the top of the valence band causing the insulating behavior of NiO (the interchanged position of the stripes in Fig. 1).

To unravel the details of electronic states in the valence band of NiO, the RIKEN researchers completed a detailed investigation using photoemission spectroscopy[1]. In these experiments, NiO was irradiated by x-rays, and the energy and number of electrons that were emitted as a consequence of the x-ray bombardment were measured. The energy and intensity distribution of the emitted electrons revealed many details of the material's electronic structure, from which the origin of the valence band structure can be derived.

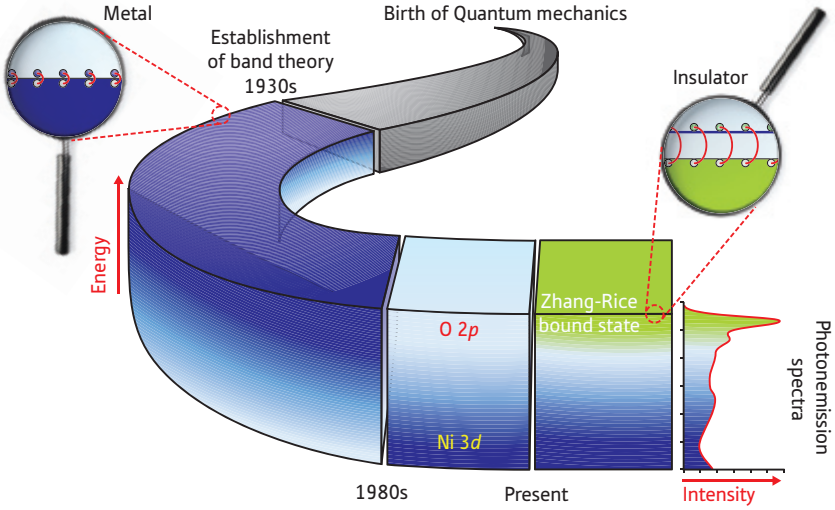


Figure 1: A history of the assumed electronic states of NiO. In the 1930s, NiO was thought to be a metal, with high energy Ni 3*d* orbital states (dark blue) at the top of the valence band providing the necessary electrons. In the 1980s, the O 2*p* orbital states were considered the origin of the actual insulating behavior of the material. Now, Zhang-Rice states are identified in photoemission spectra as the main electronic states at the top of the valence band.

In the case of NiO, "these experiments and its calculations clearly show that the top valence band is not a simple oxygen band but a so-called Zhang-Rice bound state," comments Munetaka Taguchi from the research team. These Zhang-Rice states are a hybrid between the nickel and oxygen electronic states that arise out of the complex interaction of the atoms that make up the material.

Previously, Zhang-Rice states have only been observed in the high-temperature superconductor compounds that consist of more atoms and form far more complex electronic states. Therefore, the observation of Zhang-Rice states in a basic oxide has far-reaching implications for oxide materials more generally. "Our results indicate that the Zhang-Rice states are by no means limited to specific high-temperature superconductors but are a universal behavior for many oxide materials," explains Taguchi. Once more, NiO has proven itself a model system. ■

1. Taguchi, M., Matsunami, M., Ishida, Y., Eguchi, R., Chainani, A., Takata, Y., Yabashi, M., Tamasaku, K., Nishino, Y., Ishikawa, T. *et al.* Revisiting the valence-band and core-level photoemission spectra of NiO. *Physical Review Letters* **100,** 206401 (2008).

Crystal quest brings success

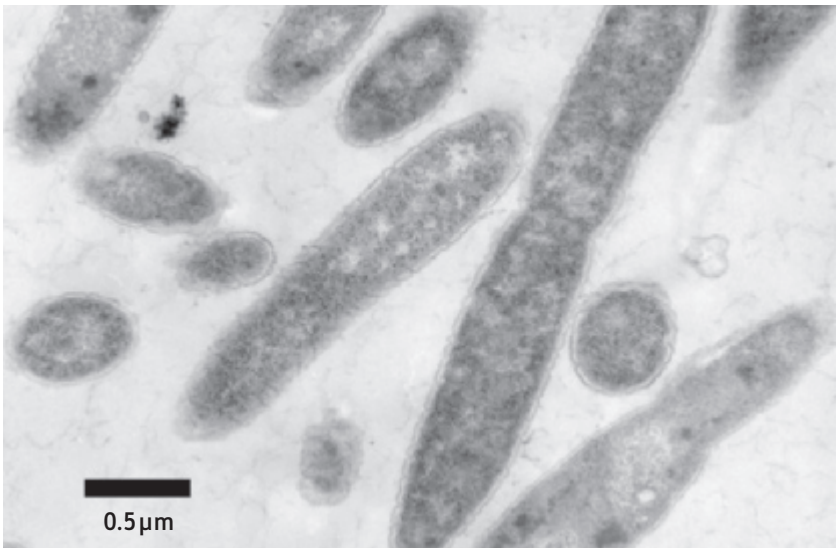
Study obtains protein structures more efficiently using a combination of techniques

The chances of obtaining crystals of sufficient quality and quantity to allow determination of three-dimensional protein structures using synchrotron radiation are significantly increased using a mix of robots geared to different crystallization techniques. That is the conclusion of a screening study by researchers in Japan, led by Seiki Kuramistu of RIKEN's SPring-8 Center in Harima, recently reported in *Acta Crystallographica*[1].

The work was part of the whole-cell project on the bacterium *Thermus thermophilus* HB8 (Fig. 1), which is found naturally in hot springs at temperatures of up to 85 °C. The aim of the project is to increase understanding of cells at a molecular level by determining the structures and functions of all proteins encoded by genes. *Thermus* was chosen as a model organism because it has a minimal set of genes which codes for about 2,000 proteins which are highly stable for analysis and more than 70% of which have human equivalents.

The standard means of determining protein structure, x-ray crystallography, involves aligning protein molecules into a lattice of repeating series of 'unit cells', and then passing x-rays through the resulting crystal. The structure of the protein is 'solved' by analyzing the resulting diffraction pattern.

But proteins are of irregular shape and the protein lattice is held together only by relatively weak electrostatic forces. So protein crystals are generally fragile and highly sensitive to environmental conditions. These must be adjusted to optimum levels for crystallization. At best it takes several hours to grow crystals suitable for data collection, but typically it takes months. Thus, protein crystallization has proved a major bottleneck in the whole-cell project.



2008 Seiki Kuramitsu

Figure 1: An electron micrograph of *Thermus thermophilus* HB8.

In an attempt to increase efficiency, the researchers used 18 sample proteins from *Thermus* to test the capabilities of robots which use different techniques to crystallize proteins—sitting-drop vapor diffusion, hanging-drop vapor diffusion and a modified microbatch technique. They also trialed a microfluidic device designed to rapidly determine the best initial conditions, but which could not produce crystals in large enough quantities for diffraction.

The research team found that both vapor diffusion robots produced diffraction-quality crystals quicker than the microbatch robot—the sitting-drop being the faster. The microbatch robot, however, was most likely to be successful. The microfluidic device outperformed the other three on both counts. On the basis of these results the researchers used a combination of a sitting-drop and a microbatch robot to successfully determine structures for 360 of 944 purified proteins for the whole-cell project. ■

1. Iino, H., Naitow, H., Nakamura, Y., Nakagawa, N., Agari, Y., Kanagawa, M., Ebihara, A., Shinkai, A., Sugahara, M., Miyano, M., *et al.* Crystallization screening test for the whole-cell project on *Thermus thermophilus* HB8. *Acta Crystallographica* **F64,** 487–491 (2008).

Metamaterials shake up electrons

New man-made materials could produce unique chaotic motion in electron beams

A team at the RIKEN Advanced Science Institute in Wako has predicted that man-made structures called metamaterials could produce instabilities in electron beams[1]. The effect could provide new methods for generating and amplifying optical signals.

Metamaterials are often known as left-handed media (LHM) because they break the right-hand rule of electromagnetism. This means that the 'envelope' of a wave—created by changes in wave height—in LHM can move in the opposite direction to the wave's overall motion. This is expected to produce phenomena similar to backward wave oscillators, which are common sources of microwave radiation (Fig. 1).

"Any system that contains two oppositely directed fluxes of information can be unstable if the coupling between the information carriers (waves and electrons in our case) is strong enough," explains RIKEN scientist Yuriy Bliokh, also at Technion-Israel Institute of Technology in Haifa.

The coupling between carriers in LHM is provided by Cherenkov radiation—a type of radiation emitted when a charged particle passes through an insulator at a speed faster than the speed of light in the insulator. It is responsible for the blue glow in nuclear reactors, and propagates from a particle beam just like the wake from a moving ship.

In LHM, Cherenkov radiation moves backwards, providing strong feedback for particles moving behind. In particular, two electron beams side-by-side could excite each other via their Cherenkov radiation, producing unstable, chaotic motion in the beams.

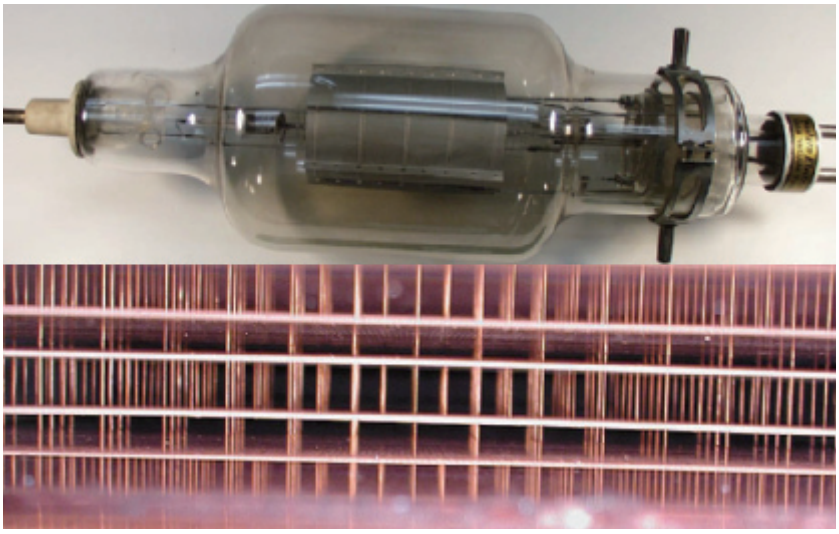


Figure 1: A backward wave oscillator (top), which generates microwave radiation by passing an electron beam through a 'slow-wave structure'. Similar devices could incorporate man-made metamaterials, or 'left-handed media' (bottom) in place of the slow-wave structure to generate tuned visible or infrared radiation.

To investigate these effects, Bliokh and RIKEN co-workers Sergey Savel'ev, also at Loughborough University, UK, and Franco Nori, also at the University of Michigan, USA, developed a model which solves the equations of motion for two electron beams passing through LHM, and calculates the total electric field generated. "Small perturbations in the beam density were introduced to represent fluctuations that can occur in the real world," says Savel'ev.

The small perturbations developed into large instabilities, causing the beam to excite itself. "The behavior resembles beam instabilities that have been discovered in both plasma physics and microwave electronics," says Nori, and could have several applications if a suitable LHM can be realized in the laboratory.

"From my point of view, the most interesting applications would be in the short-wavelength (infrared, visible light) range, because there are already so many devices in the microwave frequency band," says Bliokh. "This effect could provide tunable sources of regular or stochastic radiation. Also, when the beam current is low, the instability is not developed and the system could be used as an amplifier." ■

1. Bliokh, Y.P., Savel′ev, S. & Nori, F. Electron-beam instability in left-handed media. *Physical Review Letters* **100,** 244803 (2008).

Color to the nanoworld

New design concept for a tiny metallic lens tipped to revolutionize imaging of nanoscale objects

Researchers in Japan have developed a design concept for a device that allows imaging at scales previously impossible for optical instruments. Their advance is based on novel imaging techniques that allow optical imaging in the subwavelength regime, where the wavelength used is larger than the smallest features of the object being imaged. However, although subwavelength imaging is capable of significantly expanding the resolution of optical microscopes, a drawback of existing designs is that they only work at a single wavelength and in close proximity to a sample.

The approach developed by Satoshi Kawata's team, with members from RIKEN's Advanced Science Institute, on the other hand, allows subwavelength imaging in color and at large distances from the sample. "Such nanolenses could be used to directly image viruses or the distribution of proteins in cell membranes," says Kawata, commenting on the promise of the research.

Subwavelength imaging is based on plasmonic resonances, which are collective motions of electrons at the surface of a metal that can significantly amplify light waves in the vicinity of a metal. These amplified light fields can then be used for subwavelength imaging. However, these light fields decay rapidly away from the metal, so their use in actual imaging applications is rather limited.

Reporting in *Nature Photonics*, the researchers present a device concept that offers a promising solution to this problem[1]. Similar to a relay race, light is passed along a chain of silver nanorods (Fig. 1). A full image can be transmitted when a large number of stacked rods are bundled together.

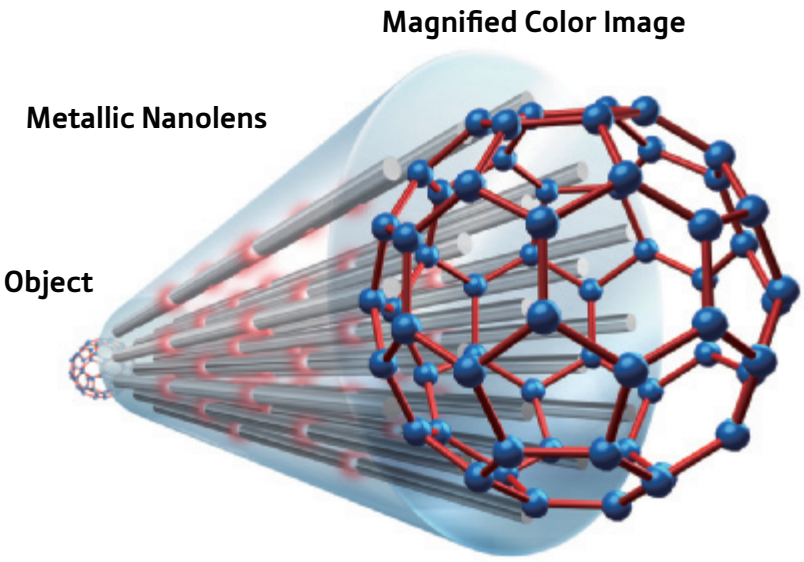


Figure 1: A metallic nanolens. Plasmonic resonances along stacked silver nanorods are capable of long-distance image transfer and magnification.

The gaps along the stacked nanorods play an important function, despite appearing cumbersome. Firstly, the plasmonic resonances in the gaps replenish the transmitted light field that would decay rapidly along longer nanorods. Secondly, the gaps perturb the plasmonic resonances along the transmission line. As calculations by the researchers have revealed, this broadens the wavelength range that the rods can transmit, and enables the transmission of different colors along the structure. Finally, magnification of the light can be achieved if the rods are arranged such that they are tapered, which gradually expands the image as it is transmitted along the rods.

With such promising predictions, the next step will be to build a device based on this concept. Whilst the researchers are continuing to improve their device design, Kawata is certain that "this invention will replace conventional lens-based optical microscopes with metallic nanolenses capable of extremely high resolution." ■

1. Kawata, S., Ono, A. & Verma, P. Subwavelength colour imaging with a metallic nanolens. *Nature Photonics* **2**, 438–442 (2008).

Critical questions

Ripples in the structure of graphene could be the key to understanding its unusual characteristics

Graphene is a two-dimensional material that consists of a hexagonal array of carbon just one atom thick (Fig. 1). Although it is essentially just a single sheet of graphite, its properties are remarkable and unique. Notably, its charge carriers behave like massless relativistic particles, and move at a speed of just 300 times less than the speed of light—many times more quickly than in silicon. This makes graphene a potentially attractive alternative to silicon as future computer chips.

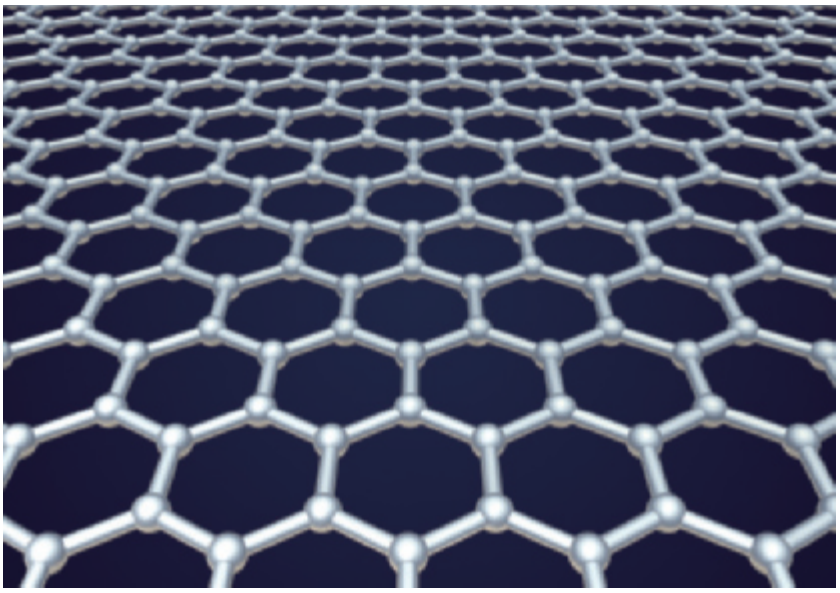
Many questions remain about graphene. A numerical study conducted by an international team of physicists including Akira Furusaki of RIKEN's Advanced Science Institute in Wako, attempts to explain the unusual quantum Hall effect that arises in graphene, and the influence of disorder of its 2D structure on its behavior[1].

The quantum Hall effect occurs in metal-like systems whose electrons are confined to move only in a two-dimensional plane. It is characterized by the emergence of plateaus in the conductance measured transverse to the flow of current through the system—known as the Hall conductance—when a large magnetic field is applied through the plane.

In graphene, the quantum Hall effect is subtly different to that in other 2D systems. Normally, the Hall conductance begins at zero and increases in exact increments, described as e^2/h, with increasing magnetic field or charge concentration. In graphene, however, the conductance changes in multiples of $4e^2/h$ and the whole characteristic is shifted by half this value.

Moreover, in most systems it is usually destroyed by disorder or by thermal fluctuations at temperatures much above absolute zero. But in graphene, it is remarkably insensitive to both, with the Hall plateaus around zero conductivity evident all the way up to room temperature.

The simulations performed by Furusaki and colleagues suggest that the robustness of the quantum Hall effect in graphene arises as a result of the relativistic nature of its charge carriers. Under certain amounts of disorder, the wavefunctions of zero-energy carrier states do not become localized in the same way as those of nonrelativistic carriers in conventional quantum Hall systems would. The researchers argue that the occurrence of such nonlocalized states—known as critical states—could explain why the initial Hall plateaus occur at +/- $2e^2/h$, rather than at zero before increasing in multiples of $4e^2/h$. Moreover, they argue that the expected occurrence of ripples in graphene's structure could be enough to cause these nonlocalized states to emerge. ■



source: Wikimedia/Thomas Szkopek

Figure 1: **Graphene consists of a single layer of carbon atoms arranged in a hexagonal array. Its structure and two-dimensional nature gives rise to its unique and potentially useful electronic characteristics.**

1. Nomura, K., Ryu, S., Koshino, M., Mudry, C. & Furusaki, A. Quantum Hall effect of massless Dirac fermions in a vanishing magnetic field. *Physical Review Letters* **100,** 246806 (2008).

Carbon gets extra bonds

Mapping of electron distribution in highly unusual hypervalent atom will advance our understanding of rare carbon compounds

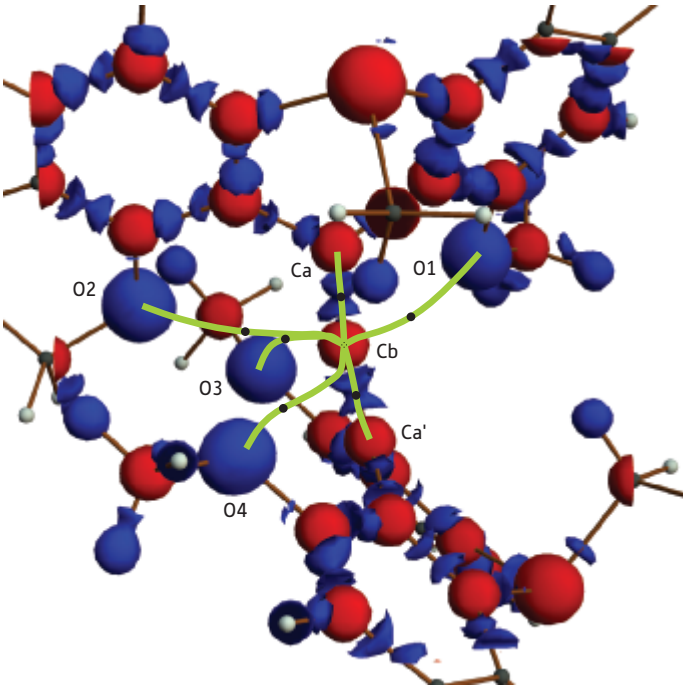


Figure 1: This map of the electron density in the molecule shows that the central carbon atom (Cb) is connected by six bonds (green).

Carbon atoms are the building blocks for millions of organic molecules, yet this variety is built on the simple rule that carbon almost always shares a total of four chemical bonds with its neighbors. Now, an international team of chemists has mapped a highly unusual compound containing a carbon atom that hooks up to six atoms at once[1].

The chemical bonds in organic compounds are made from pairs of electrons, whose negative charge sticks atoms together. Atoms with more than four chemical bonds are called hypervalent, which is common in elements such as phosphorus and sulfur, but very rare in carbon.

Hypervalent carbon atoms are not just a curiosity, explains team-member Daisuke Hashizume of RIKEN's Advanced Science Institute in Wako. "Carbon is the most important atom in chemistry," he says. When carbon-based organic molecules react, the atomic rearrangement involved usually creates short-lived hypervalent intermediates. Studying more stable hypervalent compounds can help to explain why certain chemical reactions proceed in particular ways.

Scientists at Hiroshima and Waseda Universities first created a compound containing a central carbon atom connected to two flat anthracene groups that carried a total of four dangling oxygen atoms. A chemical reaction then added positive charges to the anthracene groups, drawing electrons from the oxygen atoms to form a bond with the central carbon atom, making it hypervalent.

Then Hashizume's team—working with colleagues at the University of California, Riverside, and the Rigaku Corporation—analyzed exactly how electrons were distributed in the molecule, to confirm its bonding pattern.

The team used x-ray diffraction at RIKEN's SPring-8 Center to map the positions of atoms and the electron density in the molecule, and confirmed that the oxygen atoms were close enough to the carbon to form genuine chemical bonds. They also found that electrons originally located around the oxygen atoms had shifted towards the central carbon atom. These bonding electron pairs are spread between three atoms, as opposed to the usual two, says Hashizume.

Although some hexavalent carbon compounds are already known, this is the first time that such a detailed analysis of how the electrons are distributed around the molecule has been done. "We have confirmed all six bonds on the carbon atom experimentally, not theoretically," explains Hashizume (Fig. 1).

The results will help scientists to synthesize related compounds, says Hashizume, and to study reaction intermediates in more detail. "The next stage is to isolate and see the electronic structure of reaction intermediate species, to give us a deeper understanding of chemical reactions." ■

1. Yamaguchi, T., Yamamoto, Y., Kinoshita, D., Akiba, K., Zhang, Y., Reed, C.A., Hashizume, D. & Iwasaki, F. Synthesis and structure of a hexacoordinate carbon compound. *Journal of the American Chemical Society* **130,** 6894–6895 (2008).

Copper catalyst recycles carbon dioxide

Versatile reaction could help greenhouse gas become a more useful synthetic chemical

RIKEN chemists have developed a catalyst that should allow carbon dioxide to be used as a versatile synthetic chemical.

Carbon dioxide (CO_2) is produced whenever fossil fuels are burned (Fig. 1), and it is a powerful greenhouse gas that traps heat in our atmosphere, contributing to global warming. As such, turning the gas into a chemical feedstock, rather than allowing it to escape into the atmosphere, is an extremely appealing idea.

In fact, industry has long used carbon dioxide as a chemical building block—in the manufacture of the painkiller aspirin, for example—but its use is limited by the difficulty of breaking open its strong carbon-oxygen double bonds.

Carbon compounds activated by lithium or magnesium are often needed to attack and incorporate carbon dioxide successfully, but these reagents are extremely reactive and quite hazardous on a large scale.

Chemists have recently developed milder, boron-based alternatives, which require a rhodium catalyst to speed up the reaction. Unfortunately, this catalyst tends to break down particularly sensitive chemical groups in the product.

Zhaomin Hou, of RIKEN's Advanced Science Institute, Wako, along with colleagues Takeshi Ohishi and Masayoshi Nishiura, has now developed a copper catalyst that helps the boron compounds to react with carbon dioxide without destroying sensitive chemical groups.

This makes the reaction particularly useful for building complex molecules containing several different types of chemical group, something that would not be possible with the harsh lithium reagents. "We have tried many different metal compounds, among which the copper catalyst was the best," says Hou.

Figure 1: Carbon dioxide is a greenhouse gas which accelerates global warming—but it could also become a versatile synthetic chemical.

The team was also able to study exactly how the catalyst works, by isolating key molecules at various intermediate stages of the reaction. They found that the active copper catalyst first displaces the boron group from the starting molecule, forming a new copper–carbon bond. Carbon dioxide then inserts itself into this bond before the copper catalyst is finally removed, leaving behind a carboxylic acid ($-CO_2H$) group[1].

Various forms of the boron compounds, known as boronic esters, are commercially available, says Hou. "And they can also be easily prepared in the lab."

Hou adds that their method is also amenable to large-scale, commercial synthesis. "Since CO_2 is a renewable carbon resource, exploration of new reactions and catalysts for its efficient use is of great importance," he says. "One of our goals is to find a catalyst that can transform CO_2 in exhaust gasses of automobile vehicles or chemical plants into useful materials." ■

1. Ohishi, T., Nishiura, M. & Hou, Z. Carboxylation of organoboronic esters catalyzed by N-heterocyclic carbene copper(I) complexes. *Angewandte Chemie International Edition* **47**, 5792–5795 (2008).

Structural 'snapshots' of a protein implicated in Alzheimer's disease

New experiments reveal detailed physical features of a protein thought to exacerbate the pathology of Alzheimer's disease

A recent study describes the structure of the active form of BACE1, which is an enzyme implicated in Alzheimer's disease. BACE1 cleaves amyloid precursor protein (APP), thereby releasing amyloid β peptide (Aβ), the primary component of amyloid plaques found in the brains of patients with Alzheimer's disease (Fig. 1).

As amyloid plaques are thought by many to inflict brain cell damage that results in Alzheimer's disease, efforts are under way to design drugs to inhibit the activity of BACE1. Complicating these efforts is the fact that BACE1 seems to cleave APP in vesicles called endosomes, which sport a pH much more acidic than that of other areas of the cell or the extracellular fluid.

Structures of several BACE1 complexes have been solved using a technique called x-ray crystallography, wherein structural information is gleaned from x-rays diffracted from crystallized versions of proteins. However, never before has a structural view of active BACE1 been available. In a paper recently published in *Molecular and Cellular Biology*, Nobuyuki Nukina and colleagues from the RIKEN Brain Science Institute in Wako and the RIKEN SPring-8 Center in Harima present and analyze crystals of active BACE1[1].

To identify conditions in which crystallized BACE1 is active, the researchers soaked BACE1 crystals in acidic (pH 4.0, 4.5 and 5.0) and neutral (pH 7.0) solutions, together with synthetic APP peptides engineered to fluoresce after cleavage. In agreement with data localizing BACE1 activity to acidic endosomes, crystallized BACE1 cleaved APP at acidic but not neutral pH.

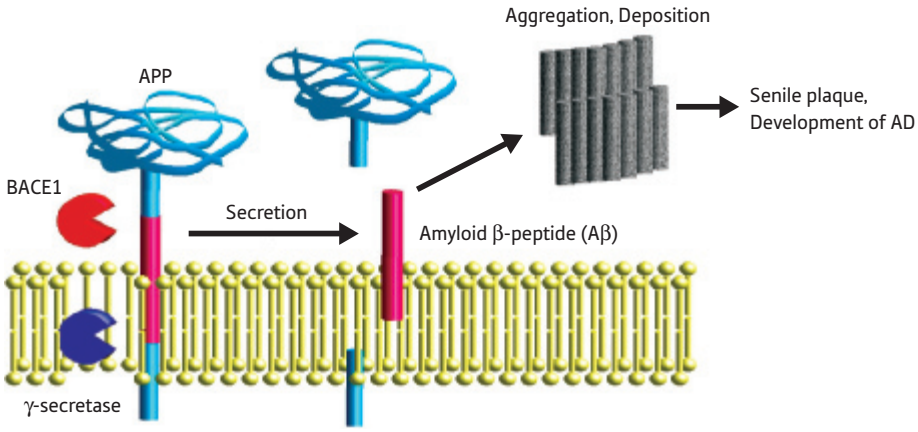


Figure 1: Schematic depicting generation of Aβ from APP. γ-secretase is another enzyme involved in the process. (AD, Alzheimer's disease.)

Comparative analyses revealed substantial differences in the shape of BACE1 crystals soaked in acidic and neutral solutions, suggesting that BACE1 undergoes structural rearrangements during activation. Most notable was the position of the 'flap' covering the active site of BACE1, which was open and closed in acidic and neutral crystals, respectively. Also observed were marked changes in the shape of the BACE1 site at which the substrate—in this case, APP—binds.

Binding of a water molecule—thought to be important in the chemical reaction through which BACE1 cleaves APP—became weaker as the pH was lowered. Whether BACE1 exists as a mix of hydrated active and dehydrated inactive forms in endosomes remains unclear.

These findings highlight the importance of considering environmental factors such as pH in structure-based design of enzyme inhibitors. "The structure of the active form of BACE1 identified here should be used for developing drugs to regulate Aβ production," says Nukina. ■

1. Shimizu, H., Tosaki, A., Kaneko, K., Hisano, T., Sakurai, T. & Nukina, N. Crystal structure of an active form of BACE1, an enzyme responsible for amyloid β protein production. *Molecular and Cellular Biology* **28**, 3663–3671 (2008).

Making the right connections

Researchers uncover a mechanism by which the brain regulates restructuring of neuronal connections during the processes of learning and memory-building

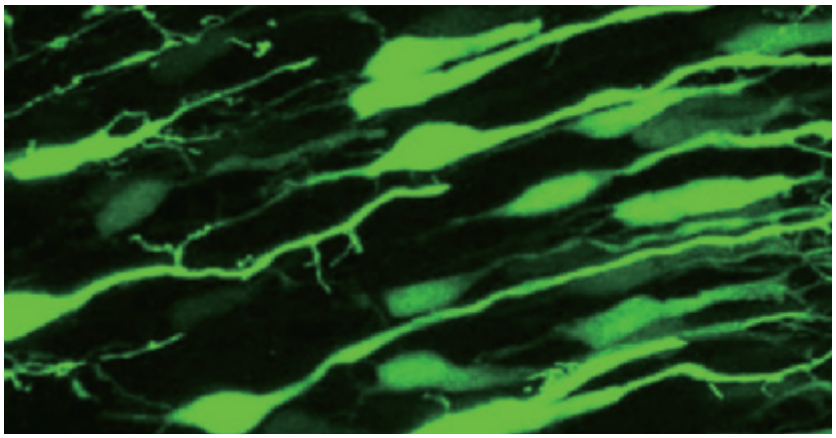


Figure 1: Neurons forming connections at embryonic day 19 in rat brain, as visualized by expression of green fluorescent protein. Prolonged activity in these neurons induces production of Plk2, a regulator of synaptic homeostasis.

Repetition is essential for acquisition and retention of new information and skills, whether one is learning to count to ten in a foreign language or dance the tango. Training induces rewiring of neurons in the learning and memory centers of the brain based on the degree to which they are actively in communication, a property known as synaptic plasticity.

"Synaptic plasticity results in changes in synaptic strength that depend critically on the correlation of activity between pre- and postsynaptic neurons," explains Morgan Sheng, formerly of the RIKEN-MIT Neuroscience Research Center but now at the Picower Institute for Learning and Memory, MIT, in Cambridge, USA. "If they act in correlation, those connections are strengthened. Conversely, consistent failure of presynaptic inputs to drive the postsynaptic cell results in weakening of those connections."

Mechanisms exist to keep this plasticity in check and prevent uncontrolled synaptic hyperactivation or deactivation, but these 'synaptic homeostasis' processes remain poorly understood at the molecular level. However, new research from Sheng's team, led by graduate student Daniel Seeburg, has provided a promising breakthrough on this front.

Previous research from Sheng's group focused on SPAR (spine-associated RapGAP), a protein that stimulates growth of dendritic spines—the projections through which postsynaptic neurons receive their signals. They found that prolonged activity in postsynaptic neurons induces production of Polo-like kinase 2 (Plk2; Fig. 1), a protein that triggers SPAR degradation and spine depletion[1]. "We thus formed the hypothesis that Plk2 might be part of a negative feedback mechanism," he says.

Their new work reinforces this hypothesis[2]. The team treated cultured neurons from the hippocampus—the brain's learning and memory center—with a drug that typically induces prolonged synaptic firing, followed by inhibition through homeostatic mechanisms. However, blocking Plk2 reduces this inhibition. The researchers subsequently found that SPAR undergoes a chemical modification that primes it for interaction with Plk2 and subsequent degradation. Eliminating this modification blocks Plk2 binding, stabilizes SPAR, and undermines synaptic homeostasis, resulting in excessive synaptic activity.

Sheng's group hopes to generate mouse strains in which Plk2 activity is eliminated or dramatically elevated to assess the role of homeostatic plasticity in brain development and the learning of basic tasks. Beyond shedding light on a mysterious neurological process, their findings could also have direct clinical implications. "An important function of homeostatic activity is preventing both inactivity and pathologically high activity, i.e. [epileptic] seizures," he explains. "Defining the mechanisms that normally provide negative feedback regulation during periods of abnormally high activity may help us to understand what goes wrong in the brain during epileptogenesis." ■

1. Pak, D.T. & Sheng, M. Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* **302,** 1368–1373 (2003).
2. Seeburg, D.P., Feliu-Mojer, M., Gaiottino, J., Pak, D.T.S. & Sheng, M. Critical role of CDK5 and Polo-like kinase 2 in homeostatic synaptic plasticity during elevated activity. *Neuron* **58,** 571–583 (2008).

A collective assault initiates motor neuron degeneration

The onset of progressive motor neuron degeneration in the disease ALS requires input from other cell types

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons that extend from the brain through the spinal cord to muscles throughout the body. The progressive degeneration of these long motor neurons occurs in ALS-affected individuals, which is caused by a diminished ability of the brain to control movement that leads to muscle paralysis and atrophy.

An international team of scientists including Koji Yamanaka and colleagues at the RIKEN Brain Science Institute, Wako, have found that damage to brain cells other than motor neurons is critical to the onset of ALS. They report their findings in the journal *Proceedings of the National Academy of Sciences*[1].

Among the factors responsible for ALS disease is mutation of a protein called SOD1, which normally scavenges for dangerous oxygen molecules that harm cells and protects cells from oxidative damage. The mutant SOD1 protein associated with ALS also functions partially as a scavenger. However, according to Yamanaka, researchers postulate that unknown toxicity produced by mutant SOD1, which is not linked to scavenging activity, causes motor neuron disease.

To test this theory, Yamanaka and colleagues genetically engineered mice to express the mutant SOD1 protein and mimic ALS disease (Fig. 1). "We previously demonstrated toxicity of the mutant SOD1 in motor neurons is a contributor of disease onset, but toxicity in other cell-types than motor neurons is also required," says Yamanaka.

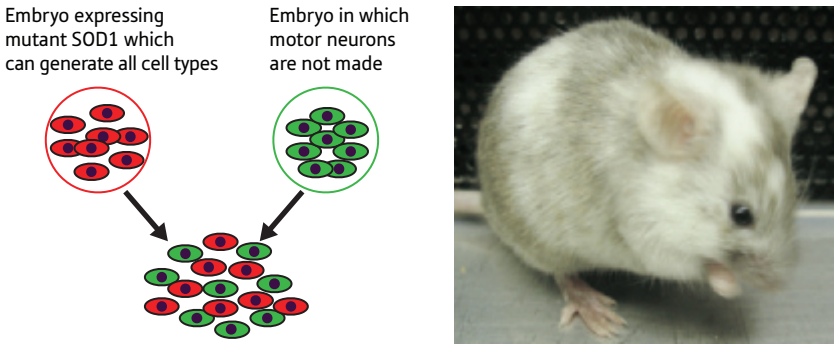


Figure 1: Testing the role of mutant SOD1 toxicity in motor neurons in ALS. Experimental design for creating new mutant mice that have mutant SOD1 expression in all motor neurons with the other cell types being a mixture of mutant and normal cells (left). Newly designed mutant SOD1 mouse (right), as described in the left panel, shows no motor neuron disease for 50 days beyond the mean life span of mouse expressing mutant SOD1 in all cells.

By selectively modifying the mice so that all motor neurons express the mutant SOD1 protein but other cell types are a mixture of mutant and normal, the team could test directly the importance of other cell types in ALS onset. Surprisingly, they found that disease onset was significantly delayed. This means the presence of normal non-motor neurons significantly delays the onset of ALS disease symptoms.

The team's findings raise several interesting issues. Expression of the mutant SOD1 protein only in motor neurons is not sufficient for early onset of ALS symptoms. Also, in mice that express mutant SOD1 in brain cells other than motor neurons and show severe ALS-like disease, the contribution of mutant SOD1 to the function of brains cells, such as interneurons, Schwann cells or endothelial cells, is strongly implicated in triggering ALS. As such, this study provides a framework for evaluating the contributions of these other cell types in promoting the degeneration of motor neurons that devastates individuals afflicted with ALS. ■

1. Yamanaka, K., Boillee, S., Roberts, E.A., Garcia, M.L., McAlonis-Downes, M., Mikse, O.R., Cleveland, D.W. & Goldstein, L.S.B. Mutant SOD1 in cell types other than motor neurons and oligodendrocytes accelerates onset of disease in ALS mice. *Proceedings of the National Academy of Sciences USA* **105,** 7594–7599 (2008).

Brain gain

Attention enhances initial brain responses to sights and sounds

Each moment that we are awake, our senses are bombarded with stimuli. Focusing our attention on the few stimuli that are important allows us to filter out the ones irrelevant to the task at hand.

The first parts of the brain to respond to objects and sounds in the environment are the so-called 'primary' visual and auditory cortex, respectively. Paying attention to sounds in one ear enhances the initial response of the primary auditory cortex on the opposite side. But previous studies have not found the same to be true in the primary visual cortex in subjects paying attention to objects in the visual field.

Now, Vahe Poghosyan and Andreas A. Ioannides at the RIKEN Brain Science Institute in Wako have found that attention does in fact modulate primary cortical responses to both auditory and visual stimuli[1].

The researchers directed five human subjects to pay attention to a pre-determined sound coming into one ear, or to a pre-determined image within a specific part of the visual field. They then presented a variety of auditory and visual stimuli, and asked the subjects to respond only to the pre-determined sound or image by raising their finger, ignoring all of the irrelevant stimuli. For example, a subject may be told to respond only to sound in the right ear, and to ignore sound in the left ear, as well as any presented images.

During these experiments, the researchers recorded the activity of different areas of the subjects' brains using magnetoencephalography (MEG), which measures magnetic fields produced by electrical activity of large populations of neurons (Fig. 1). Then, using analytical methods not used in earlier studies, they found that attention focused on pre-determined stimuli increased the activity in both the primary visual and auditory cortex almost as soon as the stimuli arrived there.

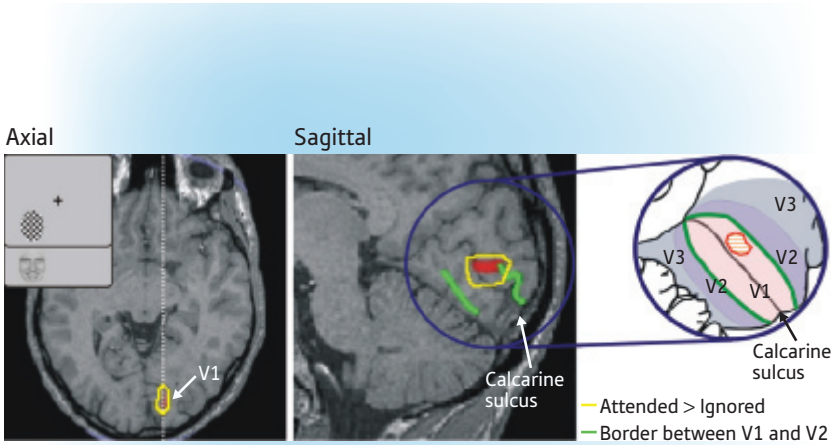


Figure 1: Brain activity recorded by MEG near the calcarine sulcus area of the brain. Approximately 55 ms after a visual stimulus (insert, top left), the first brain activity signal (left) and the first effect of attention (middle) are identified in the primary visual cortex (V1, right).

Ioannides believes that previous studies did not come to a similar conclusion about the role of attention on the primary visual cortex because these studies attributed the early MEG signals "to the primary visual cortex only, ignoring other fast activity in areas beyond the primary visual cortex. This introduced a small error in the location of the primary visual cortex, enough to 'wash away' the early attention effect on the primary visual cortex," he says.

The findings suggest that attention to a location in the visual field modulates the earliest sensory processing in the brain of stimuli appearing there. ■

1. Poghosyan, V. & Ioannides, A.A. Attention modulates earliest responses in the primary auditory and visual cortices. *Neuron* **58,** 802–813 (2008).

Uncovering hidden pathways

An investigation into the pathway by which bone-remodeling cells differentiate has yielded information about an unexpected, parallel development pathway

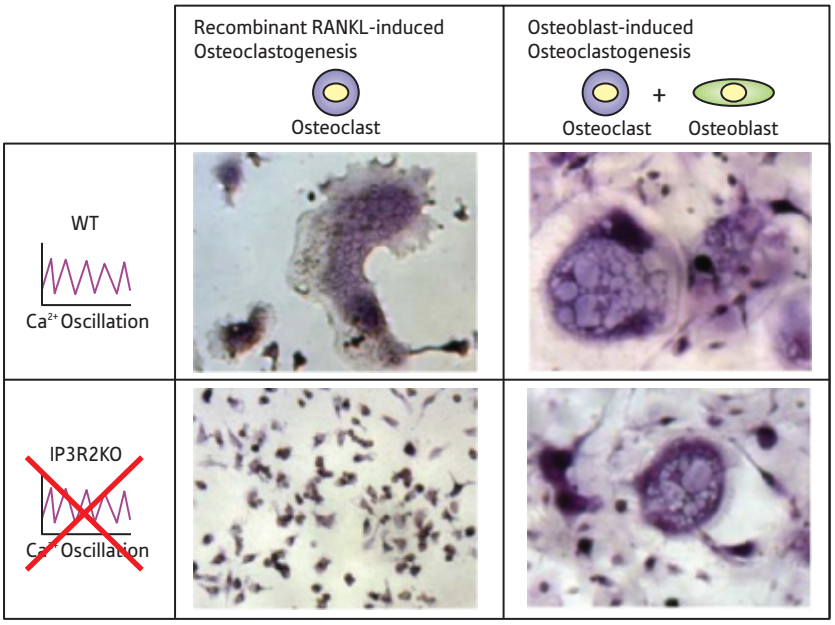


Figure 1: Slides depicting differentiation of cultured bone precursor cells, illustrating how two pathways independently control osteoclastogenesis. Panels in the top row depict normal precursor cells, whereas the panels in the bottom row depict cells lacking IP_3R—preventing intracellular calcium oscillations. Even without IP_3R, cells can still differentiate into osteoclasts via a calcium oscillation-independent pathway when co-cultured with osteoblasts (lower right panel), although both pathways need to operate for normal levels of differentiation to occur (top right panel). When both pathways are inoperative, the cells fail to differentiate (bottom left).

As with any renovation project, skeletal growth and repair require a regulated balance between demolition and construction. These processes are mediated by two classes of cells: the osteoblasts that synthesize bone, and the osteoclasts that break it down.

Uncontrolled osteoclast production can have dire consequences for skeletal integrity, and a better understanding of the process by which osteoclasts differentiate from precursor cells could lead to better therapeutic strategies for treating bone diseases. One known trigger is the osteoclast differentiating factor RANKL, which induces fluctuations in intracellular calcium levels that activate additional signaling molecules responsible for osteoclast development.

Katsuhiko Mikoshiba's team at the RIKEN Brain Science Institute in Wako has focused much of their research on the IP_3 receptors(IP_3Rs), which are important regulators of cellular calcium trafficking, and Mikoshiba became interested in exploring a potential role for IP_3R in RANKL-induced osteoclastogenesis.

In fact, his hunch was confirmed, and Yukiko Kuroda in Mikoshiba's laboratory found that a specific subclass of IP_3R is directly involved in mediating RANKL-induced calcium oscillation and inducing activation of the molecules required for osteoclast differentiation[1]. What they found next, however, was unexpected.

If calcium oscillation is an absolute requirement for differentiation, one would expect that precursor cells lacking IP_3R would fail to form osteoclasts—but in fact, when IP_3R-deficient precursor cells were cultured alongside osteoblasts, a considerable number of them successfully differentiated into osteoclasts.

Subsequent experiments confirmed that no calcium oscillation was taking place in these developing osteoclasts, providing evidence for a previously undiscovered, parallel pathway for differentiation (Fig. 1). This pathway was even observed *in vivo*, via experiments performed with mice lacking IP_3R. "Before our report, it had been believed that RANKL-induced calcium oscillation [is] essential for osteoclast differentiation," explains Kuroda and Mikoshiba. "We have demonstrated for the first time the existence of calcium oscillation-independent osteoclastogenesis."

Precursor cells undergoing osteoclast formation via the calcium oscillation-independent pathway exhibit reduced efficiency in differentiation, suggesting that the two pathways may normally work together in concert, but respond differently to health-threatening disruptions. "Many situations are known to activate osteoclastogenesis in both physiological and pathological contexts," says Mikoshiba. "We are speculating that both these two pathways contribute to osteoclastogenesis in normal bone development and that in some particular situations one pathway will be dominantly activated." With this in mind, Mikoshiba's group is now actively trying to sort out the mechanisms behind this novel pathway in order to better understand when and how it is typically activated in bone development. ■

1. Kuroda, Y., Hisatsune, C., Nakamura, T., Matsuo, K. & Mikoshiba, K. Osteoblasts induce Ca^{2+} oscillation-independent NFATc1 activation during osteoclastogenesis. *Proceedings of the National Academy of Sciences USA* **105**, 8643–8648 (2008).

Fluorescence or flexibility

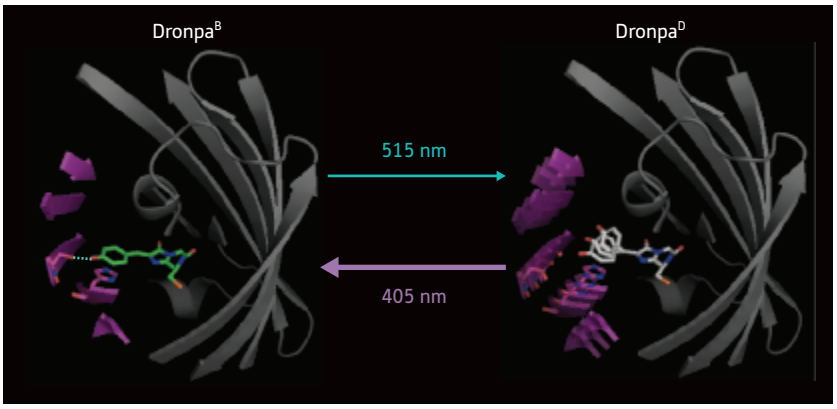
Researchers shed light on the molecular mechanism responsible for fluorescent and dark states of a genetically engineered protein

Some organic substances have a property called photochromism, meaning that their absorption spectrum, or color, changes when they are exposed to certain types of light. In particular, a new artificial protein called Dronpa shows great promise for applications because it can be switched back and forth between a 'bright' state and a 'dark' state. Now Atsushi Miyawaki, Hideaki Mizuno at the RIKEN Brain Science Institute in Wako and co-workers[1] have explained for the first time exactly what causes Dronpa to move between these two states.

Dronpa was developed by Miyawaki and colleagues by genetic engineering on a wild coral protein. Usually Dronpa absorbs light of around 503 nanometers wavelength and emits green fluorescence—the so-called bright state. However if it is exposed to strong radiation at 488 nanometers it converts into the dark state, which emits no fluorescence. The protein will switch back to the bright state if it is re-irradiated at an even shorter wavelength.

"Such reliable photochromism for Dronpa prompted us to develop it for information storage with the ability to record, erase, or read information," says Miyawaki. However to date no-one has proven exactly what causes the photochromism.

To unlock this mystery the researchers used nuclear magnetic resonance (NMR) spectroscopy to study the Dronpa molecule, which takes a cylindrical shape called a β-barrel, like other fluorescent proteins. They discovered that the bright and dark states arise from interactions between the β-barrel and the chromophore—the part of the molecule responsible for light absorption.



2008 Atsushi Miyawaki

Figure 1: Comparison of the bright (DronpaB) and dark (DronpaD) states of the Dronpa protein. In the bright state, the chromophore (green) is tethered to the molecule by a hydrogen bond (dotted blue line), while in the dark state the hydrogen bond is gone and the chromophore can vibrate.

In the bright state, the chromophore is tightly tethered to the β-barrel by a hydrogen bond (Fig. 1). This holds the chromophore in a rigid, flat configuration, so that when it is excited by light it releases its excess energy by fluorescing.

When the dark state is induced, the hydrogen bond is lost and the chromophore becomes much more flexible. Therefore it releases the excess energy by vibrating instead of fluorescing.

In other compounds that have been studied, photochromism results from physical rearrangements of atoms in the molecules. This is the first time that photochromism has been linked to structural flexibility.

"We present a new molecular mechanism for photochromism of a fluorescent protein," says Miyawaki. "The mechanism requires a special microenvironment involving a β-barrel, a structure not present in organic photochromic compounds."

Miyawaki hopes that Dronpa could eventually be used in very high-resolution optical microscopy. "The next stage will be to develop many mutants of Dronpa with different photochromic properties," he says.

1. Mizuno, H., Mal, K.T., Wälchli, M., Kikuchi, A., Fukano, T., Ando, R., Jeyakanthan, J., Taka, J., Shiro, Y., Ikura, M. & Miyawaki, A. Light-dependent regulation of structural flexibility in a photochromic fluorescent protein. *Proceedings of the National Academy of Sciences USA* **105**, 9927–9932 (2008).

How the gut manages bacteria

A previously unknown mechanism enables the immune system in the gut to respond rapidly to changes in bacteria

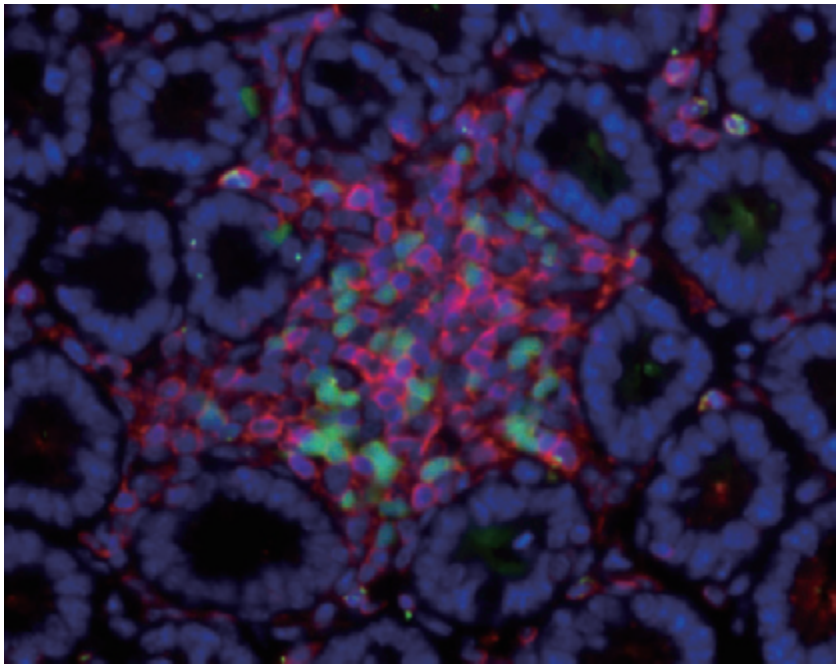


Figure 1: Adult LTi cells (green) interact with underlying stromal cells in the gut to recruit lymphocytes (red) in the small intestine. The nuclei of cells are stained in blue to reveal the gut structure.

A RIKEN-led international research group has puzzled out details of the intricate mechanism by which the immune system in the gut can respond rapidly to changes in its bacterial environment. Eventually, the work could lead to better treatment and control of gut infections and inflammatory bowel diseases.

The gut is in direct contact with the external environment and houses at least 400 different species of bacteria in vast numbers. It maintains a finely tuned immune system built around immunoglobulin A (IgA) antibodies produced by B cells to protect the body against pathogens and manage the growth of benign organisms. Previous research by other researchers unraveled a mechanism whereby T cells control the formation of these IgA-producing B cells in organized multi-cellular structures called Peyer's patches, which develop in the embryo. But such a system could take weeks to respond to invasive bacteria.

The latest work reveals a second mechanism that operates without intervention of T cells, and develops only after colonization of the intestine with bacteria, hence after birth. It involves another set of cellular structures called isolated lymphoid follicles (ILFs).

In a recent paper in the journal *Immunity*[1], the researchers, led by Sidonia Fagarasan of the RIKEN Center for Allergy and Immunology in Yokohama, detail how these ILFs piece together, providing an understanding of the newly identified mechanism. They used strains of mice bred to lack compounds significant to the development of ILFs.

The researchers noticed that the numbers and size of ILFs in the gut paralleled the level of bacteria, increasing with bacterial colonization and decreasing with the use of antibiotics. Recently, they also discovered cells in adults similar to embryonic lymphoid tissue-inducer (LTi) cells essential to the development of immune centers, such as lymph nodes and Peyer's patches.

Fagarasan and her colleagues showed that these adult LTi cells could interact with underlying stromal cells in the gut to recruit the cellular components of ILFs—B cells and antigen-presenting dendritic cells (Fig. 1). But the adult LTi cells only did this effectively in the presence of bacterial cells which stimulate an immune response, partly through the production of tumor necrosis factor. So ILFs are only formed when bacteria are present. The researchers also demonstrated that functioning ILFs could transform typical B cells that make immunoglobulin M into those that produce IgA.

"If we can understand more about these LTi cells and their interactions," says Fagarasan, "it could provide us with the potential to manipulate the gut immune system." ■

1. Tsuji, M., Suzuki, K., Kitamura, H., Maruya, M., Kinoshita, K., Ivanov, I.I., Itoh, K., Littman, D.R. & Fagarasan, S. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent Immunoglobulin A generation in the gut. *Immunity* **29**, 261–271 (2008).

How to halt immune cell activation

A new study sheds light on the molecular machinery required for reining in cellular signals that, if unleashed, could result in pathological inflammation

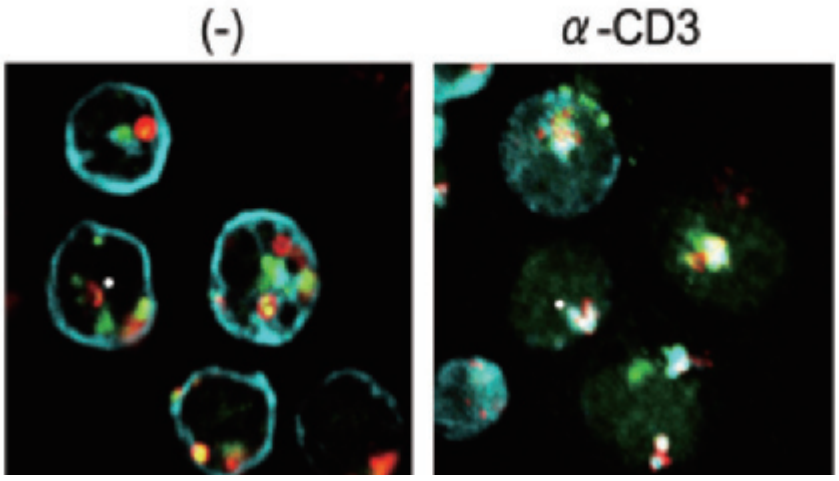


Figure 1: Interaction between TCR, CD3 and LAPTM5. Left, CD3ζ (blue) is localized on the plasma membrane whereas LAPTM5 (green) and the lysosome-associated protein LAMP1 (red) are in the lysosomes in T cells before stimulation. Right, after TCR stimulation (α-CD3), CD3ζ moves to the lysosomal compartment where it co-localizes with LAPTM5 and LAMP1 and is degraded.

Researchers in Japan have identified part of the mechanism responsible for preventing prolonged—and potentially dangerous—activation of immune cells called T lymphocytes[1]. Each decorated with a unique surface receptor (TCR) capable of detecting pathogenic foreign proteins, T lymphocytes circulate throughout the body patrolling for invading microorganisms. Upon encounter with rogue proteins, TCRs trigger—via a complex of CD3 signaling proteins—intracellular events that orchestrate release of pro-inflammatory mediators called cytokines.

As unrestrained inflammation can cause tissue damage, the immune system exerts tight control over T lymphocyte activation. During healthy conditions, TCR and CD3 proteins are constantly internalized and released back to the lymphocyte surface; this 'recycling' maintains a low level of TCR expression and thus a high 'threshold' precluding unwarranted activation. After stimulation, however, TCRs and CD3 subunits are routed towards destructive intracellular compartments called lysosomes, where they are degraded as part of a signal 'shut off' mechanism.

A team led by Ji-Yang Wang of the RIKEN Center for Allergy and Immunology in Yokohama sought to identify proteins underpinning this 'fail safe' TCR signal termination process.

Having noted in previous experiments that expression of the lysosomal protein LAPTM5 is altered after TCR stimulation, the researchers tested whether LAPTM5 is involved in turning off TCR signals. They used genetic manipulation techniques to generate mutant mice in which the *Laptm5* gene is not expressed. These *Laptm5*-deficient animals exhibited excessive T lymphocyte-driven responses to skin sensitization.

The team also found that, compared to normal T lymphocytes, LAPTM5-deficient T lymphocytes underwent more cell divisions, and released the cytokines interferon-γ and interleukin-2 more frequently after TCR stimulation. After activation, T lymphocytes lacking LAPTM5 expressed higher amounts of surface and intracellular TCR and a CD3 subunit, CD3ζ, than did wild-type T lymphocytes. Conversely, overexpression of LAPTM5 dampened CD3ζ expression.

TCR and CD3ζ proteins co-localized with LAPTM5 in lysosomes of activated T cells, and LAPTM5 physically interacted with CD3ζ (Fig. 1). These findings indicate that LAPTM5 might promote CD3ζ degradation by binding to and shuttling this protein to lysosomes.

Whether LAPTM5 cooperates with other lysosomal proteins to orchestrate CD3ζ destruction, and whether any human immune disorders are associated with mutations in *Laptm5*, remains to be determined.

LAPTM5 is the first lysosomal protein known to be specifically expressed in blood-generating (hematopoietic) cells. "In addition to its role in the negative regulation of TCR signaling, preliminary studies indicate that LAPTM5 may regulate the cell surface expression of additional immune receptors and may also function to prevent hematopoietic malignancies," says Wang. ■

1. Ouchida, R., Yamasaki, S., Hikida, M., Masuda, K., Kawamura, K., Wada, A., Mochizuki, S., Tagawa, M., Sakamoto, A., Hatano, M., Tokuhisa, T., Koseki, H., Saito, T., Kurosaki, T. & Wang, J.Y. A lysosomal protein negatively regulates surface T cell antigen receptor expression by promoting CD3 ζ -chain degradation. *Immunity* **29**, 33–43 (2008).

Pinpointing susceptibility to knee arthritis

Identification of a previously unknown gene linked to knee arthritis provides new therapeutic target

Molecular geneticists in Japan and China have identified a previously unknown gene associated with susceptibility to osteoarthritis (OA), a common disease affecting the functioning of knee (Fig. 1) and hip joints through abnormal wearing of the cushioning cartilage. The researchers have named the newly identified gene *DVWA* (double von Willebrand factor A) and suggest that it codes for a protein involved in the formation of cartilage. The discovery could lead to genetic diagnosis of some forms of knee OA, and possible development of a therapeutic drug.

More than one adult in 10 over the age of 50 suffers from OA, a painful condition that restricts movement. Genetic susceptibility to OA is largely a mystery, although a few genes are already known to be associated with it.

In a recent paper in *Nature Genetics*[1], researchers from RIKEN's Center for Genomic Medicine in Tokyo and Yokohama together with colleagues from several medical schools describe how they screened about 100,000 point mutations or single nucleotide polymorphisms (SNPs) from the Japanese SNP database to find the previously unknown gene.

Initially the researchers screened the genomes of 94 Japanese sufferers of knee OA and about 650 controls against the whole set of SNPs. About 2% of these SNPs were significantly correlated with OA. These were then tested against the genomes of an independent group of about 900 Japanese OA patients and 1,100 controls, and a third group of more than 400 Han Chinese OA sufferers and a similar number of controls. Several of the SNPs significantly associated with knee OA occurred in the *DVWA* gene. This association was independent of age, body mass index and sex.

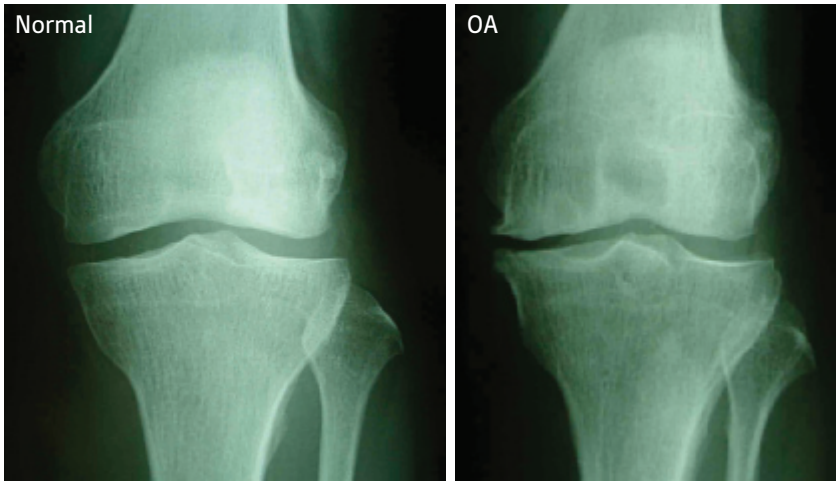


Figure 1: Radiographs of normal (left) and OA (right) knees. The medial joint space has decreased with OA and an osteophyte (bone spur) has formed.

The researchers determined in the laboratory that the DVWA protein binds to a protein building block of microtubules, β-tubulin. Microtubules are structural components of cells, which are associated with internal transport and have also been reported to play a role in the differentiation of cartilage-forming cells. Two of the SNPs of *DVWA* significantly weaken its protein product's capacity to bind to β-tubulin.

"We are now planning to check the replication of our results in other ethnic groups to examine whether *DVWA* is a 'global' gene or not," says Shiro Ikegawa, who led the research project. "And we also intend to clarify our proposed molecular mechanism as to how SNPs of the gene make people susceptible to OA." ■

1. Miyamoto, Y., Shi, D., Nakajima, M., Ozaki, K., Sudo, A., Kotani, A., Uchida, A., Tanaka, T., Fukui, N., Tsunoda, T., Takahashi, A., Nakamura, Y., Jiang, Q. & Ikegawa, S. Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. *Nature Genetics* **40,** 994–998 (2008).

Guiding the decision-making process

Identification of a novel protein involved in embryonic development leads to new insights into the first stage of neural development

In developing animal embryos, stem cells soon differentiate into three distinct layers of tissue, the primary germ layers. These are the endoderm, mesoderm and ectoderm, and each subsequently develops into a specific subset of tissues and organs. These differentiating cells follow a highly complex 'decision-making' process, and a lot of ambiguity still remains as to why, for example, an ectodermal cell became an ectodermal cell.

Yoshiki Sasai of the RIKEN Center for Developmental Biology in Kobe views the exploration of this process as a long-term mission. "Fifteen years ago, I isolated the neural inducer chordin, which induces neural progenitors from uncommitted ectoderm," he says. "However, how the uncommitted ectoderm develops from pluripotent cells has remained unknown—so it has been my 15-year-old homework to elucidate it!"

In the frog species *Xenopus laevis*—a popular animal model for developmental studies—germ-layer differentiation is triggered via two sets of signals: some derived from maternally produced factors, and others generated by the zygote itself. Previous research has identified several candidate maternal factors, but the mechanisms involved in the latter pathway have proven more elusive, and Sasai's team has focused much of their recent effort on finding zygotic factors potentially responsible for ectoderm formation.

In their most recent study, Sasai's team identified a previously uncharacterized protein, XFDL, which exhibits a marked ability to block mesoderm formation[1] (Fig. 1). Elevated XFDL levels lead to inhibition of mesoderm-specific genes, while reduced levels of the protein lead to broader expression of these genes in embryonic regions that normally form ectoderm.

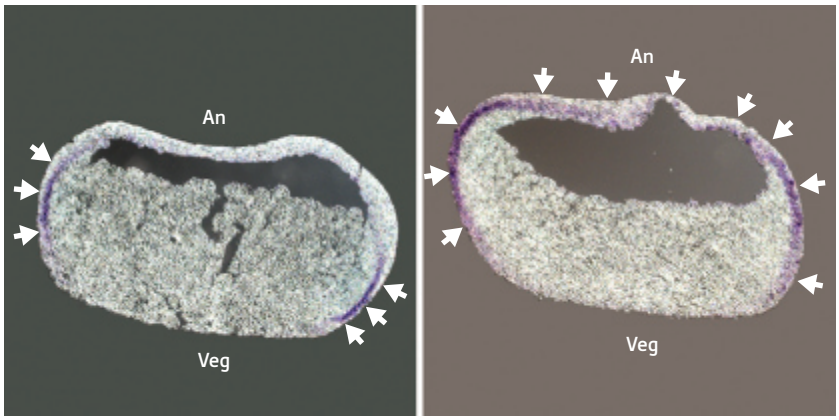


Figure 1: *Xenopus* embryos stained to indicate expression of a mesodermal marker. In the left panel, an unmodified embryo exhibits the marker in the region between the ectodermal 'animal pole' region (An) and the 'vegetal pole' (Veg), where the endoderm is formed. The embryo in the right panel has greatly reduced XFDL production, resulting in the formation of mesoderm in a typically ectodermal region of the embryo.

Subsequent experiments revealed that XFDL acts by interacting with p53, a transcription-regulating protein with a known role in mesoderm formation, and directly interfering with its ability to bind to DNA and activate its target genes. When XFDL was prevented from interacting with p53, it lost its ability to regulate ectoderm differentiation—an unexpected finding, according to Sasai. "Although p53 is implicated in the regulation of mesodermal development, we did not think it had such a profound function in the binary decision of ectodermal versus mesodermal determination," he says.

The researchers have identified two XFDL-related proteins in mice, both of which also inhibit mesoderm formation in *Xenopus*, indicating that mammalian germ layer formation may also be regulated via a similar pathway—a possibility that Sasai's team is currently exploring more closely. "Our preliminary studies suggest that this is the case at least with *in vitro* differentiation of mammalian embryonic stem cells," he says. ■

1. Sasai, N., Yakura, R., Kamiya, D., Nakazawa, Y. & Sasai, Y. Ectodermal factor restricts mesoderm differentiation by inhibiting p53. *Cell* **133,** 878–890 (2008).

Cutting loose

New research clarifies how cells rearrange from two-dimensional sheets into three-dimensional structures during embryonic development

Early in development, vertebrate embryos transition from being simple balls of cells to more complex structures comprising three distinct cellular layers, each of which will give rise to a distinct subset of tissues. Part of this transitional process, called gastrulation, involves the carefully choreographed migration of the cells that form the mesodermal layer—the precursor to bones and muscles, among other tissues.

An important step in mesoderm formation is the epithelial–mesenchymal transition (EMT), in which two-dimensional (epithelial) sheets of cells rearrange into more complex three-dimensional (mesenchymal) structures. EMT is itself a complex multi-stage process, and even after decades of research, ambiguity remains about how it is coordinated.

Now, new findings from a team at the RIKEN Center for Developmental Biology in Kobe, led by Guojun Sheng and postdoctoral fellow Yukiko Nakaya, has provided valuable insights into the initial stages of EMT[1]. Earlier research by Nakaya had shown interesting effects of changes in expression of a protein called RhoA on mesoderm formation, and so the team decided to follow up by examining its role in EMT in developing chick embryos.

Sheng, Nakaya and colleagues found that EMT appears to begin with the breakdown of the basement membrane (BM), a matrix of proteins including laminin and fibronectin that provide structural support for epithelial sheets. They also found that retention of the BM within the epiblast—the embryonic cells actively involved in gastrulation—coincides with expression of RhoA at BM-facing cell surfaces; conversely, the absence of such RhoA expression in mesoderm precursor cells provides an indicator of BM disintegration (Fig. 1).

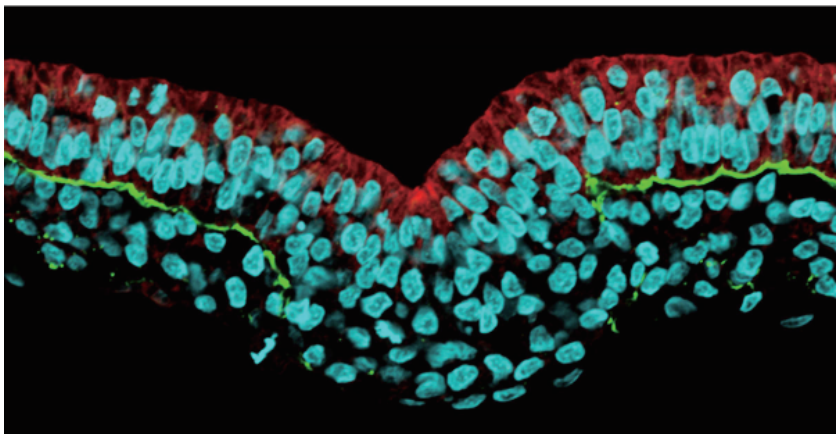


Figure 1: Section from a gastrulation-stage chick embryo. Cell nuclei have been stained cyan, with red and green stain indicating the presence of RhoA and laminin, respectively, illustrating the loss of expression RhoA that coincides with the loss of the basement membrane.

These findings were further supported by experiments in which RhoA expression was boosted or reduced, revealing a direct dependence of BM stabilization on local RhoA expression. This appears to result in part from RhoA's effects on microtubules, long protein-based cables that compose part of the cellular infrastructure. The researchers found that loss of RhoA activity specifically disrupts microtubules near the BM-facing surface of epiblast cells, effectively severing their anchors to the BM as a logical first step before their reformation into more sophisticated three-dimensional assemblies.

Sheng was pleased to observe that their observed mechanism fits into a rational model for embryonic development. "The most surprising outcome," he says, "is that the sequential steps of EMT in gastrulation fit beautifully with how one would imagine the mesoderm cells should come off from the epiblast." ■

1. Nakaya, Y., Sukowati, E.W., Wu, Y. & Sheng, G. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nature Cell Biology* **10**, 765–775 (2008).

Using supramolecules to bring about a revolution in skilled manufacturing

Tatsuo Wada

Chief Scientist, Supramolecular Science Laboratory
Advanced Science Institute

As typified by the microfabrication of semiconductor circuits, the current mainstream manufacturing process for information equipment employs methods in which hard materials are cut and arranged according to a detailed design to produce devices with the required functions. From the viewpoint of protection of the global environment, these methods cannot be considered sustainable because they often require large amounts of energy and enormous resources. On the basis of a new principle that uses soft properties of organic materials called 'supramolecules,' the Supramolecular Science Laboratory has devised functional materials, with the aim of bringing about a revolution in skilled manufacturing.

Materials that can detect only areas of movement

Tatsuo Wada, Chief Scientist, and members of his laboratory have developed a prism device that can detect only the moving areas in an image (Fig. 1). "When a more developed version of this prism device is applied to a surveillance camera, it will contribute significantly to crime prevention, because the device can quickly and automatically detect only the moving areas in an image. For example, when the device is installed in a parking lot, it can capture images of people or cars entering or leaving the place," says Wada. The prism device consists of organic materials that use supramolecules, which are molecules that act as a single molecule and perform specific functions by combining or splitting off in various ways.

"Semiconductor circuits must be microfabricated onto silicon semiconductors so that they can fulfill the required functions. In contrast, we can dispense with those processing techniques because supramolecules can change their orientations and geometric shapes flexibly under external influences such as light, heat, or an electric field, or allow specific kinds of light to pass through them, or cause specific kinds of light to be reflected. We call this soft-optoelectronics."

The prism device that Wada and members of his laboratory have developed produces a black-and-white strip pattern (interference pattern) when a beam of noninformational laser light (reference light) interferes with a beam of laser light passing through a target object (Fig. 1). The changes in refractive index based on the interference pattern play the role of memory because they remain unchanged for a short period, depending on the materials. The pattern prevents the same information from passing through it and allows only changed information to pass through when the next beam of laser light that has passed through the target object enters the prism device. Thus information on the moving images only can be detected automatically by this 'novelty filtering'. The principle of operation is completely different from conventional methods in which computers are used to verify the difference between images before and after an event.

Making minute changes spread to the whole structure

In this way, supramolecules can contribute to creating functional materials that process information by a nontraditional principle of operation. Wada is currently advocating a new idea called 'molecular information science,' which deals with the application of supramolecules. "Positive and negative charges are treated as an information carrier in the semiconductor devices, whereby the shape, size, or orientation of each molecule can be treated as information in supramolecular technology. Thus molecular information science is based on the new idea that changes in information can be transformed into overall changes in the property of a material. This allows the science to be applied to information processing devices."

The following are some study examples of molecular information science that originated in the Supramolecular Science Laboratory, which has served as a meeting place for researchers in synthetic chemistry and solid state physics.

One example is an application of a rotaxane molecule, which consists of a ring-shaped molecule with a dumbbell-shaped molecule passing through it (Fig. 2). Rotaxane is a supramolecule in which ring and dumbbell-shaped molecules act as a single molecule. The supramolecule, created by Wada and members of his laboratory, has a benzene ring on each of the ring and dumbbell-shaped molecules. A single crystal of rotaxane was formed and an X-ray crystallographic structural analysis was conducted to investigate how the structure of the supramolecule changes with changing temperature.

At 30 °C, all the benzene moieties in the ring and dumbbell-shaped molecules are parallel to each other as a result of π–π interaction. However, the dumbbell-shaped molecules start rotating with increasing temperature, and all the benzene rings of these molecules in the whole crystal become fixed at a certain angle through π–CH interaction at 128 °C.

"We expected an activated motion of the molecules and rotation of the dumbbell-shaped molecules at higher temperatures; however, these kinds of local change are usually observed in the individual molecules of a crystal," says Wada.

Why did such minute changes spread to the whole structure? "We have clarified the reason through joint research activities with the excellent researchers in X-ray crystallographic structural analysis at RIKEN. When a pair of adjacent dumbbell-shaped molecules started rotating, this motion was transmitted to neighboring supramolecules one after another through supramolecular interaction by a domino effect until the motion had spread to the whole single crystal. This was something we had not expected."

A plate of the single crystal placed between mutually orthogonal deflecting plates allows green light to pass through it at room temperature, but allows orange light to pass through it at 128 °C. The supramolecule changed its geometric shape on the ångström scale (one ten-billionth of a meter, or 0.1 nm), and the minute change spread to the whole single crystal, changing its light-transmission properties. The single crystal returned to its original state when the temperature was returned to 30 °C. "Scientists have advocated the concept that minute molecular changes can affect the physical properties of a material. However, we think that this is the world's first experiment that has actually verified the effect of ångström-scale molecular changes on physical properties. This is a result that will surely encourage researchers who are making strenuous efforts to synthesize new molecules, and in doing so are trying to create functional materials with nontraditional properties."

Another study example is an experiment to investigate whether a single molecule that triggers motion on the ångström scale can move 1,000 nematic liquid crystal molecules. The azobenzene compound that Wada and members of his laboratory created changes its geometric shape slightly depending on the wavelength of light used to irradiate it (Fig. 3). When the compound is mixed with liquid crystal, the liquid crystal is arranged in a helical structure. The azobenzene compound is then irradiated with a certain wavelength of light to change its geometric shape. This changes the arrangement of the liquid crystal, decreasing the helical pitch by 40%. As a result, the color of reflected light (its interference color) is changed. Furthermore, a different wavelength of light can be used to return the arrangement to its original state.

"It seems that the material can be applied to light-control mirrors. Current information devices, 'digital multi-mirror devices,' incorporate many small mirrors manufactured by semiconductor microfabrication technology, and these mirrors are controlled by small actuators (drive units). These mirrors are very expensive, but the cost is reduced by mass production. Considering the problems of the global environment and resources, we are no longer living in an age of mass production. The application of supramolecules will raise the possibility of producing those mirrors effectively at a reasonable cost."

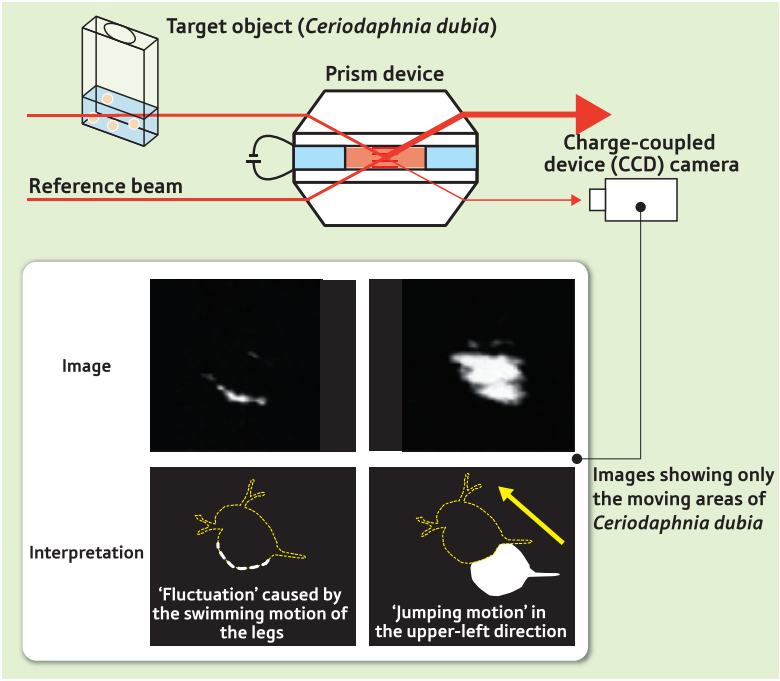


Figure 1: Prism device that detects only the moving areas.
Interference change patterns are created in the prism device. Only the moving images that move within 0.6 s are transferred through the device to the CCD camera. The above images are taken using *Ceriodaphnia dubia* as the target object. They show only the moving parts of the organism.

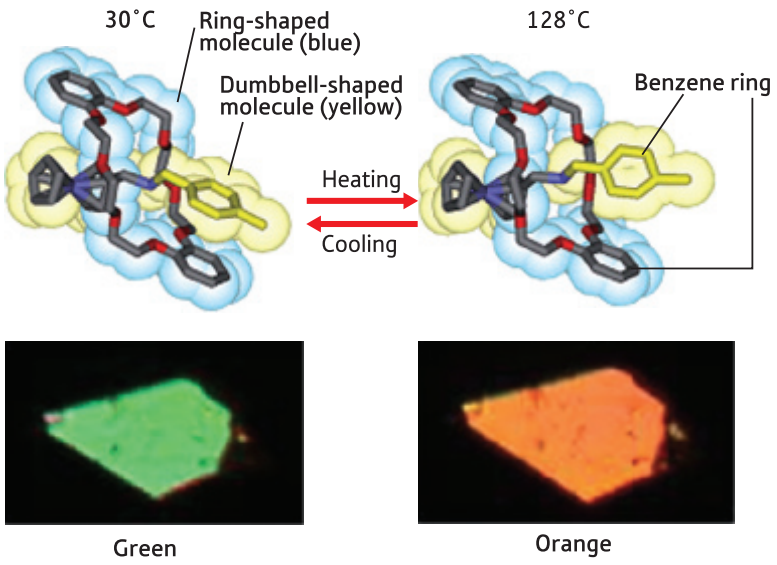


Figure 2 : Rotaxane, in which minute changes spread to the whole structure.
A dumbbell-shaped molecule rotates when heated, and the benzene ring of the molecule stops rotating at a certain angle at 128 °C. The ångström-scale change transmitted to the whole structure of a single crystal in a domino effect can change how light is transmitted. A plate of the single crystal placed between mutually orthogonal deflecting plates allows green light to pass through it at room temperature, and orange light at 128 °C.

Developing highly efficient organic solar cells

"In the future, I want to develop a supramolecule that moves like an inchworm. Thus we need to learn a lot from living organisms because an organism is a collection of supramolecules." For example, a large number of proteins interact with each other in a cell, and cells ingest the materials they require from the outside or discard unnecessary materials. In other words, proteins are supramolecules with the function of mobility because they can move or carry other molecules or materials. Following the example of the function of mobility, Wada and members of his laboratory are conducting research to create movable supramolecules and to use them in skilled manufacturing.

One research example is the development of highly efficient organic solar cells. Today, mainstream solar cells made from silicon have a photoelectric conversion efficiency of about 30% for single crystals and about 10–13% for amorphous silicon solar cells. There are now high expectations that solar cells could be used for the generation of electric power as a means of mitigating global warming and energy problems. Production of the single-crystal silicon solar cell, in particular, requires an enormous amount of energy because a high temperature is necessary to melt the material. Thus the single-crystal silicon solar cell highlights the problem of the balance between the energy required for production and the energy generated by the solar cell. There are hopes for the development of solar cells made of organic materials because these can be manufactured at low temperatures using less energy. Most organic solar cells developed so far, however, have a conversion efficiency of only 1% or lower.

Wada and members of his laboratory are developing materials that can automatically change into a particular molecular arrangement when irradiated with light. In this arrangement, the molecules that carry positive and negative charges can move freely, thereby efficiently converting light energy into electrical energy. "We want to achieve a conversion efficiency of 10–13%, which is comparable to the amorphous silicon solar cell. Organic solar cells are expected to have a range of applications because they are flexible, and large solar panels can be produced economically. For example, the application of organic solar cells to the material for a tent will be extremely useful when used in disaster areas where the electricity supply has been cut off.

"In the future, I want to carry out computations using movable supramolecules," says Wada, talking about new horizons for supramolecules. "For example, I have a dream of using a supramolecule like the rotaxane molecule that I mentioned earlier, and manipulating a ring-shaped molecule for calculation in the same way as we move a bead on an abacus."

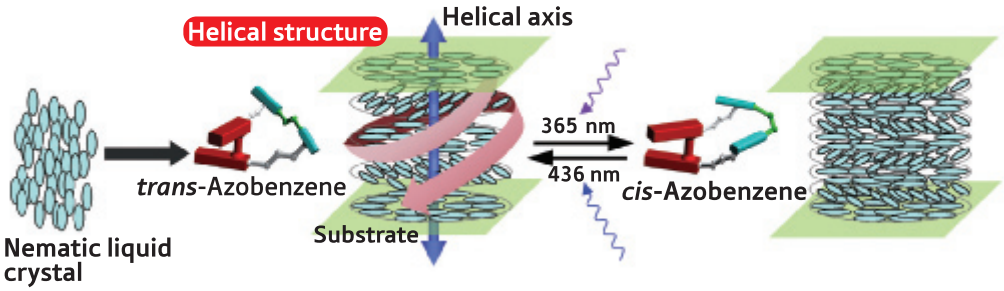


Figure 3: Azobenzene compounds, in which minute changes spread to the whole structure.
An azobenzene changes its three-dimensional structure from *trans*- to *cis*-isomerization when irradiated with ultraviolet radiation at a wavelength of 365 nm. The ångström-scale change triggers rearrangement of the whole structure of the liquid crystal, decreasing the helical pitch by 40%. The helical structure returns to its original arrangement when irradiated with light at a wavelength of 436 nm.

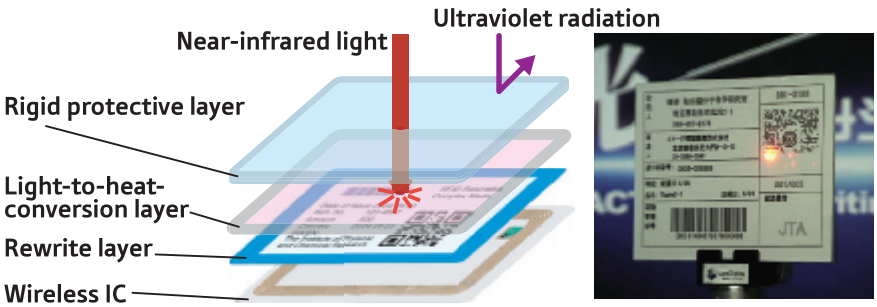


Figure 4: Non-contact change of information displayed on an IC card.
Development of a light-to-heat-conversion layer that can quickly convert the energy of near-infrared laser beam into heat has enabled the non-contact rewriting and erasure of information displayed on the card.

Non-contact change of display

Finally, the following is a study example at an advanced stage. Today, wireless integrated-circuit (IC) card tickets such as Suica and PASMO have become popular. The IC card user can pass through a ticket gate using the 'touch-and-go' system just by holding his or her IC card over a scanner installed at the ticket gate. The section of railway line valid for a particular commuter pass can be displayed on the surface of these IC cards, but the 'touch-and-go' system does not make it possible to rewrite or delete information displayed on the card. This is because color development on the IC cards relies on a thermochromic material. Thus we need to insert an IC card into a thermal printer to print the data displayed on the card.

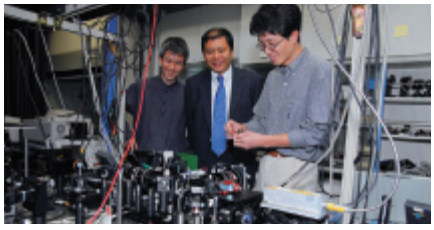
Wada and members of his laboratory successfully developed a light-to-heat-conversion layer that can quickly convert near-infrared laser light into heat, and this has enabled the non-contact rewriting and deletion of information displayed on the card. "This technique can also be applied to logistic labels (Fig. 4). This technique is expected to lead to resource saving because it contributes to reducing the labor and cost required for sticking on and removing labels. Thus we are developing the technique for practical use in the future."

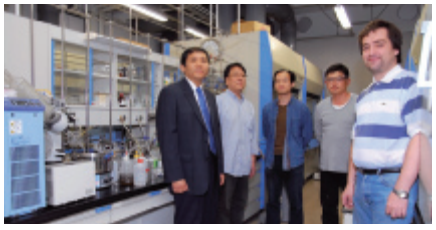
Molecules excellent in functionality are also beautiful in shape

Wada was brought up near the previous Kagoshima Airport in Kamoike Kagoshima City. "I used to watch the piston-engined aircraft, and adored the beauty of their planes," he says, looking back on the old days. "I visited many aviation museums. Of course I hate war, but even today I am interested in the Zero combat plane (Type Zero Carrier Fighter). The plane looks and feels to me like a machine-tooled artifact, which still boasts a sophisticated shape and elegant beauty. In contrast, the F4F 'Wildcat' gives the impression of a mass-produced machine although it was a worthy rival for the Zero combat plane. Thus, I think the Zero wins the title as far as elegant utility is concerned."

Wada insists that geometric shape is also important when creating a new molecule. "Molecules with excellent functionality also have beautiful shapes. First of all, we launch a new program of research, to create new molecules that are more beautiful than those in the past. The molecules will naturally join together to fulfill advanced functions. I want to create as many of these molecules as possible."

Those molecules with their beautiful geometric shapes will contribute significantly to bringing about a shift from manufacturing that necessitates the consumption of large amounts of energy and enormous resources, to environmentally friendly manufacturing based on the concept of energy saving and resource saving. ■





Tatsuo Wada, Chief Scientist, and members of his laboratory.
Currently, there are 17 members of the Supramolecular Science Laboratory. Two experimental laboratories for solid state physics (upper) and synthetic chemistry (lower) are located next to each other. The scientists are conducting basic study and research for practical applications combining the two research areas, trying to create new functional materials.

About the researcher

Tatsuo Wada was born in Kagoshima in 1956. He received BEng, MEng, and DEng degrees in applied chemistry from the University of Tokyo, Japan, in 1979, 1981, and 1984, respectively. He synthesized the β-forming polypeptide containing photoconductive chromophores and determined their carrier mobilities. He joined RIKEN (the Institute of Physical and Chemical Research) as a research scientist in the Biopolymer Physics Laboratory in 1984 and he was engaged in research on the application of ion implantation into conducting polymers. After the Frontier Research Program (FRP), RIKEN, was launched in 1986, he was in charge of organic nonlinear optical material research in the Laboratory for Nonlinear Optics and Advanced Materials (1986–1991) and in the Laboratory for Nano-photonics Materials (1991–1999). He succeeded in the generation of blue light by second-harmonic generation (SHG) and sum-frequency generation (SFG). using organic crystals DIVA and studied the enhanced third-order nonlinear optical responses of vanadyl phthalocyanine thin films. He also developed monolithic photorefractive materials and demonstrated optical image processing. In 2000, he became a Chief Scientist of the Supramolecular Science Laboratory and proposed the new concept of 'soft-optoelectronics materials'. His current research interests cover supramolecular photonics and molecular information science.

Kobe symposium integrates math and life

RIKEN recently sponsored a symposium at the Center for Developmental Biology in Kobe on 'cell and tissue scale' research in the life sciences to explore directions for the next generation of study. The symposium was especially directed toward complex, multiscale, multidimensional phenomena, and focused on the integration of the mathematical and life sciences.

The life sciences have advanced greatly in the past 10 years. In particular, genomic, stem cell and brain sciences are evolving quickly, and have shown remarkably rapid progress. Such research generates huge amounts of data on biomolecules. The next stage in life science research will be determining how these many molecules assemble into cells, and how various cells form tissues to generate dynamic life phenomena. In addition to such preceding studies as genome informatics and molecular dynamics of protein, research on complex cell behavior and tissue formation now demand the integration of mathematical, computing, live imaging and life science.

The symposium, which ran from September 2–3, covered a variety of pertinent topics, including: research centered on the 'scale of cell and tissue', with the aim of integrating cell biological studies with mathematical sciences; system biomechanics; quantitative approaches to the analysis of cell behaviors and structures; and three-dimensional modeling of biological tissue. RIKEN researchers and other invited researchers gave a presentation regarding these topics, and 23 speakers and about

160 participants conducted an active discussion from various standpoints.

In addition, RIKEN researchers gave a talk on their work installing the next-generation supercomputer known as the petaflops computer and strategies for its use. The computer is situated at Port Island in Kobe, and completion is planned for the end of 2012. ■

Integrated terahertz spectra database opens to the public

The first integrated database of terahertz data in the world opened on September 15 at RIKEN and the Next Generation Network Center at the National Institute of Information and Communications Technology (NICT) (www.thzdb.org). By consolidating the terahertz spectra of various materials, such as research reagents and paint materials, the database allows researchers to access a wide variety of useful data for use in both basic research and practical applications.

Terahertz radiation has recently been found to be extremely useful, as it can penetrate such materials as clothing, paper, wood, plastic and ceramics, but cannot pass through metal or water. This makes it potentially extremely useful in imaging and non-destructive testing, as well as in security and for finding illegal drugs.

When materials are irradiated with terahertz light and then examined with a spectroscope, the absorption spectrum is different depending on the material—this is the material's 'fingerprint spectrum'. The new database contains hundreds of these fingerprint spectra, which researchers can use to identify materials, such as medicines, illicit drugs or agricultural chemicals, as well as reagents and chemicals contained in the material.

Moreover, containers that are opaque to visible light, such as bags and envelopes, are often transparent in the terahertz spectrum. This makes it possible to find dangerous articles without opening the container, by combining the unique nature of the sample's spectrum with the penetrative properties of terahertz light. This enables, for example, non-destructive inspection of luggage and food packaging.

Art historians are also using terahertz data to ascertain the appearance of early drafts of paintings under many layers of paint. The fingerprint spectra of over 200 kinds of paint materials were included in the database.

The integrated terahertz database was constructed by a research consortium comprising the RIKEN Advanced Science Institute and NICT. RIKEN and NICT researchers integrated data on the terahertz spectra of about 500 kinds of materials from previous databases and modified it to make it easier to use.

RIKEN is also cooperating with outside organizations, including Italy's National Agency for New Technologies, Energy and the Environment, to improve and expand the data, and aims to have about 2,000 entries by 2010. ■

Bridging research begins on pollen disease vaccine

Researchers at the RIKEN Research Center for Allergy and Immunology (RCAI) have begun bridging research for a vaccine to prevent and treat pollen disease caused by cedar pollen. This is the first pollen disease vaccine developed so far, and consists of fused cryptomeria (cedar) pollen antigens created by genetic engineering.

Pollen disease is a serious and growing problem in Japan, where an estimated 16% of the population is suffering from symptoms of the allergy, many of whom are living in cities. Besides the discomfort experienced by sufferers, the condition causes work absences and other social problems. Some people have even suffered anaphylactic shock from cedar pollen. Until now there has been no treatment, except for allopathy, such as antihistamines to suppress the symptoms of itchy eyes, runny nose and sneezing. The new vaccine offers the hope of a cure, or at least a way to stimulate the immune system to regulate the allergic response caused by pollen disease.

Yasuyuki Ishii and his colleagues at RCAI's Vaccine Design Research Team synthesized two kinds of principle cryptomeria pollen antigens using genetic engineering techniques. They found from animal trials that the vaccine was effective in both treating the condition and reducing the likelihood of anaphylactic shock, a risk when natural cryptomeria pollen is present.

The bridging study aims to expand research into clinical applications in humans, to determine dose levels and toxicity levels. Human clinical trials are still needed, but a vaccine to stimulate the immune system to fight cedar pollen disease appears to be in sight. ■

Dr. Yuji Kamiya
Director of Growth Regulation Research Group
RIKEN Plant Science Center
Suehirocho, Tsurumi-ku, Yokohama, Japan

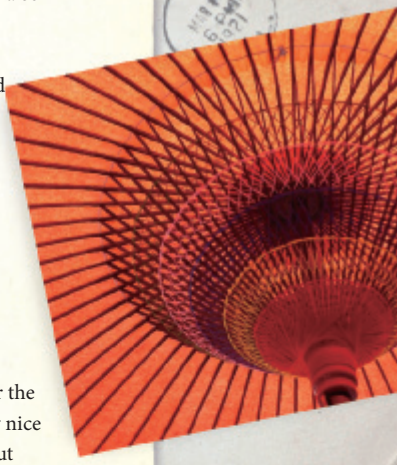
Dear Kamiya-sensei,

As you know my professional work is on plant hormones and in particular gibberellins, which are closely related to Japan and even more to your laboratory. It is now about 15 years since I first worked with you in Japan, which was within the Frontier Research Program (a great program that you initiated to advance plant hormone research worldwide). I acknowledge that my career has profited a lot from the meetings you organized, from the international atmosphere, and from the open-minded discussions with you and your laboratory members.

Perhaps I never told you, but your laboratory holds particularly beautiful memories for me because I met my wife there. She is from Portugal.

During my recent stay at the RIKEN Plant Science Center,I enjoyed the many exciting discussions on projects that are underway in your laboratory.

It was also my pleasure to discuss important new discoveries recently made in your and Shinjiro Yamaguchi's laboratories, including new ultra-sensitive techniques for detecting plant hormones. Successfully, we initiated collaborations between our groups. Moreover, I enjoyed the seminars held at RIKEN Plant Science Center. I also had the opportunity to present and discuss our current research results.



Currently, we are analyzing the level of gibberellins, an endogenous plant hormone that is necessary for seed germination, at different developmental stages of pumpkin seeds. We hope this work will serve as a model to better understand development in other plant species.

Visiting Japan also offered my family and me the opportunity to meet our friends and colleagues and to visit three different research institutions: RIKEN, the private university Nihon University, and the public university Ehime University. I am impressed by the level of scientific research performed at all three institutions and how engaged co-workers are. Moreover, RIKEN Plant Science Center is very international and communication is no problem. Seminars, lectures, discussions, all are held in English.

Coming back to Japan after 10 years was a wonderful experience for all of us. We are very grateful to you for the opportunity to show the root of our family to our two kids (three and five years old). For us it is particularly nice to report about life in Tokyo that is very safe and enjoyable for families. Orientation for foreigners is easy. But most of all we were overwhelmed by the hospitality and friendship that we received from everyone we met and, in particularly, from you and your laboratory members.

Thank you very much.

Yours,
Theo Lange
Institute of Plant Biology of the Technical University of Braunschweig
Brunswick, Germany

Introducing

RIKEN podcast

produced by Nature Publishing Group

presents a selection of topical research from RIKEN, Japan's leading research organisation.

vol.5

In this month's episode, we interview researchers about:

The nanoworld in colour

A new kind of optical microscope designed by RIKEN researchers is tipped to revolutionise imaging of nanoscale objects.

Focus on phosphate

Masses of new data on protein phosphorylation could help scientists improve plant immunity.

Size matters

Why bigger is often better when it comes to cell size in plants.

Visit the site to listen to the FREE RIKEN Podcast!

www.nature.com/rikenpodcast

RIKEN Podcast is sponsored by

SHIMADZU

RIKEN | npg nature publishing group

RIKEN Global Relations Office
2-1, Hirosawa, Wako, Saitama, 351-0198, Japan TEL : +81 48 467 9443 FAX : +81 48 462 4715
rikenresearch@riken.jp www.rikenresearch.riken.jp