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Hot on the scent of neural wiring

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Waiting for the light to change

A new technique installs fluorescent 'traffic lights' in living cells, enabling researchers to tell whether or not they are actively dividing

Cells live their days on the fixed schedule of the cell cycle, which regulates when—and if—they divide. Some cells cycle rapidly and, as a result, divide actively. For others, the cell cycle is stalled; these will not divide, but may instead differentiate into mature cells that no longer reproduce.

Understanding cell cycle behavior of individual cells in complex tissues would be valuable in studying processes like embryonic development and tumor formation, but this has proven difficult. Existing techniques for visually monitoring cell cycle state generally require either the use of chemical stains that act as indicators of DNA replication, or direct tinkering with cellular behavior through the use of specialized pharmacological agents. These methods have proven useful for basic cell cycle studies, but more sensitive and precise indicators will be required for scientists hoping to identify and characterize patterns of cell cycle behavior in complex tissues.

"Considerable progress has been made towards understanding the mechanism of cell cycle progression in single cells," explains Atsushi Miyawaki of the RIKEN Brain Science Institute in Wako. "However, little is known about how the cell cycle is coordinated with differentiation, morphogenesis, and cell death in a multicellular context."

To ensure tight regulation of cell division, cells use a variety of strategies to maintain close control over levels of proteins involved in the cell cycle. For example, the proteins Cdt1 and Geminin regulate chromosomal DNA replication during the 'S' phase of the cell cycle. These molecules have opposing effects on DNA replication, and their levels fluctuate accordingly throughout the cell cycle—Cdt1 levels are highest in the 'G_1' phase, directly before DNA replication, but as cells transition into S phase, Cdt1 levels fall and Geminin levels rise, remaining high until the cell returns to G_1. Rather than regulating this process by modulating gene activity, cells exert their control over Cdt1 and Geminin at the protein level, using a process called 'ubiquitination' that precisely targets unwanted proteins for destruction.

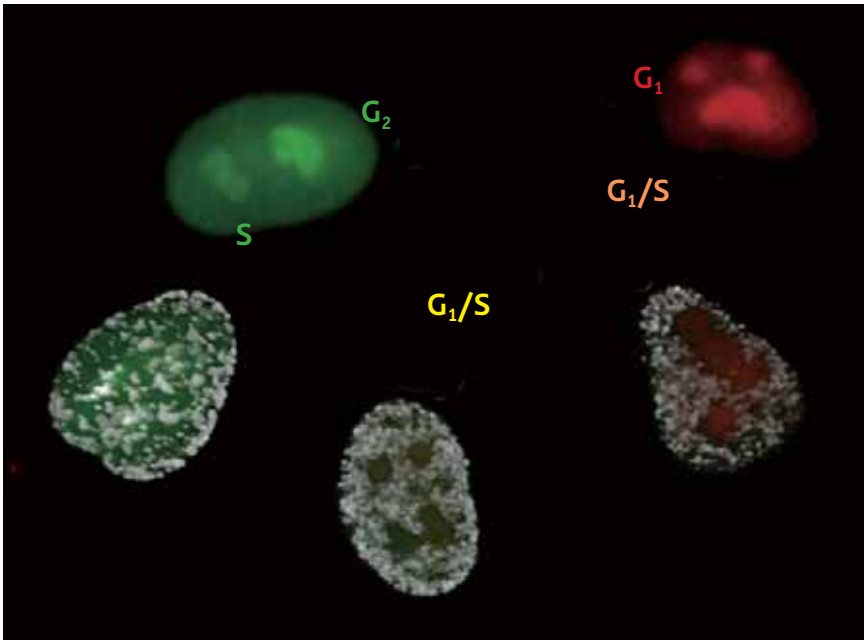


Figure 1: The Fucci cell cycle visualization method. Cells in phase G1 fluoresce bright red. As they transition into the S phase and begin DNA replication, Cdt1 levels are dramatically reduced while Geminin levels increase. This results in faint yellow fluorescence early in G_1/S that soon gives way to robust green fluorescence, which lasts until the cell re-enters G1 phase.

Fucci labels light up the cell cycle

Miyawaki and colleague Asako Sakaue-Sawano took advantage of this carefully regulated process to develop a surprisingly precise visual indicator of cell cycle status in living cells, a technique they call Fucci[1]. In Fucci, which is short for 'fluorescent ubiquitination-based cell cycle indicator', cells are genetically modified to express Cdt1 and Geminin with red and green fluorescent tags, respectively. As a result, the nuclei of cells in S and G_2 phases glow green, indicating active division. Cells that are in G_1, on the other hand, will glow red—this can indicate a temporary cessation of division, or permanent cell cycle arrest due to terminal differentiation. Due to the timing of the red-to-green conversion in the nucleus, cells re-entering active division will glow yellow as their DNA begins to replicate, providing an additional color indicator for cell cycle activity (Fig. 1).

As an initial test of their system, the team examined changes in the cell cycle profile of Fucci-expressing epithelial cells in a simulated wound-healing

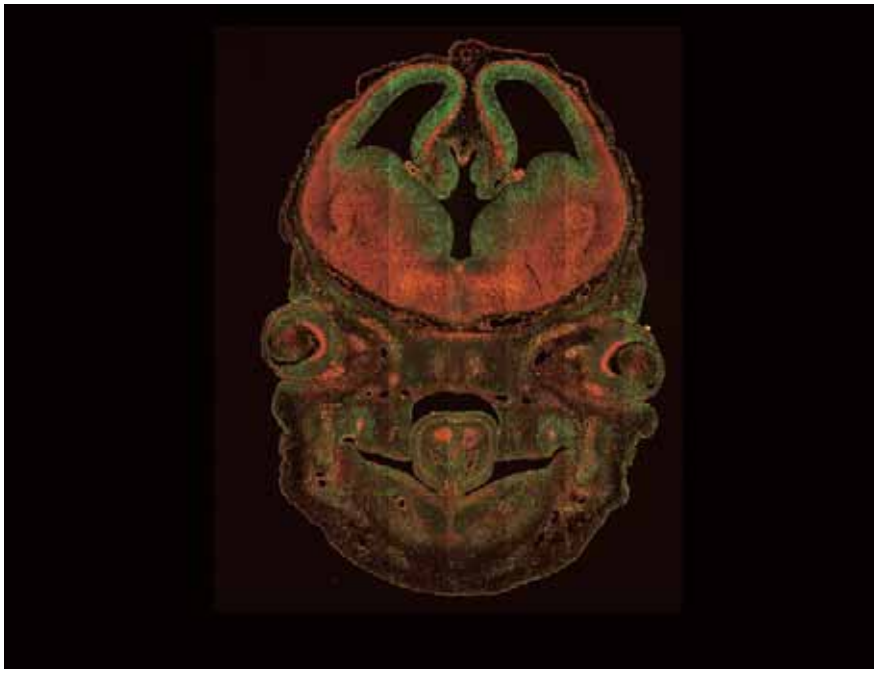


Figure 2: Sectional image of the head from a Fucci-expressing transgenic mouse embryo, illustrating how cell growth in the developing brain is controlled with cell differentiation. Cell growth and differentiation are indicated by green and red signals, respectively.

scenario. The cells initially exhibited green fluorescence as they divided to spread across the glass surface on which they were cultured, but began to turn red as they approached a critical level of crowding, indicating that cell division was being arrested. However, the introduction of a lesion in this cell monolayer led to the appearance of a burst of green fluorescence at the edges of the wound as the cells began dividing anew to initiate healing.

Applications beyond the cell cycle

Miyawaki and colleagues subsequently demonstrated that their method is suitable for intravital imaging studies, in which cellular processes are directly observed within a living animal. In a first round of experiments, cancer cells expressing Fucci were injected into nude mice, enabling the researchers to monitor changes in the cell cycle profiles of the foreign cells as they replicated and began to spread metastatically. Based on these findings, the team has already begun applying Fucci as a means for examining candidate anticancer drugs and their impact on tumor cell division and migration.

Fucci also offers a powerful means for studying embryonic development. Miyawaki and colleagues generated genetically modified mice that express the Fucci proteins in every cell, and used these to characterize the cell-cycle behavior of neural progenitor cells within the developing rodent brain (Fig. 2). The fluorescent proteins were sufficiently bright for the investigators to collect time-lapse image series, enabling them to assemble detailed profiles of the division, migration and differentiation behavior of large numbers of cells at once. Miyawaki believes that future studies could combine the Fucci system with other fluorescent labels specific for various cytoplasmic proteins, making it possible to perform more exclusive cell cycle analyses of specific cell subtypes.

Indeed, one of the greatest strengths of Fucci lies in its flexibility, and since their initial publication Miyawaki's team has made considerable progress in building on their technique. These advances include expanding the range of colors available for use in Fucci, thereby extending the technique's compatibility with other fluorescent indicators, and developing Fucci constructs that operate effectively in zebrafish—another popular model for studying vertebrate development. Their work has also been assisted by parallel efforts at developing new software tools for image analysis. "We have developed special software that will facilitate cell cycle analyses using Fucci," says Miyawaki, "as well as some programs that allow us to track cell lineage very efficiently." ■

1. Sakaue-Sawano, A., Kurokawa, H., Morimura, T., Hanyu, A., Hama, H., Osawa, H. Kashiwagi, S., Fukami, K., Miyata, T., Miyosi, H. *et al.* Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* **132,** 487–498 (2008).

About the researcher

Atsushi Miyawaki was born in Gifu, Japan, in 1961. He received his MD in medicine at Keio University School of Medicine in 1987 and his PhD in signal transduction at Osaka University School of Medicine in 1991. He served as a researcher and then as an assistant professor in the Institute of Medical Science, the University of Tokyo, from 1991 to 1998. His main research was focused on calcium signaling. He also joined the Department of Pharmacology at the University of California, San Diego, with a long-term fellowship from the Human Frontier Science Program and worked as a research pharmacologist from 1995 to 1998, to investigate technological innovations in fluorescence imaging. In 1999, after returning to Japan, he set up the Laboratory for Cell Function Dynamics at the Brain Science Institute (BSI) of RIKEN. Since 2004 he has been directing the Advanced Technology Development Group of BSI. Earlier this year, he was promoted to the position of vice director of BSI. His primary research goal is to better understand how biological functions are controlled in space and time.

Watching DVDs in real-time

High-speed x-ray diffraction provides a better view of rewritable digital recording media

Digital versatile discs (DVDs) are one of the most convenient ways of storing large amounts of information. But despite two decades of development since the discovery of the materials on which they are based, many of the details of how they work remain unclear. Researchers from RIKEN SPring-8 Center, CREST and Matsushita Electric Industrial, led by Yoshihito Tanaka and Masaki Takata, are filling the gaps in our understanding of these materials, by using state-of-the-art time-resolved x-ray diffraction analysis to watch their atomic structure change as they are written.

Rewritable DVDs are based on so-called 'phase-change materials'. Information is written to these materials by changing their atomic structure using a laser. The initial material structure of a blank DVD is amorphous. Irradiating it with a laser causes this structure to change to a crystalline state or to remain in its amorphous state, depending on laser power. Because the optical reflectance of the two states is different, this information can be easily read from the disc by measuring this reflectance with a lower power laser.

Tanaka and colleagues have combined high-speed x-ray diffraction with *in situ* time-resolved laser reflectometry to simultaneously probe the structural and optical properties of two different DVD materials, $Ge_2Sb_2Te_5$ (GST) and $Ag_{3.5}In_{3.8}Sb_{75.0}Te_{17.7}$ (AIST)[1] (Fig. 1). This has enabled them to track the microscopic processes that take place in these materials, in real-time, as they change from an amorphous to a crystalline state.

They found that initially, the reflectance of the materials drops sharply followed by a similarly sharp increase. The researchers attribute this to an initial smoothing of the surface followed by the onset of crystal formation. They then observed a gradual increase in both the reflectivity and emergence of diffraction peaks corresponding to the materials' crystalline state, proving the close relationship between their properties and structure.

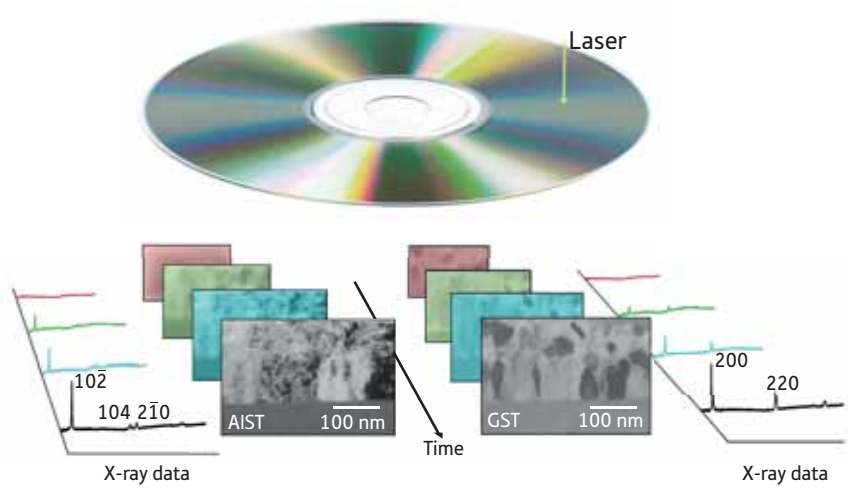


Figure 1: Cross-section images of a DVD disc together with the time-resolved x-ray diffraction data for the crystallization process by laser irradiation. The phase-change materials investigated are AIST and GST.

Differences in the width of the diffraction peaks between the two materials and their evolution over time led the researchers to propose two subtly different models of the mechanisms taking place in each. In GST they suggest that large crystal grains form over the entire volume of the material from the moment the process starts, while in AIST the process begins with the formation of small crystallites in different parts of the material, which gradually grow and merge to form larger grains.

The level of detail enabled by this and future studies is likely to make a valuable contribution to the development of better and faster DVD materials. ■

1. Fukuyama, Y., Yasuda, N., Kim, J., Murayama, H., Tanaka, Y., Kimura, S., Kato, K., Kohara, S., Moritomo, Y., Matsunaga, T., Kojima, R., Yamada, N., Tanaka, H., Ohshima, T., Takata, M., Time-resolved investigation of nanosecond crystal growth in rapid-phase-change materials: correlation with the recording speed of digital versatile disc media. *Applied Physics Express* **1,** 045001 (2008).

A vision of order

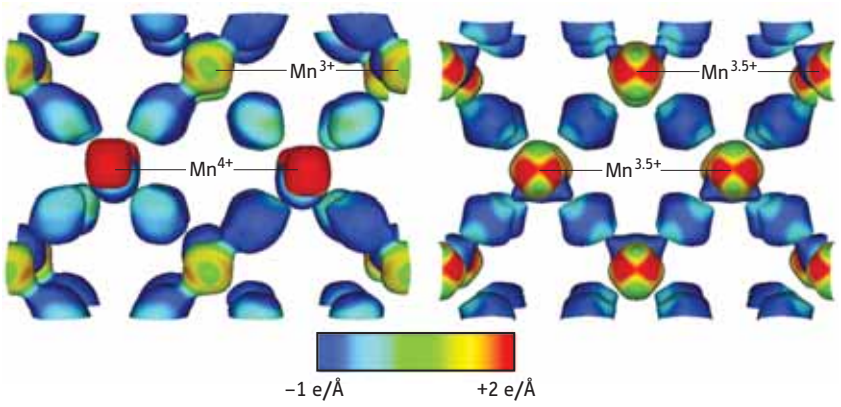
A technique for analyzing x-ray diffraction data enables the charge ordering of exotic electronic materials to be observed directly

The electrical properties of all solid-state materials are governed by the way that electronic charge is distributed throughout their atomic structure. Now Kenichi Kato from the RIKEN SPring-8 Center, in Harima, and his colleagues have developed a technique for directly visualizing the distribution of charge within certain manganese oxides, which is a valuable tool for learning more about charge ordering in these and other materials[1].

The charge ordering behavior of many rare-earth manganese oxide compounds holds a particular fascination for solid-state physicists. Under certain conditions of magnetic field and temperature, the electrons within such materials distribute themselves in unusual stripe-like patterns. This can dramatically change their magnetic and electronic properties. As well as being of fundamental interest, such effects could enable the development of sensitive sensors for high density magnetic storage applications. But the physical mechanisms responsible for this behavior are not yet understood completely.

To better study such phenomena, Kato and colleagues developed their technique for analyzing the way in which the distribution of charge within a material scatters synchrotron x-ray radiation. In doing so they have visualized the fine details of the charge distribution and electrostatic potential in an exotic electronic material known as half-doped manganite $Nd_{1/2}Sr_{1/2}MnO_3$ and how these change with temperature.

At a temperature of 300 K (26.85 °C), the material exhibits a disordered charge distribution which gives rise to metallic electrical behavior. But at a much lower temperature of 18 K (-255.15 °C), the researchers observe that the charge distribution in certain planes of the material exhibits a zigzag pattern. More significantly, the amount of information that their technique provides enables them to determine exactly how the atoms within the structure of the material are bonded to each other under both conditions. In the disordered state, they find that the manganese atoms bond to neighboring oxygen atoms with a valency of +3.5. Whereas in the ordered state, they find they bond with a valency of either +3 or +4 depending on their position (Fig. 1).



Kenichi Kato

Figure 1: Image of the electrostatic potential on the electron density isosurface (contours in the atomic structure corresponding to a constant electron density of 0.8 e/Å^3) of the ordered insulating state (left) and disordered metallic state (right) of the half-doped manganite, $Nd_{1/2}Sr_{1/2}MnO_3$. Manganese (Mn) atoms bonded to neighboring oxygen atoms with a valency of 3+ and 4+ in the ordered state, and 3.5+ in the disordered state, are labeled.

The fine detail enabled by the technique even surprised the researchers. "At first we were not sure how clearly we would be able to observe the charge ordering state. So the level of clarity with which the technique showed up even very small variations in charge distribution was quite unexpected," says Kato. "But the ability to observe how electrons behave in this way should enable materials scientists to design better materials for practical applications." ■

1. Kato, K., Moritomo, Y., Takata, M., Tanaka, H. & Hamada, N. Visualization of charge ordering in a half-doped manganite by an electrostatic potential analysis. *Physical Review* **B 77,** 081101R (2008).

A hot connection with spin

A particular spin topology in solid-state materials has a strong influence on thermally generated electron transport

Chirality, or handedness—where an object cannot be superimposed on its mirror image—is found in many physical systems. In the case of electron spin—the smallest magnetic field generated by an electron—the chirality shown by some compounds can be quantified by the solid angle subtended by three nearby spins, and can be controlled by the application of a magnetic field (Fig. 1).

Shigeki Onoda from the RIKEN Advanced Science Institute (formerly the Discovery Research Institute), Wako, and colleagues from Japan and Hungary have studied the effect of spin chirality on the transport properties in solid-state materials[1]. Spin chirality has been shown to have an effect on other transport properties, for example in the so-called anomalous Hall effects[2], in which no temperature gradient is involved.

"Spin chirality bears a fictitious magnetic field and bends the electron motion driven by the temperature gradient, [thus] introducing a transverse current," explains Onoda. "The direction in which the electron motion is bent depends on the sign of the spin chirality."

In their study, the researchers focussed on whether spin chirality influences the Nernst effect, which describes the generation of an electric current in the direction perpendicular to that of a temperature gradient.

Onoda and colleagues studied a series of compounds named pyrochlore molybdates. Because some members of this family show spin chirality and others do not, they could make direct comparisons.

The comparisons between results on different compounds highlighted the effect of the spin chirality. Specifically, compounds with spin chirality showed an anomalous Nernst effect in a specific temperature range (20–30 K (-253.15–-243.15 °C)), while a compound with no spin chirality showed no effect.

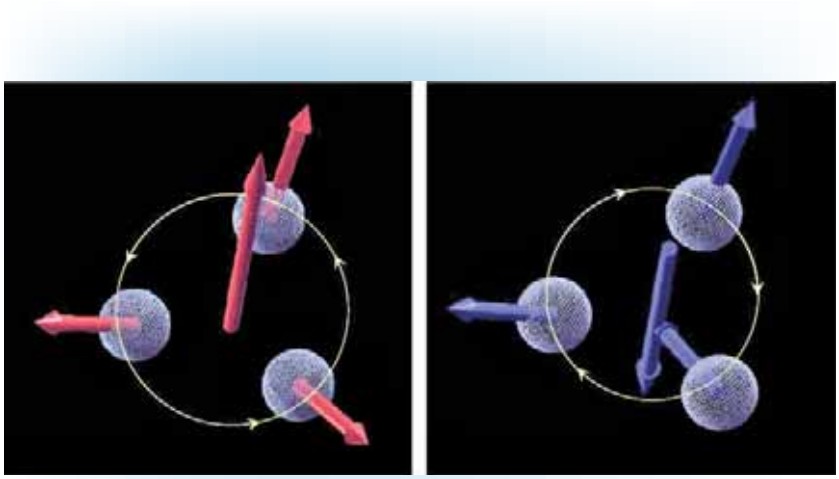


Figure 1: Schematic representation of spin chirality. Reversing one of the spins (right) results in reversing the spin chirality.

The importance of the result goes beyond the specific case of the class of materials studied. "The observation of this fundamental phenomenon has revealed that a fairly large fictitious magnetic field can be generated in materials by controlling the low-energy degrees of freedom of the spin chirality," says Onoda. "The sign of the spin chirality controls that of the transverse heat/electric current. This is unlike the usual cases of Nernst effects, where [the sign of the transverse current] is exclusively determined by that of the temperature gradient and the applied magnetic field or the magnetization in particular materials." According to the researchers, their study reveals that spin chirality is really a new and promising basic quantity in electron transport phenomena. ■

1. Hanasaki, N., Sano, K., Onose, Y., Ohtsuka, T., Iguchi, S., Kézsmárki, I., Miyasaka, S., Onoda, S., Nagaosa, N. & Tokura, Y. Anomalous Nernst effects in pyrochlore molybdates with spin chirality. *Physical Review Letters* **100,** 106601 (2008).
2. Taguchi, Y., Oohara, Y., Yoshizawa, H., Nagaosa, N. & Tokura, Y. Spin chirality, Berry phase, and anomalous Hall effect in a frustrated ferromagnet. *Science* **291,** 2573–2576 (2001).

Slower switching for quantum coherence

The performance of quantum computing can be improved by operating logic gates slowly

Over the past decade scientists have made rapid progress towards building a quantum computer, but many obstacles still remain. One important requirement is that researchers must achieve extremely accurate control of the electrical signals used in quantum logic gates. A research team based at the RIKEN Advanced Sciences Institute (formerly the Frontier Research System) in Wako has proposed a new method for operating quantum logic gates that could help reach the required accuracy[1].

Calculations in quantum computing are performed on units of quantum information called qubits, which can be implemented in superconductors. The slightest noise or interference from the external world can cause qubits to lose their quantum information and revert to classical behavior. This problem, known as decoherence, has been partly overcome by 'adiabatic' quantum computing in which the quantum algorithm is designed to keep the whole system in a relatively robust state throughout the calculation.

The researchers' proposal could reduce decoherence further by avoiding the need for fast logic gates acting on qubits. Their alternative approach involves slowly transferring populations of qubits between selected quantum states (Fig. 1).

The quantum algorithm is not changed in the new proposal. Franco Nori of RIKEN and the University of Michigan in the USA, explains: "The only thing that has changed is the way that the gates are operated. Instead of using so-called pi-pulses, which require very accurate electrical signals to manipulate the qubits, we propose using slow population transfers, which are not affected by imperfections in the manipulation signal."

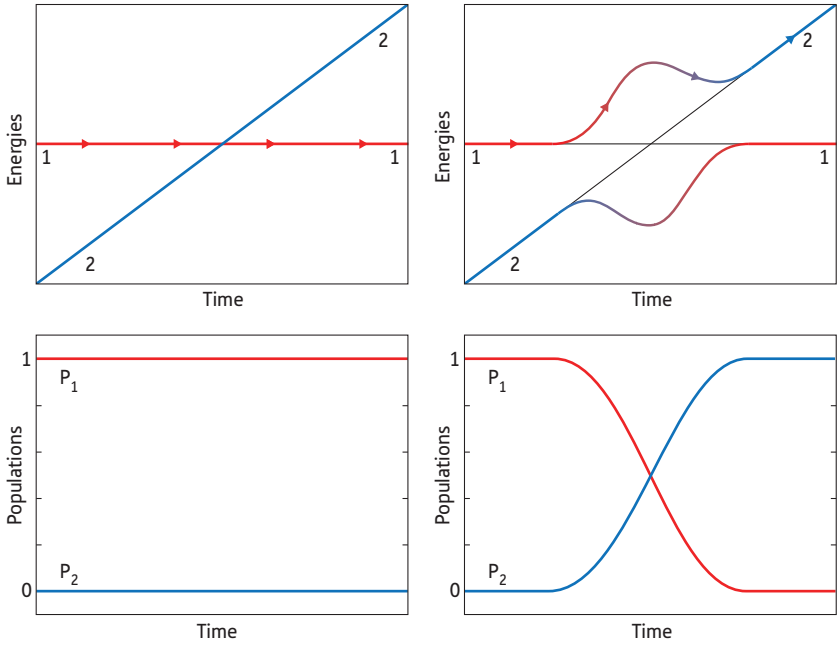


Figure 1: The slow and robust transfer of a qubit from one quantum state (1) to another (2). The temporal evolution of the energy levels (upper panels) and the populations of those energy levels (lower panels) are shown for a two-state quantum system. The population transfer from one quantum state to another occurs only in the circuits with an electromagnetic (coupling) pulse (right-hand panels).

The qubits must still be very accurately controlled, but now the strict requirement is shifted to a different step in the quantum gate, sometimes referred to as the phase gate. This step is easier to perform than the population transfer. Thus, the strictest accuracy requirement is now imposed on a step where the available technology allows accurate design.

"When you manipulate quantum states slowly, nature works on your side and protects the quantum state against imperfections in the control signals," says Lian-Fu Wei of the RIKEN team, now at Southwest Jiaotong University in Chengdu, China.

Team-member Sahel Ashhab summarizes the approach by saying: "We identified a difficulty in the requirement of accurate control pulses and asked the question: can we perform quantum gates without having to deal with this?" The theoretical analysis has shown that it is indeed possible to do so, and the researchers hope that experimental tests of their idea will be performed in the near future. ■

1. Wei, L.F., Johansson, J.R., Cen, L.X., Ashhab, S. & Nori, F. Controllable coherent population transfers in superconducting qubits for quantum computing. *Physical Review Letters* **100,** 113601 (2008).

Rubbery reactions

It only takes the addition of a simple aluminum compound to a new polymerization system to switch between rubber polymers

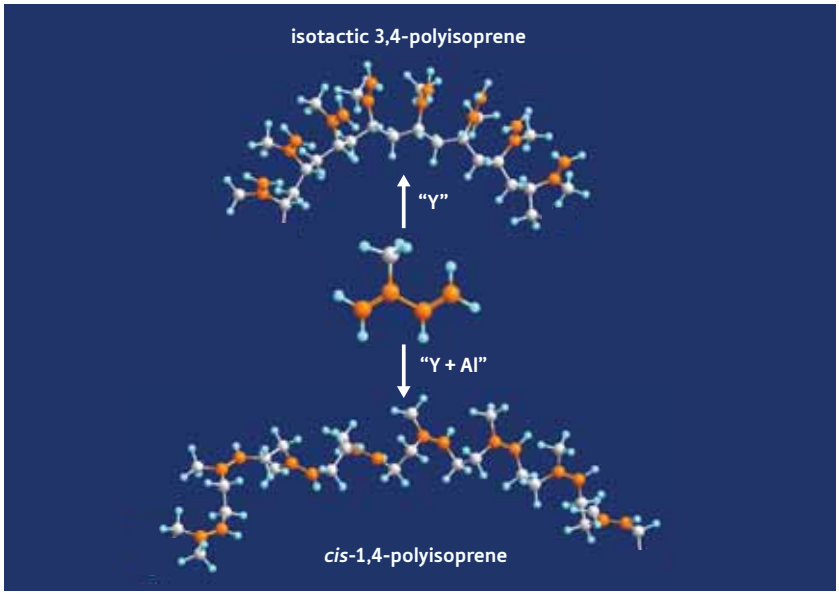


Figure 1: Isoprene (center) can form the new polymer, isotactic 3,4-polyisoprene (top) using an yttrium-based catalyst, but when trimethyl aluminum is added in sufficient quantity, 1,4-*cis* polyisoprene (bottom), the main component of natural rubber, is produced.

Researchers from RIKEN Advanced Science Institute in Wako, using a rare earth-based catalyst, have produced a novel polymer or chain of isoprene molecules with unique properties. In addition, much to their surprise, they found their polymerization system can be dramatically switched, simply by adding an aluminum-based compound, to produce a different isoprene polymer which is the main component of natural rubber.

The two polymers vary in which of the carbon atoms of the four-carbon isoprene molecules connect together to form the chain. The rubber chain (1,4-cis polyisoprene) is formed by joining the two end carbons of the isoprene molecules, whereas in the new polymer the molecules are joined at two adjacent carbons in a particular three-dimensional arrangement (isotactic 3,4-polyisoprene) (Fig. 1). It leaves the other two carbon atoms double-bonded on a side chain all along the same side of the main or polymer chain, a construction which allows a range of groups with different chemical functions to be added easily. The researchers are collaborating with a chemical company on employing this facility to transform 3,4-polyisoprene into further useful polymers.

The research group has been exploring how catalysts based on rare-earth metals—a group of elements which includes scandium, yttrium and the lanthanides—can be used to produce high-performance synthetic rubber[1]. "We need a chemical way to produce rubber," says project leader Zhaomin Hou. "Not only is the natural supply limited, but a big advantage of chemical synthesis is that it allows us to modify the properties of rubber in the laboratory."

In their latest paper published in *Angewandte Chemie International Edition*[2], the researchers discuss their work with a catalyst made up of an yttrium ion with bulky nitrogen-based groups called amidinates attached. The shape of the catalyst restricts the polymerization reaction to the two adjacent carbons at positions 3 and 4 in the isoprene molecule. The result is the new polymer 3,4-polyisoprene. But when they added trimethyl aluminum ($AlMe_3$), widely recognized as a co-catalyst which can potentially increase the activity of the catalyst system, they found the reaction began to change.

At levels below two $AlMe_3$ molecules to one catalyst molecule they were still producing 3,4-polyisoprene, but by five $AlMe_3$ molecules to one catalyst molecule, 98% of their product was rubber (1,4 *cis*-polyisoprene). "The finding that an aluminum alkyl compound can switch the selectivity of isoprene polymerization suggests that we should reconsider the role of aluminum compounds in such catalyst systems," Hou says. ■

1. Zhang, L., Suzuki, T., Luo, Y., Nishiura, M. & Hou, Z. Cationic alkyl rare-earth metal complexes bearing an ancillary bis(phosphinophenyl)amido ligand: A catalytic system for living *cis*-1,4-polymerization and copolymerization of isoprene and butadiene. *Angewandte Chemie International Edition* **46,** 1909–1913 (2007).
2. Zhang, L., Nishiura, M., Yuki, M., Luo, Y. & Hou, Z. Isoprene polymerization with yttrium amidinate catalysts: Switching the regio- and stereoselectivity by addition of $AlMe_3$. *Angewandte Chemie International Edition* **47,** 2642–2645 (2008).

Watching chemical reactions molecule by molecule

Electrons trigger precision bond breaking

RIKEN scientists have developed a method to control and study a chemical reaction in a single molecule. The technique could eventually help to fabricate advanced electronic devices molecule by molecule.

Chemistry involves moving the electrons in chemical bonds around to create new arrangements of atoms, and firing an electron at a molecule is one way to trigger that reaction.

Scientists such as Yousoo Kim of RIKEN's Advanced Science Institute, Wako, use scanning tunneling microscopes (STMs) to study these reactions in more detail[1].

Normally used for taking atomic snapshots, an STM contains a sharp conducting tip that delivers electrons to jump a few billionths of a meter onto a nearby surface. But these electrons can also be used to trigger chemical reactions, causing target molecules to vibrate until they fall apart.

Kim and his colleagues, led by RIKEN's Maki Kawai and including collaborators from the University of Tokyo and Osaka University, have now shown that a chemical bond can be broken apart in this way by exciting different bonds in the same molecule[2].

"This is the first observation of an indirect pathway of bond breaking, which demonstrates the effective energy flow between two different vibrational modes," says Kim.

The scientists studied how molecules of dimethyl disulfide ($CH_3S–SCH_3$) on a copper surface split into two methyl thiolate (CH_3S) fragments (Fig. 1), and found that vibrations in the carbon–hydrogen bond, induced by an STM electron, could drive the reaction.

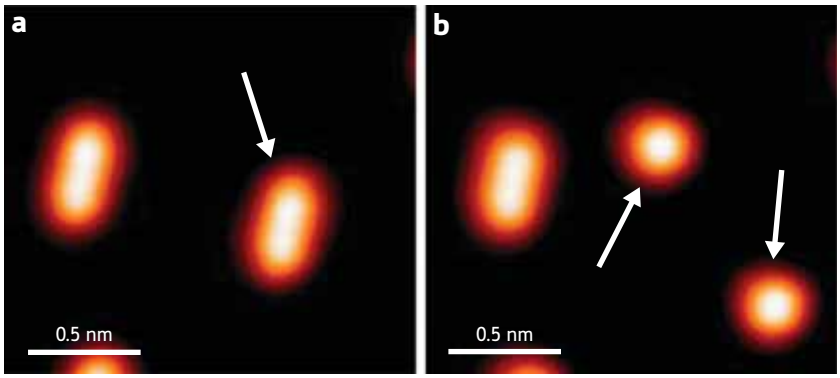


Figure 1: Splitting molecules. (a) Individual dimethyl disulfide molecules appear as ellipses in these STM images; (b) after injecting an electron, the molecule splits apart into two methyl sulfide fragments.

"The fact that we can utilize a new, indirect pathway to induce bond breaking could provide wider approaches for controlling and identifying surface chemical reactions," adds Kim.

The STM's electrons can also induce the methyl sulfide fragments to hop around on the copper surface. "This provides practical way to form a self-assembled monolayer of CH_3S molecules in a controlled manner—it is molecule by molecule assembly," explains Kim.

The choice of dimethyl disulfide was significant, according to Kim. Similar sulfur-based compounds are widely used to create self-assembled monolayers—just one molecule thick—by breaking apart the sulfur–sulfur bond. Self-assembled monolayers on metal surfaces are showing promise as starting points for molecular-scale electronic devices.

"The most significant part of the present work is that these dynamic process—bond breaking and lateral hopping motion—can be induced in a controllable way by tuning the energy of the injected tunneling electrons," says Kim. "This will provide a possible general basis for designing the architecture of molecular electronics." ■

1. Anon. How to make a molecule move. *RIKEN RESEARCH* **2,** 11–12 (2007).
2. Ohara, M., Kim, Y., Yanagisawa, S., Morikawa, Y. & Kawai, M. Role of molecular orbitals near the Fermi level in the excitation of vibrational modes of a single molecule at a scanning tunneling microscope junction. *Physical Review Letters* **100,** 136104 (2008).

The giddy round of molecular timing

RIKEN synchrotron radiation sheds light on a dynamic reaction cycle

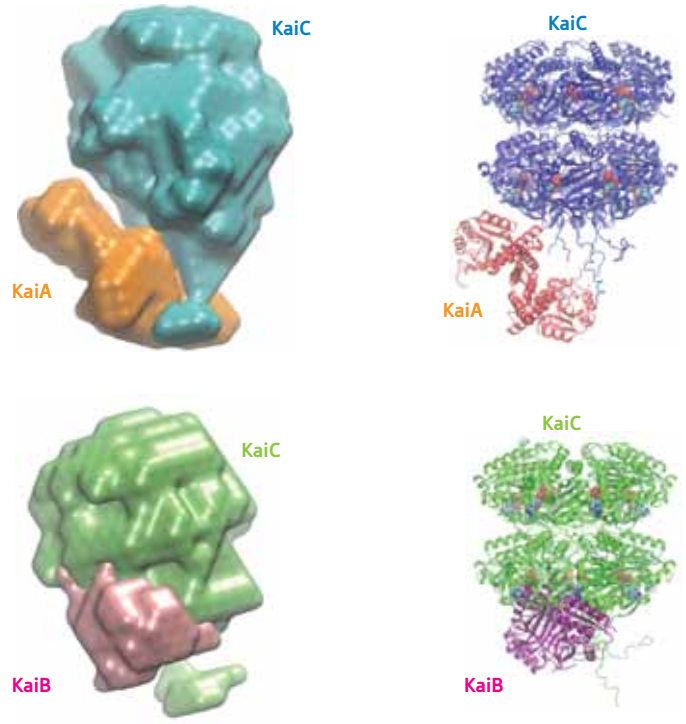


Figure 1: Low-resolution envelope models (left) and superimposed crystal structures (right) of the KaiA:KaiC complex (top) and the KaiB:KaiC complex (bottom).

Japanese molecular biologists have determined the structures of a chronological sequence of complexes formed in an oscillating interaction among three proteins that acts as the timing mechanism for the circadian clock of blue-green algae, now known as cyanobacteria. This is the first time researchers have been able to observe such a biochemical system in motion.

Cyanobacteria are the simplest organisms known to have a biological clock. Earlier studies have shown the timing system relies on three clock proteins KaiA, KaiB and KaiC. When these three proteins are incubated with the phosphate-donor adenosine triphosphate, a stable cycle is established whereby KaiC gains and releases phosphate in reactions involving the other two Kai proteins.

Now, in a paper in *Molecular Cell*[1], researchers from the Japan Science and Technology Agency (JST), Nagoya University, and the RIKEN SPring-8 Center report they were able to use the powerful small-angle x-ray scattering (SAXS) beamline BL45XU at SPring-8 in Harima to follow the dynamic oscillatory reaction in real time. The intensity of forward scattering provides a sensitive measure of the average molecular weight of the compounds in solution and can be used to track the reaction cycle. The SAXS data could also be solved to provide low-resolution models of the complexes formed by the interactions of the proteins (Fig. 1).

Previous x-ray crystallographic studies determined that each of the proteins occurs as a multi-subunit structure, consisting of two subunits in the case of KaiA, four subunits in KaiB and a six-subunit barrel-shaped structure in KaiC. By determining the structures of the complexes formed when either KaiA or KaiB interact with KaiC, the researchers were able to show that the KaiC binding sites for the other two proteins were close enough for KaiA and KaiB to interfere with each other. KaiA binds to KaiC more quickly than KaiB, but the KaiB:KaiC interaction is thermodynamically more stable. During its interaction with KaiA phosphate is incorporated into KaiC, making the complex more attractive to KaiB which strips the phosphate away, making it more attractive to KaiA.

The oscillation cycle is driven initially by the assembly and disassembly of the KaiA:KaiC and KaiB:KaiC complexes, the researchers say, but subsequently is driven by the addition and removal of phosphate from KaiC. The researchers suggest two possible reaction cycles which fit their data. "The next target of our work is to determine which of our two possible schemes is more realistic," says project-leader Shuji Akiyama of JST and RIKEN. ■

1. Akiyama, S., Nohara, A., Ito, K. & Maéda, Y. Assembly and disassembly dynamics of the cyanobacterial periodosome. *Molecular Cell* **29,** 703–716 (2008).
2. Akiyama, S., Nohara, A., Ito, K. & Maéda, Y. Real-time small-angle x-ray scattering observation of assembly and disassembly dynamics of cyanobacterial periodosome. *SPring-8 Research Frontiers* 24–25 *2007*.

Complexes full of energy

A crystal structure of a complex that generates energy shows scientists the way towards treatments for metabolic disorders

A team of scientists from Japan has determined the structure of a protein complex known to be important in metabolic syndrome, which describes people who may be obese or have high blood pressure and are at risk from heart disease and stroke. Metabolic syndrome affects around one in four adults in the western world.

All organisms convert and store excess calories as fatty-acids for use during times of famine or emergencies. However it is this storage system in humans that can lead to weight gain and increased blood pressure resulting in metabolic syndrome.

A key enzyme in the production of fatty-acids is acetyl-CoA carboxylase (ACC). Biotin carboxyl carrier protein (BCCP) forms part of this ACC molecule that is activated by another enzyme known as biotin protein ligase (BPL). One possible approach to treatments for metabolic disorder would be to interfere with the activation of the BCCP fragment of ACC preventing the formation of fatty-acids and avoiding obesity.

To enable development of treatments scientists need a complete understanding of what the activation complex looks like between the fragment BCCP and the activation enzyme BPL. Now, Naoki Kunishima and colleagues from the RIKEN SPring-8 Center in Harima have successfully determined the structure of this key complex[1].

It is clear from the extent of the study by Kunishima and the team that determining the structure of this complex has not been easy. Complexes such as this one are inherently unstable as they are essentially intermediates in a reaction. Previous work from the group determined the structure of BPL and, using this information, the group designed eight mutants that could potentially form a complex but would only react slowly with BCCP.

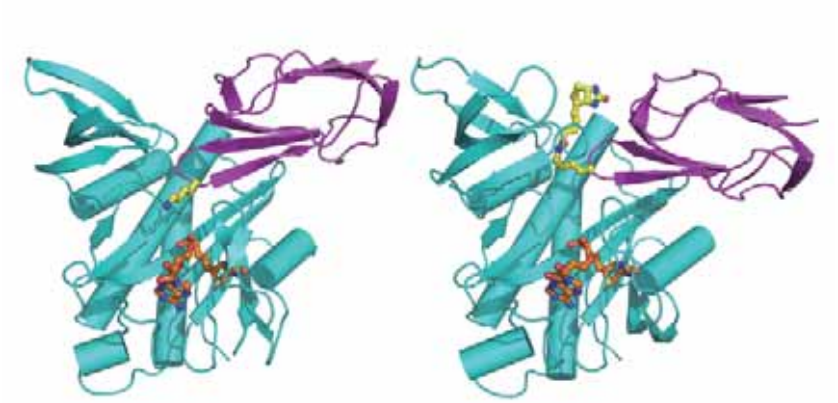


Figure 1: The crystal structure of the complex between the activation enzyme, BPL (cyan), and BCCP (magenta), a fragment of a key enzyme in the production of fatty-acids. The structures show how BPL activates BCCP: before reaction (left) and after reaction takes place (right).

After a considerable number of trials, a few of the mutants formed crystals that were analyzed using specialist synchrotron radiation facilities capable of solving very detailed structures. Referring to Figure 1, Kunishima notes "in one of the crystals, surprisingly, we observed two different states of BCCP activation: one of the complex in the [unactivated] state before the reaction and the other complex showed the activated state after the reaction."

Kunishima and the team are planning two new projects as a result of this research. The first project will investigate complexes of other protein mutations to create a general method to assist the structural determination of difficult target proteins. In the second project, the team will look to develop drug targets to treat metabolic syndrome. ■

1. Bagautdinov, B., Matsuura, Y., Bagautdinova, S. & Kunishima N. Protein biotinylation visualized by a complex structure of biotin protein ligase with a substrate. *The Journal of Biological Chemistry* **283,** 14739–14750 (2008).

Transport proteins make special deliveries

Two proteins responsible for ushering molecular cargo into the nucleus also play an unexpected role in making targeted deliveries during cell division

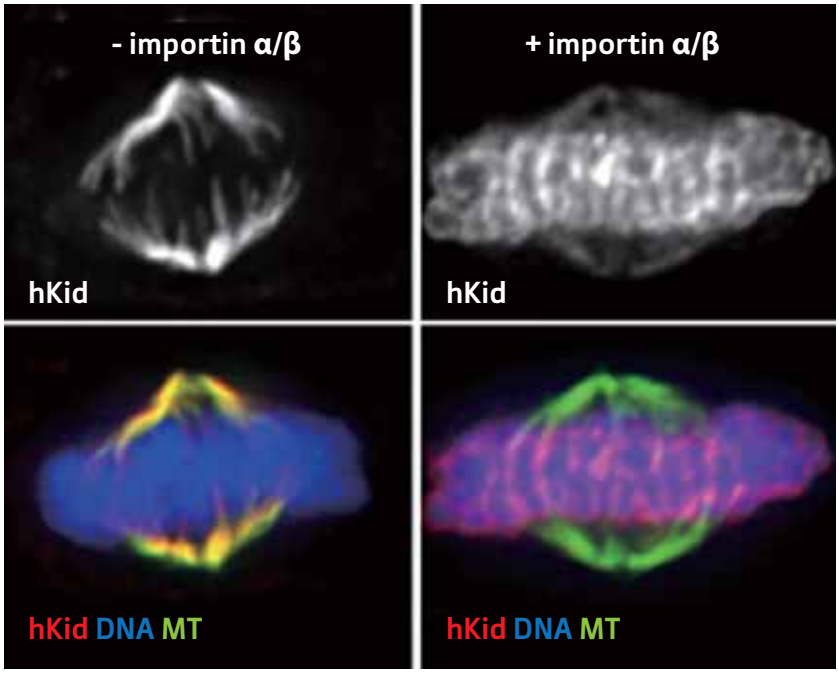


Figure 1: Disruption of hKid association with mitotic chromosomes in the absence of importin-α and -β. In the absence of the importins, hKid remains preferentially bound to the mitotic spindles (top left); following the reintroduction of importin-α and –β, hKid dissociates from the spindles and binds to the chromosomes, which are aligned at the center of the dividing cell (top right). The bottom panels show the same two images with distinct fluorescent labels for hKid (red), chromosomal DNA (blue), and the spindle microtubules (green).

Any cytoplasmic protein requiring access to the nucleus typically needs an escort, in the form of specialized proteins that bind to cargo molecules and mediate their entry through pores in the nuclear envelope.

Two proteins involved in this trafficking process are the importins, α and β, which form a complex with target proteins and then shuttle them into the nucleus. Once inside, the importin–cargo complex is bound by the protein-nucleotide complex Ran-GTP, which triggers cargo release and the subsequent shuttling of the importins back into the cytoplasm.

During the early stages of mitosis, the process of cell division, the nuclear envelope disintegrates and the chromosomes are aligned in the center of the dividing cell by assemblies of long protein filaments known as spindles. One of the proteins that manage this process is hKid, which binds both to the spindles and to the chromosomes themselves.

Previous research has shown that hKid is transported by importins, but it has remained unclear how this association is relevant to the mitotic process. "Nobody had thought about importins having any role in targeting proteins to mitotic chromosomes, especially when there is no nuclear envelope present," explains Naoko Imamoto, an investigator at the RIKEN Advanced Science Institute (formerly the Discovery Research Institute) in Wako. Nonetheless, this surprising finding is exactly what Imamoto's team uncovered in their most recent work[1].

They identified two nuclear localization signals (NLS)—the 'address labels' used by importins—on hKid. Without these NLS, or in the absence of the importin proteins, the ability of hKid to stably and efficiently associate with chromosomes was markedly reduced in mitotic cells (Fig. 1). Under these conditions, hKid instead showed an increased preference for binding the spindles, suggesting that the importins serve a double role: disrupting hKid spindle-binding, and targeting the protein to the chromosomes. They subsequently showed that this process is in turn expedited by the local production of Ran-GTP at the chromosome, which enables efficient release of hKid once the importin complex is appropriately positioned nearby.

Imamoto suggests that these findings may be part of a bigger picture, in which transport proteins are not merely ushers for mitosis-related factors, but deliverymen as well. "This is a new phenomenon," she says. "We certainly think that the system found in this current study—the targeting of proteins onto mitotic chromosomes by transport factors—could also be adopted by many other mitotic chromosomal proteins. Therefore, we will examine the generality of this phenomenon." ■

1. Tahara, K., Takagi, M., Ohsugi, M., Sone, T., Nishiumi, F., Maeshima, K., Horiuchi, Y., Tokai-Nishizumi, N., Imamoto, F., Yamamoto, T., Kose, S. & Imamoto, N. Importin- β and the small guanosine triphosphatase Ran mediate chromosome loading of the human chromokinesin Kid. *Journal of Cell Biology* **180**, 493–506 (2008).

Bugs helping bugs

Scientists get their first glimpse into the workings of the complex bacterial community residing within the termite gut

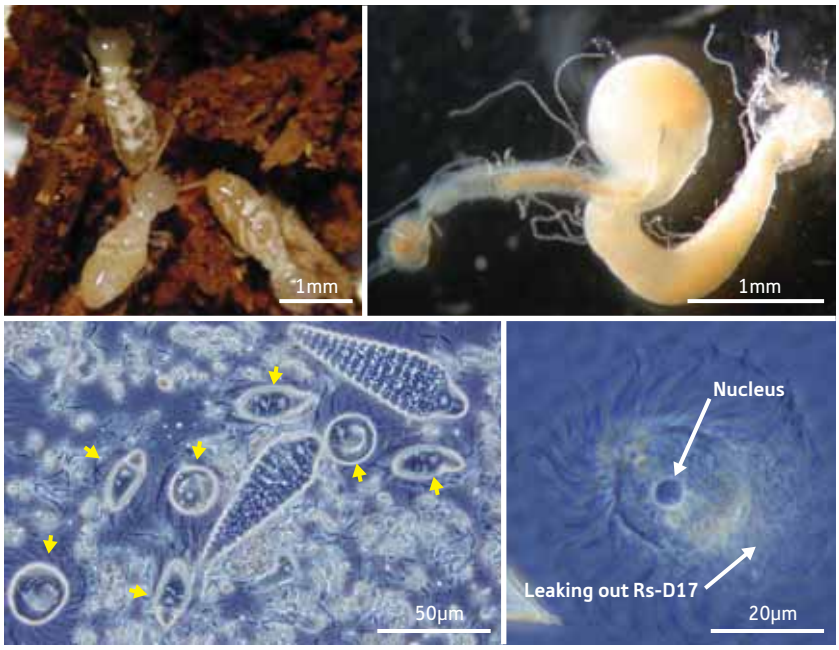


Figure 1: Rs-D17, a gut microbe isolated from the termite species *Reticulitermes speratus* (top left). Within the gut (top right) of the termite is a thriving ecosystem of microbial species (lower left). The Rs-D17 bacterium can be seen leaking out of a gut protist cell in which it resides (lower right).

Left unchecked, a band of termites can eat their way through a house, but they can't do it alone. Within every termite is a thriving ecosystem of bacteria that maintain an essential symbiotic relationship with their hosts, taking shelter within the insect's gut and contributing to its survival by various means, including facilitating the digestion of plant matter.

Little is known about these symbiont species, as these bacteria have proven virtually impossible to cultivate in the laboratory and thus difficult to characterize in detail. "One termite gut harbors several hundred species of microbes," explains Yuichi Hongoh, a postdoctoral fellow in Moriya Ohkuma's research group at RIKEN's Advanced Science Institute in Wako, "and it is not realistic to collect a large enough amount of a specific species to analyze with general methodologies."

Fortunately, recent years have seen the development of new tools for obtaining detailed genomic sequence information from limited sample quantities and Hongoh, in partnership with the RIKEN Bioinformatics And Systems Engineering division (formerly the RIKEN Genomic Sciences Center), in Yokohama, was able to take advantage of these new amplification and sequencing methods to finally assemble the first comprehensive genomic sequence from a termite gut symbiont species[1].

The team analyzed Rs-D17 (Fig. 1), a representative species that is thought to account for approximately 4% of all termite gut bacteria. The analysis revealed that the Rs-D17 genome consists of a single chromosome containing 761 putative protein-coding genes, as well as several smaller non-chromosomal DNA molecules.

The researchers were surprised to find that a large percentage (>15%) of the genes identified were actually pseudogenes, non-functional evolutionary descendants of known genes. Although these pseudogenes no longer generate protein, comparative analysis of the genes lost or maintained by Rs-D17 yielded some valuable hints about how the bacterium evolved to adapt to its host environment over time.

In general, Rs-D17 appears to have sacrificed its regulatory and cellular defense mechanisms in favor of maintaining robust pathways for synthesizing organic molecules required by the termite. "This indicates that this bacterium has been 'domesticated' by the host like an organelle, which synthesizes the nitrogen compounds critically deficient in wood food material," says Hongoh.

He indicates that the team is now looking to extend their strategy to assemble genomic maps for additional symbiont species, in the hope of clarifying the complex relationships between the various members of this gut ecosystem. "By combining these data," concludes Hongoh, "our understanding of termite gut symbiosis will be greatly enhanced."■

1. Hongoh, Y., Sharma, V.K., Prakash, T., Noda, S., Taylor, T.D., Kudo, T., Sakaki, Y., Toyoda, A., Hattori, M. & Ohkuma, M. Complete genome of the uncultured Termite Group 1 bacteria in a single host protist cell. *Proceedings of the National Academy of Sciences, USA* **105,** 5555–5560 (2008).

Assisted development

A silencer of a genetic switch steers the production of killer versus helper immune cells

New work by Japanese researchers forwards our understanding of how transcription factors—proteins that turn genes on and off—direct the production of operationally discrete populations of immune cells called T lymphocytes.

Responsible for detecting and eliminating pathogens and transformed cells, T lymphocytes exist in two varieties. Helper T lymphocytes, which bear the CD4 surface marker and require interaction with immune proteins called MHC class II molecules during development, release soluble mediators and promote antibody production from another type of lymphocyte, the B cell. Cytotoxic T lymphocytes, which tote the CD8 surface marker and require contact with MHC class I proteins, directly kill infected and transformed cells.

For many years, the molecular mechanisms regulating T lymphocyte precursor 'commitment' to the helper versus cytotoxic lineage remained mysterious. However, recent work designated Th-POK as a transcription factor necessary and sufficient for the development of helper T lymphocytes.

Now a team, led by Ichiro Taniuchi, at the RIKEN Research Center for Allergy and Immunology in Yokohama has identified additional transcription factors influencing T lymphocyte lineage commitment. Their work was recently published in *Science*[1].

The researchers focused on Runx proteins, which are regulators of gene transcription and are expressed in T lymphocyte precursors. Using gene targeting techniques, the team generated mice that lack Runx3 and express a nonfunctional version of Runx1. These mutant mice exhibited a dearth of $CD8^+$ T lymphocytes. Surprisingly, many $CD4^+$ T lymphocytes in these animals developed even in the absence of MHC class II proteins, like mice expressing elevated amounts of Th-POK. This finding led the team to conclude that impaired Runx activity results in 'redirection' of killer T lymphocyte precursors into the helper lineage (Fig. 1).

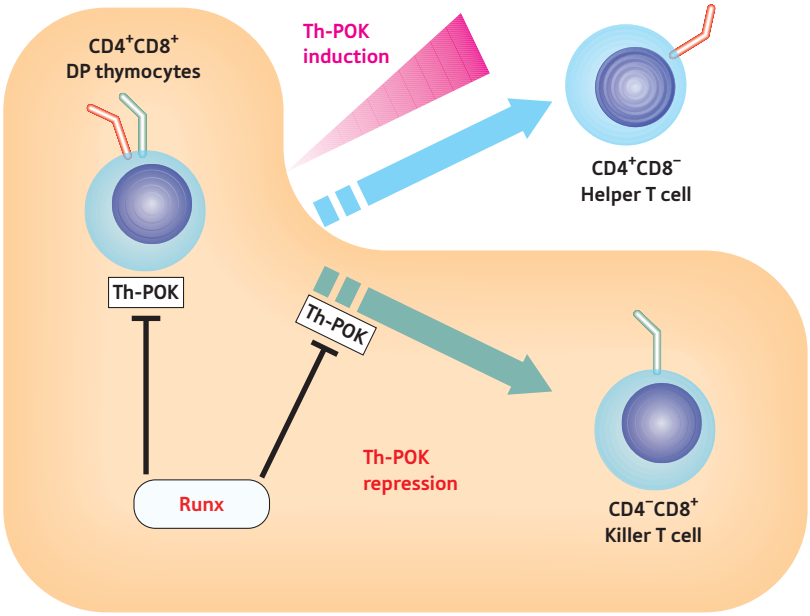


Figure 1: Transcription factor network directing lineage commitment of $CD4^+CD8^+$ lymphocyte precursors (thymocytes). Repression of Th-POK—a transcription factor that promotes development of $CD4^+$ helper T lymphocytes—by Runx transcription factor complexes is essential for the generation of $CD8^+$ cytotoxic, or killer, T lymphocytes. Red and green shapes depict CD4 and CD8, respectively.

Noting that Th-POK expression was elevated in Runx mutant T lymphocyte precursors, the researchers hypothesized that Runx proteins actively suppress Th-POK transcription. Confirming this theory, the team pinpointed a Runx-binding site in the DNA sequences responsible for controlling Th-POK expression. Removal of this Runx binding site—referred to as the Th-POK silencer—from the mouse genome resulted in uniformly high expression of Th-POK and near complete loss of cytotoxic T lymphocyte populations.

Future efforts will be directed towards understanding the upstream signals that promote Runx and thus suppress Th-POK activity.

"The Th-POK silencer may distinguish between signals triggered by MHC class I and class II, and thus act as a nuclear 'sensor' to detect and translate signals emanating from the cell surface," says Taniuchi. ■

1. Setoguchi, R., Tachibana, M., Naoe, Y., Muroi, S., Akiyama, K., Tezuka, C., Okuda, T. & Taniuchi, I. Repression of the transcription factor Th-POK by Runx complexes in cytotoxic T cell development. *Science* **319,** 822–825 (2008).

Tracing lymphocyte development signaling pathways

Two related proteins are essential for proliferation and survival of immune cell precursors

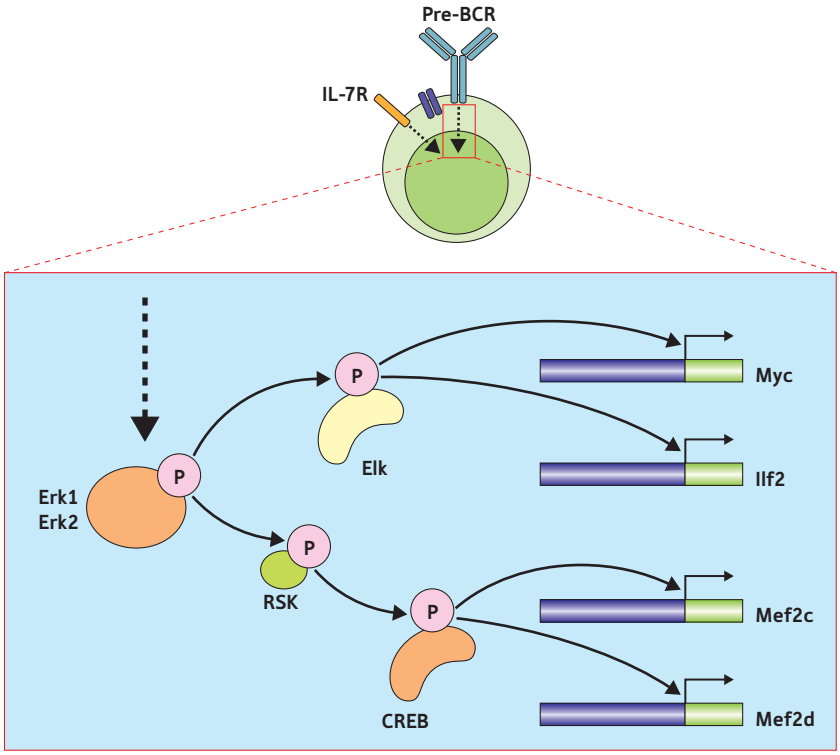


Figure 1: The proteins Erk1 and Erk2 control a transcription factor network that allows B lymphocyte progenitors to traverse the pre-BCR developmental checkpoint.

Researchers have pinpointed the proteins required for transduction of signals directing the development of B lymphocytes, a type of immune cell. Responsible for producing antibodies capable of neutralizing invading microbes, B lymphocytes develop in the bone marrow and, in mature form, circulate throughout the body through the blood and lymphoid organs.

Before exiting the bone marrow, B lymphocyte precursors are propelled through a series of distinct developmental stages. Progression through early stages depends largely on signals triggered by extracellular growth factors, whereas passage through some later stages requires a signal from the pre-BCR, a receptor expressed on the surface of B lymphocyte precursors.

Pre-BCR signals culminate in the induction of gene transcription in the nucleus. Although the cell-surface proteins responsible for initiating pre-BCR signals have been identified, Tomoharu Yasuda and co-workers at RIKEN's Research Center for Allergy and Immunology in Yokohama set out to identify proteins further down the pre-BCR signaling pathway.

The team focused on the related proteins Erk1 and Erk2, which transduce signals by phosphorylating, or 'tagging' downstream target proteins. Using genetic techniques, the researchers generated mice in which Erk1, Erk2 or both Erk1 and Erk2 are deleted or 'knocked out' on demand[1].

Compared to normal mice or those lacking either Erk1 or Erk2, mice lacking both Erk1 and Erk2 exhibited an almost complete block in development beyond the stage during which pre-BCR signaling occurs. Despite normal surface pre-BCR expression, these 'double knockout' precursors showed impaired proliferation and survival.

Consistent with the defects of the double-knockout cells, B lymphocyte precursors treated with an inhibitor of Erk activity expressed lower amounts of immediate early genes (IEG), which are required for population expansion.

As Erk1 and Erk2 do not directly bind to DNA, the team searched for DNA-binding factors that link Erk proteins with IEG upregulation. Noting the presence of binding sites for the factors Elk and CREB in the DNA regions controlling IEG expression, the team showed that pre-BCR-induced Erk activation results in Elk and CREB phosphorylation (Fig. 1).

Illustrating the importance of Erk activity in B lymphocyte development, forced expression of mutant forms of Elk and CREB that lack Erk phosphorylation sites blocked pre-BCR-induced population expansion.

"Pre-BCR signals transmitted through Erk and downstream transcription factors are critical for pre-B cell proliferation," says Yasuda. "Drugs able to promote or suppress such molecular activity may be useful for treatment of acute lymphobastic leukemia or immune deficiency disorders." ■

1. Yasuda, T., Sanjo, H., Pagès, G., Kawano, Y., Karasuyama, H., Pouysségur, J., Ogata, M. & Kurosaki, T. Erk kinases link pre-B cell receptor signaling to transcriptional events required for early B cell expansion. *Immunity* **28,** 499–508 (2008).

Choosing the right path

A new study of blood cell development could help put an end to a decade-old controversy about immune cell differentiation

Blood contains many different cell types, all of which develop from a common stem cell precursor via a multi-stage differentiation process. T cells and B cells, immune cells that respond to foreign particles in the body, belong to a class known as lymphocytes, and have long been assumed to follow a similar developmental course. This 'classical model' of differentiation predicts that stem cells will differentiate into common lymphoid progenitors (CLPs), which become T and B cells, or myelo-erythroid progenitors, which form myeloid cells—such as macrophages—and red blood cells (Fig. 1a).

It was therefore quite surprising when immunologists Hiroshi Kawamoto and Yoshimoto Katsura were unable to detect CLPs from blood cell progenitors in the fetal liver[1]. Their 1997 study suggested a 'myeloid-based model' of differentiation (Fig. 1b), in which the ability to form myeloid cells is retained by all blood cell progenitors. However, another group's subsequent discovery of putative CLPs in adult bone marrow[2] left immunologists with a controversy on their hands: do fetal and adult blood cells develop along different pathways?

New work from Kawamoto's group at the RIKEN Research Center for Allergy and Immunology, Yokohama, represents important progress towards resolving this mystery[3]. Kawamoto and colleagues developed a new culture system that enabled them to gauge the ability of progenitor cells from different developmental stages to form T and myeloid cells. The researchers found that these progenitors could form macrophages even at stages of differentiation where B cell formation was no longer a possibility, regardless of whether the cells were adult or fetal in origin. Similar results were obtained in live mice when these later-stage progenitor cells were grafted into the thymus.

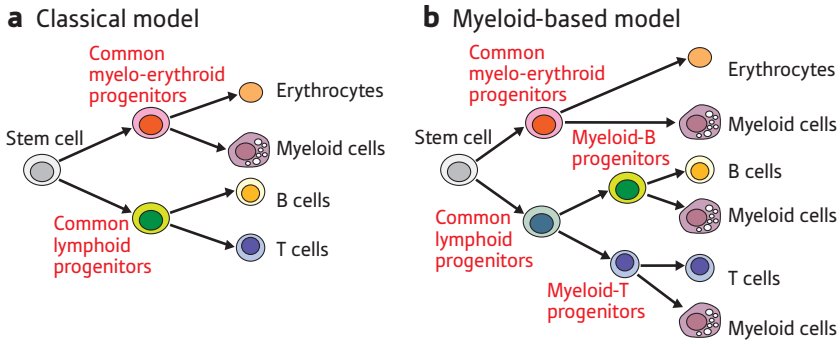


Figure 1: Two models of blood cell differentiation. (a) In the long-standing 'classical model', stem cells can differentiate into myelo-erythroid progenitors, which can form myeloid cells and erythrocytes (red blood cells), or CLPs, which can form B and T cells but not myeloid cells. (b) Kawamoto and colleagues' findings support a 'myeloid-based model', in which CLPs are not a requisite intermediate stage. Instead, stem cells develop into common myelo-lymphoid progenitors and then into myeloid-B or myeloid-T progenitors, all of which retain the ability to form myeloid as well as lymphoid cells.

Kawamoto believes that these findings support a myeloid-based model of development for both fetal and adult immune cells, and argues against the need for CLPs. "Many researchers have taken it [for] granted that T cells and B cells are very closely related, and thus both lineage cells [are] generated through a common pathway," he says. "Our findings—that T cells and macrophages are generated from progenitors that have lost B cell potential—refute this dogma."

These findings should shed new light on T cell development. "Now that we have certified that the myeloid-T bipotential stage exists in hematopoiesis, we are in the best place to start to clarify the molecular mechanisms of myeloid-T lineage commitment," he says. "These studies will give us answers on what environmental and intrinsic factors determine the identity of T cell lineage." ■

1. Kawamoto, H., Ohmura, K. & Katsura, Y. Direct evidence for the commitment of hematopoietic stem cells to T, B and myeloid lineages in murine fetal liver. *International Immunology* **9,** 1011–1019 (1997).
2. Kondo, M., Weissman, I.L. & Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* **91,** 661–672 (1997).
3. Wada, H., Masuda, K., Satoh, R., Kakugawa, K., Ikawa, T., Katsura, Y. & Kawamoto, H. Adult T-cell progenitors retain myeloid potential. *Nature* **452,** 768–772 (2008).

Finding the roots of a mysterious disease

A new study pinpoints a specific gene variation linked to a major cause of pediatric acquired heart disease

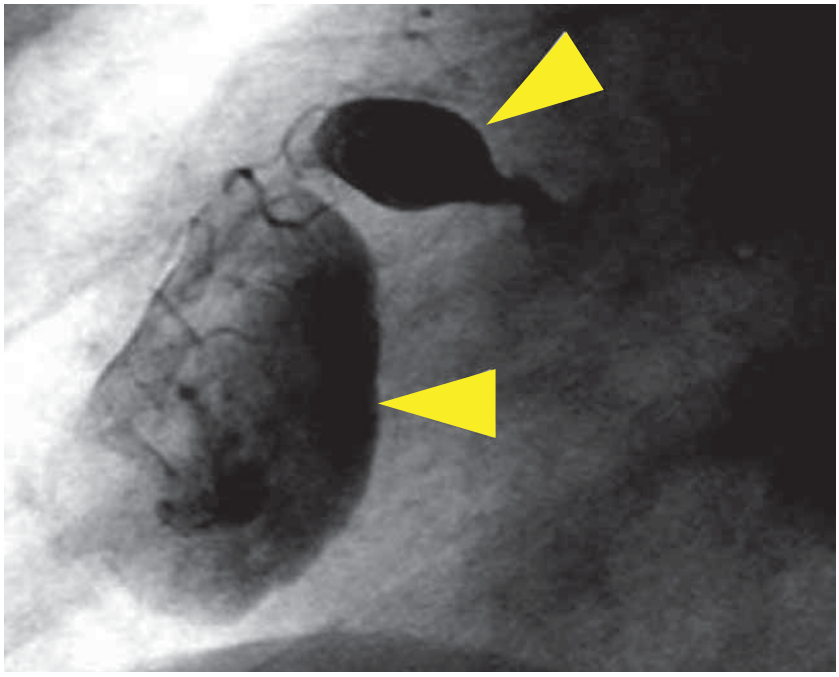


Figure 1: Consequences of untreated—or treatment-resistant—Kawasaki disease can include the formation of giant coronary aneurysms.

Kawasaki disease (KD) is an inflammatory disorder of the blood vessels, most frequently observed in infants and young children. Left untreated, KD can have severe health consequences (Fig. 1), and it is currently the leading cause of acquired heart disease for children in the developed world.

KD was first discovered by Tomisaku Kawasaki over 40 years ago, yet little is known about its root causes. Some evidence has suggested a microbial origin for KD; more recent findings indicate a genetic basis, however, and geneticist Yoshihiro Onouchi was among the first to closely explore this possibility.

As a student, Onouchi worked with Kawasaki to collect DNA samples from related KD patients in an effort to identify relevant genetic markers. Now, as a researcher at the RIKEN Center for Genomic Medicine in Yokohama, Onouchi has again partnered with Kawasaki and other scientists from Japan and the US to identify the first gene variant linked with KD pathology[1].

Since KD is most prevalent in Japan, the researchers began by investigating pairs of affected Japanese siblings. They looked for tiny DNA sequence variations that might be associated with the disease, and identified nine variants that could be potentially linked to KD. By performing a parallel analysis of the inheritance patterns for these variations in Americans of European ancestry—among whom KD is much rarer—the team narrowed the number of candidates down to four.

They became particularly interested in one variation, in the gene encoding the signaling protein ITPKC. Onouchi's team found that ITPKC appears to play an important role in the suppression of T cell activity—an unexpected role for this protein. The gene variant they identified appears to result in a significant reduction in ITPKC production, leading to an increase in T cell activity that could represent the underlying cause for the inflammatory component of KD pathology. "I think one of our most important findings is the characterization of *ITPKC* as an immune gene," says Onouchi.

Subsequent genetic analysis of a cohort of American patients has shown that this *ITPKC* variant is linked with resistance to standard KD therapies, and the investigators are now looking for similar patterns in Japanese patients. These findings may also lead to new therapeutic approaches. "We will try to validate the effectiveness of the immunosuppressant cyclosporine A as an alternative treatment," says Onouchi, "as this compound targets the pathway in the T-cells in which ITPKC acts as a negative regulator." ■

1. Onouchi, Y., Gunji, T., Burns, J.C., Shimizu, C., Newburger, J.W., Yashiro, M., Nakamura, Y., Yanagawa, H., Wakui, K., Fukushima, Y. *et al.* *ITPKC* functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. *Nature Genetics* **40**, 35–42 (2008).

Gene family reunion

Japanese plant biologists isolate a gene controlling plant size, flowering and development

Differentiation of cells into specific types and patterns is a crucial feature of development in multicellular organisms; and in plants, advantageous changes in cell differentiation can help them to survive under changing environmental conditions.

The plant gene *CAPRICE* encodes a small protein that promotes differentiation of epidermal cells into root hair cells or hairless cells. Three additional genes that are similar to *CAPRICE* promote differentiation of these cells. These three homologs are said to act redundantly, because without them, the physiology of the plant does not appear to change.

Now Rumi Tominaga and a team at RIKEN's Plant Science Center in Yokohama have isolated a fourth *CAPRICE*-like gene named *CPL3* from the popular model plant, *Arabidopsis thaliana*. *CPL3* shares some functions with *CAPRICE* and the other homologs, but as reported recently in *Development*[1], the team has discovered that *CPL3* boasts several characteristics that make it distinct and crucial to plant development and growth mechanisms.

Cell differentiation was affected in plants with extra copies of *CPL3* resulting in overproduction of root hairs, as was observed previously for the other genes in the *CAPRICE*-like family. The team suggests that *CPL3* redundantly regulates epidermal cell differentiation into hairless or root hair cells along with the other *CAPRICE* homologs. Although *CPL3* has diverged partially from the *CAPRICE*-like gene family by having its own distinct functions, retaining its redundant functions may represent an important evolutionary position for the plant. Because the so-called redundant functions provide the potential for an adaptive response to changes in the environment, they also provide the plant with a strategic plan for survival.

Figure 1: Normal (left) and mutant (right) *Arabidopsis thaliana* plants. The mutant plants are much larger than normal plants.

Using mutant plants in which the function of *CPL3* was disrupted, the researchers found this gene to have profound influences on flowering time and plant size. Mutant plants lacking *CPL3* flowered earlier than normal plants and were much larger (Fig. 1). Endoreduplication is the process of making copies of DNA in specialized cells without undergoing cell division and is thought to provide a mechanism for increasing cell size. The team found that *CPL3* controls flowering development, epidermal cell size, and aspects of cell-hair growth through the regulation of endoreduplication. The team will investigate *CPL3* in other plants or crops "because a useful plant that grows larger [through] the regulation of endoreduplication might be obtained," says Tominaga. ■

1. Tominaga, R., Iwata, M., Sano, R., Inoue, K., Okada, K. & Wada, T. *Arabidopsis CAPRICE-LIKE MYB 3 (CPL3)* controls endoreduplication and flowering development in addition to trichome and root hair formation. *Development* **135,** 1335–1345 (2008).

Wired for smell

Brain scientists unlock the combination of molecules used to connect up the olfactory system

Figure 1: The location of BIG-2-linked glomeruli (red) in the olfactory bulb compared with two other guidance molecules (blue and green).

Japanese researchers have unraveled details of the molecular guidance system that ensures the sensory nerves of the smell or olfactory system are correctly wired into the brain. A set of guidance molecules steers the developing nerve process or axon growing from the cell body of an olfactory sensor neuron (OSN) to a spatially specific globular tangle of axons known as a glomerulus in the olfactory bulb (OB). The smell sensor is connected through the glomerulus to the central nervous system.

This targeting system looks to be similar throughout the body's sensory systems and many of the same molecules are employed. Deleterious mutations in the genes of these guidance molecules can result in incorrectly wired sensory systems with serious developmental consequences.

Earlier work by many different research groups had shown that each OSN carries only one type of smell receptor. The axons from cells of the same receptor-type converge on a few spatially fixed glomeruli on the surface of the OB.

In order to find out more about how this targeting was achieved, researchers from RIKEN's Brain Science Institute in Wako worked with a molecule known as BIG-2 found on the axons of some mouse OSNs. In a recent paper in *Neuron*[1], they report finding that BIG-2 was associated with a small range of specific olfactory receptors. On the OB, axons carrying BIG-2 on their surface connected strongly to one group of glomeruli, weakly to another group, and not all to a third group in a mosaic pattern. There appeared to be no correlation between this binding pattern and those of other axon targeting molecules (Fig. 1). And in mice genetically engineered to lack BIG-2, the axons usually associated with BIG-2 linked to glomeruli outside the normal fixed positions.

When the researchers considered the results of their work together with studies into similar systems in zebrafish, they proposed a four-step hierarchical model for the establishment of nerve circuitry in the olfactory system—the initial outgrowth regulated by one set of molecules; navigation towards the OB by another; targeting to the appropriate district of the OB by a third; and then the final convergence onto specific glomeruli which is governed by combinations of several guidance molecules, including BIG-2.

"We now want to trace the connections from the olfactory bulb deeper into higher olfactory centers of the brain, and begin to relate the whole system to olfactory-mediated behaviors," says project-leader Yoshihiro Yoshihara. ■

1. Kaneko-Goto, T., Yoshihara, S., Miyazaki, H. & Yoshihara, Y. BIG-2 mediates olfactory axon convergence to target glomeruli. *Neuron* **57,** 834–846 (2008).

Rodents rake for rewards

A RIKEN study shows that rodents can be trained to use tools just as well as primates

In the past, the use of tools enabled humans to adapt to different ecological niches. The resulting new experiences presented more learning opportunities, extending our brain capacity.

Now researchers at the RIKEN Brain Science Institute in Wako have trained degus, a medium-sized rodent native to Chile, to use tools. The study shows that degus could be a useful animal model for directly observing how tool use modifies brain and behavior[1].

"Rodents are smaller and less costly to maintain than primates," explains project-scientist Kazuo Okanoya. "Also, techniques for genetic manipulations are more advanced for rodents and we could modify these existing procedures for degus."

The researchers originally chose to study degus because they noticed that the animals are playful, highly inquisitive, and have good manual skills (Fig. 1). Adult degus were placed in a chamber with openings that the degus could put their hands through. Food was placed just beyond the animals' reach, while a rake-like tool was placed within their reach.

To begin with, the food was placed behind the rake's blade, so that all the degus had to do was pull the rake handle. Once they had mastered this, the task was made more difficult by placing the food in front or to one side of the tool. The degus soon began to try out new movements such as pushing the tool or wiggling sideways. With more practice the movements merged into one smooth trajectory and the degus were seen to stare at the food rather than at the tool, suggesting that the tool was becoming an intuitive extension of their own arms.

Kazuo Okanoya

Figure 1: The RIKEN researchers chose degus for tool-use training after noticing their high level of curiosity and manual dexterity.

Furthermore, the degus adapted to tools of different sizes, shapes and colors, and quickly learned to ignore tools that didn't work. This shows that the animals gained a mental appreciation of the tool's function.

The results for degus compare favorably with previous studies on primates. The researchers expect that tool use produces new connections between different areas of the brain, leading to improved hand–eye co-ordination. "We are confident that other rodents could also be trained to use tools, although it might take longer than for degus," says Okanoya.

In the future, Okanoya would like to study the links between tool use and voice. "We think the site where these two activities overlap could be a precursor for the brain areas related to language production," he says. "Thus, the current tool use study can lead to the study of the origin of language." ■

1. Okanoya, K., Tokimoto, N., Kumazawa, N., Hihara, S. & Iriki, A. Tool-use training in a species of rodent: the emergence of an optimal motor strategy and functional understanding. *PLoS ONE* **3,** e1860 (2008).

Sleepy neurons keep the brain awake

RIKEN researchers are revealing the subtle differences in brain activity when we are awake or asleep

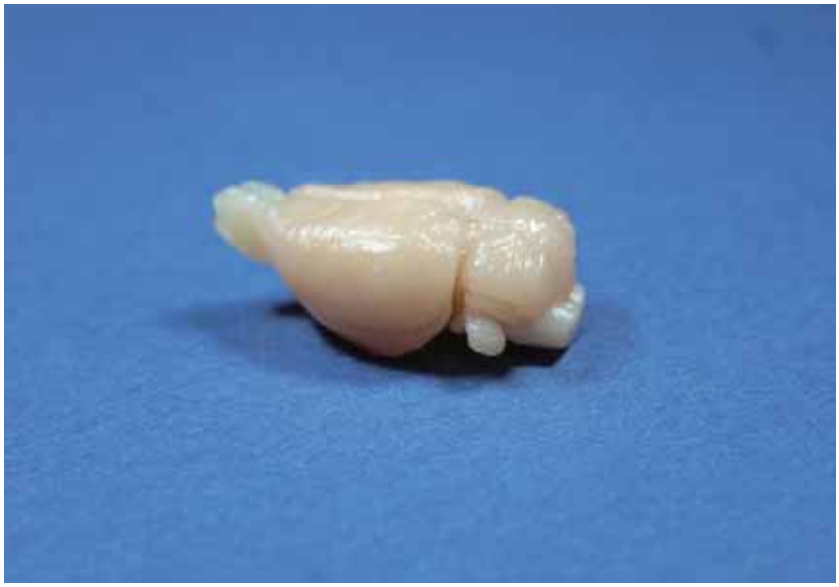
One of the reasons it is often hard to wake up in the morning may be that the brain has to suddenly make huge changes in the way it operates. Some new mechanisms by which the brain reacts to different patterns of electrical neuron firing when we are awake or asleep have now been uncovered by Tohru Kurotani, at the RIKEN Brain Science Institute in Wako, and his co-workers[1].

"All living neurons have 'membrane potential'—a voltage difference between the outside and inside of the cell," explains Kurotani, who also works at Nagoya University. "When neurons are excited by some stimuli, the membrane potential increases." Neurons 'fire' when the membrane potential passes a certain threshold.

While we are awake, neurons tend to maintain a high membrane potential and fire repetitively, but while we are sleeping deeply the membrane potential and neuron firing show slow oscillations, called slow-wave sleep. "At the risk of oversimplification, the neurons also 'sleep' as we sleep," says Kurotani.

In their latest study, the researchers gave strong anesthetic to rats that were either awake or in slow-wave sleep, and cut brain slices from their visual cortex (Fig. 1). The slices were used to monitor 'inhibitory post-synaptic currents' (IPSCs), which act to lower the membrane potential in a neuron and make it less likely to fire. The IPSCs were found to be larger in rats that had been sleeping.

The researchers believe that while the rats were awake, the firing pattern attenuated the synapses that carry inhibitory signals, but during slow-wave sleep the signals could get through. More specifically, the different firing patterns appear to affect routes by which calcium ions can pass into the cell.



Tohru Kurotani

Figure 1: Rat brain as used to monitor neuron activity in awake or sleeping rats.

"The physiological meanings of distinctive brain activity patterns during slow-wave sleep or awakening have been unclear because it was technically difficult to perform *in vivo* experiments," says Kurotani. "We have overcome this problem by using brain slice preparations and mimicking the distinctive firing patterns during slow-wave sleep or awakening."

In the future Kurotani hopes to investigate different phases of sleep, such as REM sleep. In these phases our brains may continue to process information, consolidating the memories we gained while awake.

"During [REM sleep], the brain is in an awake-like state, and is thought to be dreaming—possibly keeping some different sort of consciousness," he explains. "If the dynamics of the brain during different sleep states is revealed, it might provide a clue to explain consciousness."

1. Kurotani, T., Yamada, K., Yoshimura, Y., Crair, M.C. & Komatsu, Y. State-dependent bidirectional modification of somatic inhibition in neocortical pyramidal cells. *Neuron* **57,** 905–916 (2008).

Exploring biological phenomena using the world's fastest computer system

Makoto Taiji

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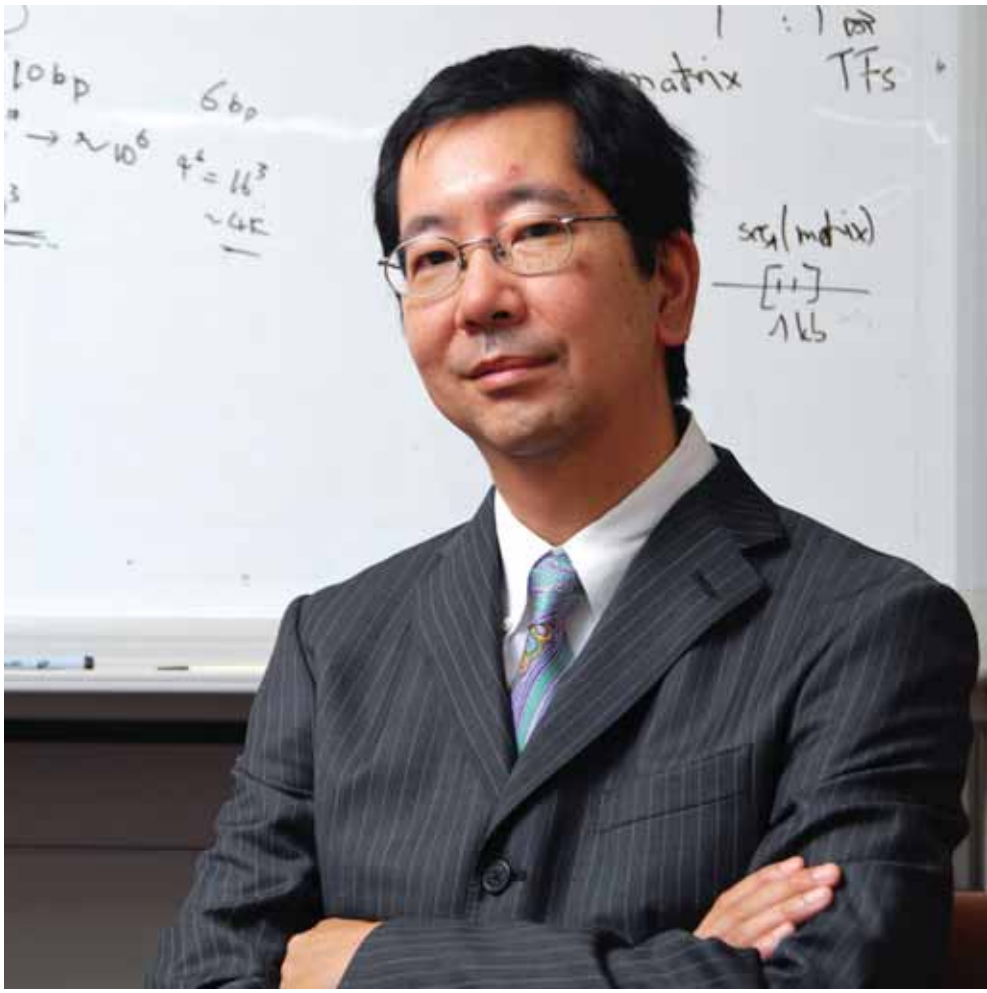


The fastest special-purpose computer system for molecular simulations in the world is available at the RIKEN Advanced Science Institute (ASI). Developed by Makoto Taiji, group director of the Computational Systems Biology Research Group, ASI, the MDGRAPE-3 system is capable of calculating interatomic forces and simulating their motions. As such, MDGRAPE-3 is expected to make significant contributions to faster development of new drugs with higher efficacy, and the understanding of biological molecules, such as proteins, at the atomic level. The following is an interview with Taiji about the prospects for life sciences explored with MDGRAPE-3, and the secret story of its development.

MDGRAPE-3, the world's fastest computer system

When the elevator door opens, a booming sound can be heard everywhere on the fifth floor of the West Building in the RIKEN Yokohama Institute. It comes from the room in which the MDGRAPE-3 is installed (left panel in Fig. 1).

In June 2006, the MDGRAPE-3 system marked the world's highest speed of 1 petaflop. The petaflop is a unit of measurement for the processing speed of a computer system; each petaflop is equivalent to 10^{15} (one quadrillion) floating point operations per second. MDGRAPE-3 is capable of doing one quadrillion calculations per second. This performance is about 20,000 times faster than the latest personal computers. "Even now MDGRAPE-3 remains the only computer system that has achieved a calculation speed of 1 petaflop," says Taiji, the developer of the system.

To understand biological phenomena at the atomic level using a computer system is the goal of the Computational Systems Biology Research Group led by Taiji. "It is our policy to undertake a full range of work, from hardware production to software development, and applied research," he says. "There are almost no other research groups like us worldwide."

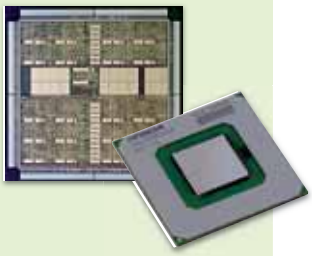
Personal computers in routine use are 'general-purpose' computers, capable of doing a broad range of different types of calculations.

MDGRAPE-3 system

With its 1 petaflop calculation speed, the system consists of 400 MDGRAPE-3 boards and 100 Intel parallel server units. Currently, part of the system operates as a component of the RIKEN Super Combined Cluster Supercomputer System installed in the Advanced Center for Computing and Communication at the RIKEN Wako campus.

MDGRAPE-3 board

Each board is configured with 12 MDGRAPE-3 chips.



MDGRAPE-3 chip

The LSI (left) is 15.7 mm^2 with a line width of 65 nanometers. The MDGRAPE-3 system incorporates a total of 4,800 chips. The chips are manufactured by Hitachi.

Figure 1 : MDGRAPE-3 special-purpose computer system for molecular dynamics simulations.

The MDGRAPE-3 system comprises a combination of general-purpose computers and special-purpose computers dedicated to calculating the forces exerted between atoms that constitute materials. Chemical binding forces, electrostatic forces, and intermolecular forces are exerted on atoms, which move according to equations of motion that describe the relation between these forces and the response of the atoms. Because any material comprises a vast number of atoms, the forces exerted on individual atoms cannot be calculated to simulate their motions without powerful computers.

Taiji characterizes the MDGRAPE-3 system by three aspects. "First," he explains, "MDGRAPE-3 became the first computer in the world to achieve a 1-petaflop calculation speed. Second, the cost performance is high. Our system has been developed with about one billion yen, which is only a tenth of the money spent in developing an ordinary general-purpose large computer system used at a university or research institute. Third, the power consumption is small."

The key to his success in achieving the world's fastest processing speed lies in the use of broadcast-based memory architecture. A great many calculations can be done at one time because each memory device contains multiple computing units connected in parallel. In the MDGRAPE-3 system, as many as 720 calculations can be performed simultaneously with one 'LSI'—an ultrafine electronic circuit mounted on a semiconductor substrate that serves as the core of a computer system. The number of calculations per LSI is limited to around 16 with the use of a current ordinary general-purpose processor.

The amount of heat generated per unit surface area by the latest processor is as much as that on the surface of a nuclear reactor containment vessel at a nuclear power station. It is critical to minimize power consumption by reducing this heat. The power consumption of MDGRAPE-3 is 200 kilowatts per hour, one hundredth the level of a general-purpose large computer system. Even so, a huge cooling system is required, and it is this that produces the loud booming sound on the floor where MDGRAPE-3 is installed.

Faster development of new high-efficacy drugs

The development of the MDGRAPE-3 system began in 2002 as part of the Protein 3000 Project, Japan's national project to explore protein structures and functions. "The structures and functions of many proteins have been elucidated. Now importance must be given to handling the huge amounts of information obtained," points out Taiji. "For this, MDGRAPE-3 was necessary."

One expectation of the MDGRAPE-3 system is in the development of new drugs. Proteins have pockets known as active sites, to which particular molecules bind to allow the proteins to function. If the structure of a disease-related protein is clarified, it will be possible to find molecules that bind to the active sites of the protein efficiently, and to design molecular shapes that facilitate the binding.

Each protein has more than a thousand drug candidate molecules. The conventional approach to selecting the most suitable molecule comprises simulations by the docking method. Candidate molecules are applied to protein active sites one by one, and the molecules that exactly fit are selected. However, Taiji points out a problem with the docking method. "In the docking method, proteins are handled as solid matter with fixed shapes, and the handling of water, the most important molecule for all organisms, is inadequate." He explains that proteins in action in the

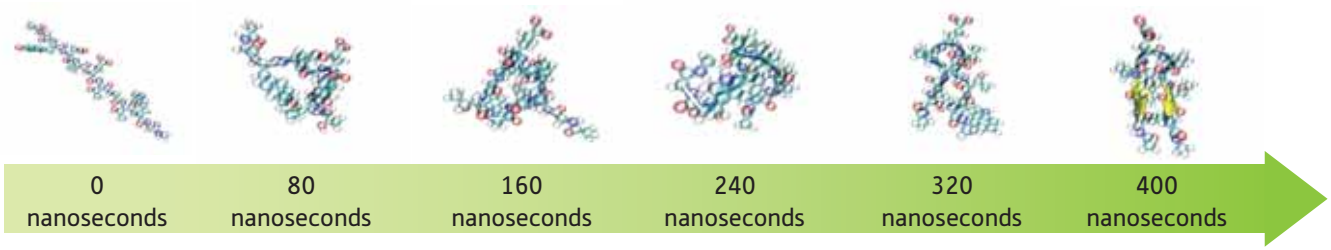


Figure 2 : Simulation of the protein folding process.
Chignolin is a small protein consisting of 10 amino acid residues. The process by which the protein is three-dimensionally folded from the state of amino acids in sequence is simulated. Each particle indicates an atom. Although water molecules do not appear here, their motions were also included in the calculations.

body are constantly oscillating in the presence of thermal energy. "Even if a candidate molecule is found to exactly fit an active site, this does not always mean high affinity, and the effect of water must be considered in determining the action of the molecule."

MDGRAPE-3 is capable of simulating the dynamics of proteins and candidate molecules, including water, at the atomic level, to enable the accurate detection of binding molecules. "Simulations are often used to understand functions," says Taiji. "And my goal is to predict functions, and to control those functions by designing molecular shapes."

A protein comprises a sequence of amino acids arranged according to genetic information. It cannot function normally unless the amino acid sequence is accurately folded. However, much remains unknown about the way in which the protein is folded. Taiji and his colleagues have succeeded in simulating the folding of Chignolin, the world's smallest artificial protein, which consists of only 10 amino acid residues (Fig. 2). The protein in the elongated state bends and elongates repeatedly while oscillating, until it gradually reaches the right shape. This represents a major step toward the functional elucidation of protein folding.

The MDGRAPE-3 system won the Gordon Bell Prize Honorable Mention (Peak Performance division) in 2006. This world-class commendation for the performance of high-speed computers is sponsored by the Association for Computing Machinery. "As a researcher engaged in developing computer systems, I am very happy to receive the prize," says Taiji with a smile. His winning article concerns simulations of the process by which peptides from the yeast protein Sup35 aggregate in water to form needle-like crystals (Fig. 3). The motions of 17 million atoms, including the surrounding water, were calculated. "Protein aggregation is involved in the onset of nervous diseases such as Alzheimer's disease and Parkinson's disease," he adds. "I am working with the expectation that my work will help elucidate the mechanism for the onset of nervous diseases."

The secret story of the development of MDGRAPE-3

"I was optimistic that it would go well," says Taiji, looking back on the early days when he began developing the MDGRAPE-3 system. However, the reality was quite different.

Development of a computer system begins with the design of the LSIs. "I devoted myself to sitting in front of a computer day after day writing LSI programs," says Taiji in retrospect. "That year was very hard. At one time, because I was always thinking in computer language, I found myself unable to talk with my family when I returned home from the office."

The electron travels at a speed of 200,000 kilometers per second—two thirds the velocity of light. "As the circuit operating speed has been increasingly getting faster, the speed of the electron must now be taken into account when designing LSIs." The LSI for MDGRAPE-3 measures 15.7×15.7 mm^2 (right panel in Fig. 1). Taiji says that sometimes he had to keep in mind the speed of electrons moving from one end of the LSI to the other.

In December 2003, the design of the LSI was completed. Taiji proceeded to prepare the MDGRAPE-3 board with the LSI mounted on it, and attempted to check its performance. At this stage, however, a problem arose. "The chip did not work well despite my efforts." Although the accuracy of the circuit connections and the normal performance of the chip had been verified by computer, the chip was failing. Redesigning the LSI would cost around an additional one hundred million yen. "At that time, I noticed my heart beating and my blood pressure rising," adds Taiji. Several days later, the fault was located, not in the chip, but in the software, and the project proceeded smoothly.

The MDGRAPE-3 chip (right panel in Fig. 1) is capable of calculations at 230 gigaflops. This performance is more than 30 times higher than the then fastest Intel Pentium 4 chip, and boasts the world's highest performance per single chip.

Twelve of the MDGRAPE-3 chips were mounted on a board (middle panel in Fig. 1). At that stage, many problems arose. Budget cuts, also brought trouble, but the 1 petaflop speed was achieved in June 2006. "All my efforts became worthwhile," says Taiji. Capable of 1 petaflop calculations, the MDGRAPE-3 system is configured with

400 boards carrying the MDGRAPE-3 chips and 100 Intel server units. Special-purpose computers and general-purpose computers are combined, as with the first computer system Taiji constructed. "People of an earlier generation might think about how to construct the system as a whole," he says. "But I grew up in the age of personal computers. Since I had been fond of building radios and electronic gadgets since my childhood, I began constructing a computer system with a light-hearted curiosity—to add something to the existing personal computer."

The first computer system Taiji constructed was a special-purpose machine for simulating a magnet known as m-TIS in 1987, when he was a graduate student. Later, he joined the development project for GRAPE, the special-purpose computer system for astronomical simulations at the University of Tokyo. GRAPE is capable of calculating gravitational forces between stars to simulate the motions of celestial bodies. By replacing stars with atoms and gravitational forces with electromagnetic forces and intermolecular forces, MDGRAPE-3 was born.

When he was a graduate student, Taiji specialized in laser spectroscopy. His doctoral thesis concerned measurement of changes in Rhodopsin, a protein in the eye, when exposed to light. Although constructing computer systems was no more than a hobby, he entered the field of computer systems when about to leave graduate school. At present, he is challenging the simulation of proteins using the world's fastest computer system, which he developed himself. Twenty years have elapsed since he built his first computer. "I am surprised at what I have achieved. In those days, 1 petaflop calculations were nowhere in my mind."

The problem concerns time

"Our next goal is to extend the simulation time," says Taiji. Binding to other molecules, folding, and other phenomena in proteins occur on timescales of microseconds (10^{-6} seconds, that is, one millionth of a second) or above. They cannot be simulated unless calculations are made at intervals of one femtosecond (10^{-15} seconds, that is, one quadrillionth of a second) in accordance with the speed at which protein atoms move, and the results are integrated from these calculations. A large gap of nine orders of magnitude exists between a femtosecond and a microsecond. Even with the use of MDGRAPE-3, it takes one day to simulate a microsecond phenomenon. One possible solution to this problem may be to increase the computing speed, but this is subject to limitations. Taiji says that he is thinking of another approach. "I am going to perform multiple short-timescale simulations and join them together, so I can watch long-timescale phenomena."

We asked him about any future plans for MDGRAPE-4. "Yes, I am planning to construct a general-purpose computer system," answers Taiji. His plans include the newly introduced concept of a 'tile processor', which comprises LSI-mounted chips arranged like tiles. The planned system is expected to perform at about 10 times higher speeds than existing systems while ensuring general-purpose quality with the tile arrangement, and completion is scheduled in four years. "Computer systems are experimental apparatus. I want to construct a more sophisticated system that will beat all the others."

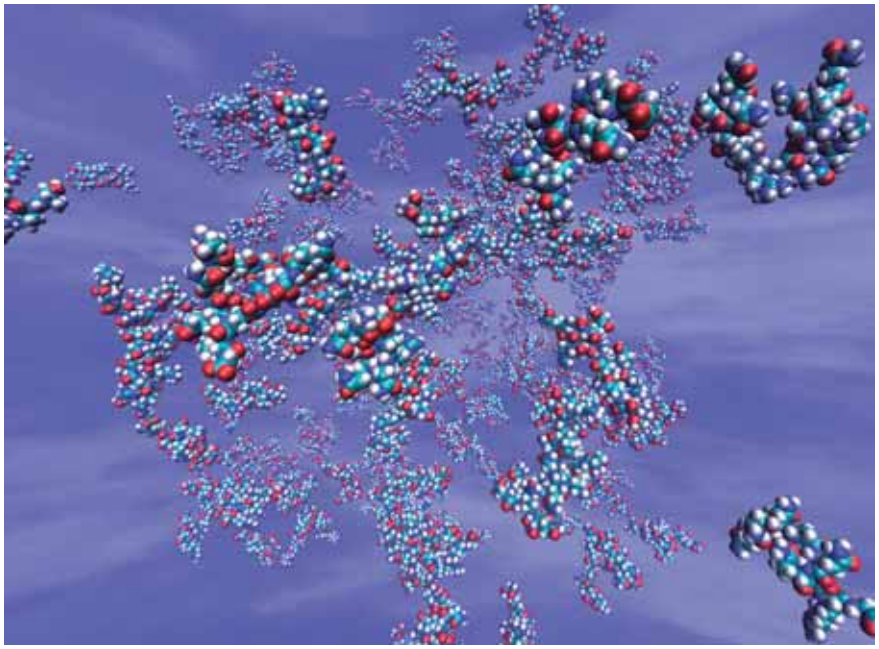


Figure 3: Simulation of the protein aggregation process.
The process by which part of the yeast protein Sup35 aggregates in water to form needle-like crystals is simulated. Although water molecules are not shown here, the motions of 17 million atoms, including water, were calculated (background cloud is an image).

About the researcher

Makoto Taiji was born in Tokyo, Japan, in 1964. He graduated from the Department of Physics, Faculty of Sciences, the University of Tokyo, in 1986, and obtained his PhD in 1991 from the same university. From 1991 to 1996, he was the assistant professor of the Graduate School of Arts and Sciences at the University of Tokyo. From 1997 to 2002, he was the associate professor of the Institute of Statistical Mathematics. He joined the Genomic Sciences Center, RIKEN, as a team leader and was promoted to deputy project director in 2007. Currently he is the group director of the computational systems biology research group at the RIKEN Advanced Science Institute. His research focuses on the applications of high-performance computing in life sciences, and dedicated computer architectures for scientific simulations. He was awarded the Gordon Bell Prize in 1995 for developing a 'Special-Purpose Machine', and in 2006 he received an Honorable Mention for the same award for the 'Peak Performance' achieved by the computer he developed.

RIKEN Science Session at ESOF2008

On July 21, RIKEN hosted a Science Session titled, 'Drought-tolerant plants: Helping the world to cope with global warming' at EuroScience Open Forum (ESOF) 2008 in Barcelona, Spain.

The session was organized by Kazuo Shinozaki, director of RIKEN Plant Science Center in Yokohama, who has for years been studying plant responses to drought stress at the molecular level. At the session, he described recent research on the functions of stress-inducible genes, regulation of gene expression in response to drought stress, and identification of many drought-inducible genes and their functions in a model plant, Arabidopsis thaliana.

Two European researchers who specialize in this field of research also joined the session: Montserrat Pagès from CSIC-CRAG (CSIC-IRTA-UB), Spain, who gave a talk on drought tolerance in corn, and Dorothea Bartels from the Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Germany, who presented her research on drought tolerance in resurrection plants in dry land areas.

Reflecting the growing interest in problems related to global warming, the conference hall was almost full, with about 100 participants attending, including young researchers, journalists, and people from industry. There was plenty of lively discussion on the theme, and even a joint research proposal from a Kenyan researcher, whose country is suffering from the problem. One young Spanish post-doc researcher said, "I was surprised to know that there exists a common molecular mechanism between plants and geese, the animal I am studying." She added, "I think it's very important for scientists to have debates like this one at ESOF on their research. We really have to try to do it more."

RIKEN also had an exhibition booth, and one of the SciArt exhibits, displaying shoes designed with images of stained cells or microorganisms, attracted a lot of attention.

Science session

Shoes designed with images

RIKEN establishes its first research lab in South Korea

RIKEN, in cooperation with the Fusion Technology Center (FTC) of Hanyang University in South Korea, has set up its first research base in that country. The center, which started operations on July 1, 2008, is part of continuing efforts at RIKEN to build research networks with Asian nations.

The Flucto-Order Functions Asian Collaboration Team at the RIKEN Advanced Science Institute is working in cooperation with researchers at Hanyang University. Together they are developing electronic devices that utilize molecular fluctuation and instability—phenomena that have until now been obstacles to functionality—to develop new functional materials and information processing technologies.

This research field acknowledges the limits of nanotechnology and aims at the establishment of a new 'post-nanotechnology' field of research, which will be pursued in cooperation with scientists at Hanyang University.

Earlier, on April 10, an agreement was signed with Hanyang University, with the aim of promoting cooperation in research, exchanges of personnel and information, and nurturing the next generation of researchers from among Asian university graduates. Opening the research base at the FTC enhances the cooperation of RIKEN and Hanyang University, and expands the spirit of cooperation among Asian countries, including China, India, and Singapore.

The opening ceremony was held on July 1, at the new 12 story FTC facility at Hanyang University, with officials from many of the participating organizations, including RIKEN, in attendance.

RIKEN hosts a conference on plant metabolomics

The 5th International Conference on Plant Metabolomics was held for four days from July 15 at the Pacifico Yokohama annex hall in Yokohama. The conference has been a forum for plant metabolomics researchers from all over the world to meet every one to two years since 2002. It was held in Asia for the first time this year.

Metabolomics is a new science that plays a key role in understanding cellular systems. An organism's metabolome is the set of all metabolites produced by its cells, and metabolomics seeks to identify and measure these products and apply them to various fields, including biology, medicine, pharmacology, and agriculture.

Plants collectively produce a huge variety of chemical compounds, which are used in food, drugs, industrial processes, and energy generation. Over 200,000 different metabolic products are believed to be produced in the entire plant kingdom, a diversity surpassing that of animals and even microorganisms.

The conference was held under the sponsorship mainly of the International Plant Metabolomics Advisory Committee and the domestic organization committee, with co-sponsorship from the RIKEN Plant Science Center. Several domestic organizations such as the Research Foundation for Pharmaceutical Sciences also sponsored the conference. About 250 researchers in total attended the conference, including 110 from 24 foreign countries.

Lecture topics included metabolite analysis, bioinformatics, integration with other omics science and systems biology, applications to basic biology, and directions for further applied research. A half-day workshop, 'Plant Metabolism and its Regulation' hosted by the Japan Science and Technology Agency was held at the same time.

A volunteer group from Yokohama gave performances of Japanese music and dance, and conference participants went on a half-day excursion to Sankei-en and the RIKEN Yokohama laboratory on the final day, which was very popular, especially among the participants from abroad.

Professor Cees van Leeuwen
Laboratory Head
Laboratory for Perceptual Dynamics
RIKEN Brain Science Institute
Wako, Saitama, Japan

Dear Professor van Leeuwen,

Before moving to Japan I never ate any fish really, and the mere idea of eating it raw sent chills up my spine. Living in Japan couldn't be all that hard I thought. I had been to France, Germany and England before, and had even seen New York! I'd simply pick up the language the same way I picked up French or German, right? And, my holidays in European countries were fun, so blending in with the Japanese and making new friends couldn't be all that hard either.

How wrong could I be? I never fully realized everything would be so different in Japan, and after one of the toughest years in my life and a pressing home-front, I left the country slightly disillusioned.

After things cooled down, all was properly rearranged and you will remember that I returned two years later to discuss a collaboration agreement between the university where I worked and your Laboratory for Perceptual Dynamics. Now we have just completed this collaboration and should produce our first joint publication shortly.

Under this arrangement, I led a group of Amsterdam-based students to assist me with experiments. In this setting I discovered how much impact that single year working for you has had. I preferred to make my students the first author when publishing papers, and I held weekly meetings to have all members of the lab communicate with one another. I gave them freedom in their working hours and the directions they chose for their experiments. It was these actions that eventually led to the success of our agreement.

I trust that in these lines you recognize the way you led the Laboratory for Perceptual Dynamics. As we say in Dutch "a good example makes good follow-ups" and now, I sincerely realize that it was my year at RIKEN—under your supervision—which has inspired me to do so. I try to maintain the high level of work I learned in your lab and have many international contacts, much like the diversity I was acquainted with at RIKEN. That year taught me many good lessons.

I still eat raw fish regularly, and try to speak Japanese whenever possible. This year, for the first time, I occasionally catch a memory of Japan slipping into my consciousness accompanied by sense of nostalgia, maybe even homesickness.

Though it was sometimes hard, it is now good to realize how far reaching the impact of that single year has been. I am very happy I worked for you at RIKEN, and very happy at the productive collaboration agreement we have recently had. I am looking forward to a continuation of our activities.

I wish you, your family, and the members of the Lab for Perceptual Dynamics good health, happiness and prosperity.

Yours sincerely,

Daniel van den Berg
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RIKEN, Japan's flagship research institute, conducts basic and applied experimental research in a wide range of science and technology fields including physics, chemistry, medical science, biology and engineering. Initially established as a private research foundation in Tokyo in 1917, RIKEN became an independent administrative institution in 2003.

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