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Stressed plants fight back

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Cloning: Back from the ice

RIKEN researchers have produced healthy cloned mice from cells taken from bodies frozen for 16 years

Many scientists, not to mention science fiction writers, have pondered the notion of resurrecting extinct animals frozen in permafrost, such as the woolly mammoth. To date, this has been assumed impossible because the frozen fossils contain no live cells, and their DNA is irreparably damaged by ice crystals.

Now, cloning techniques may have progressed to the point where resurrection from permafrost could become reality. Teruhiko Wakayama at the RIKEN Center for Developmental Biology in Kobe and co-workers have produced healthy mice cloned from bodies that were frozen for 16 years at -20 °C without any preserving treatment—simulating the conditions in permafrost[1].

These findings could greatly benefit researchers working to conserve endangered animals, because it implies that species could be cloned from dead animals simply kept in the equivalent of kitchen freezers.

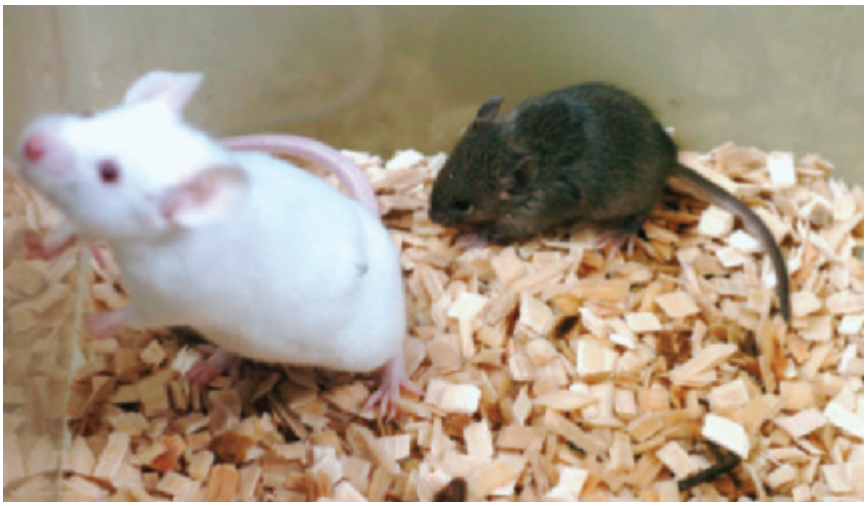


Figure 1: A cloned mouse (brown) with its foster mother (white). The clone was produced from brain cells frozen for 16 years.

Masters of cell manipulation

Wakayama and his team are making a name for themselves by developing techniques that could help rescue life from unlikely sources. For example, as reported in *Current Biology*[2], they managed to produce embryonic stem cells from eggs that had failed to become fertilized during IVF treatment. Such eggs are usually discarded in fertility clinics, so researchers could avoid some of the ethical issues that surround stem cell research. Now the researchers have expanded their techniques to create new life from completely frozen samples.

The secret of the team's success is that they can very precisely control a common cloning technique called somatic cell nuclear transfer, or SCNT. During SCNT, the nucleus from a somatic (non-reproductive) cell of the donor animal is inserted into a living egg that has had its own nucleus removed. The procedure has proven successful not only in laboratory mice but also in farm animals, starting with the famous Dolly the sheep.

Many scientists have assumed that SCNT can only be successful with high-quality donor cells taken from living animals. This is because the cloning typically involves complete fusion of the donor cell and the egg, which requires the cell membranes to be intact. Membranes are nearly always damaged by freezing, but Wakayama and co-workers remained hopeful that at least some genetic material would survive a long time in the freezer.

"The general cloning method involving cell fusion is only available for live cells," explains Wakayama. "We invented a new injection method in which the nuclei from dead cells are directly injected into the egg."

The researchers first performed SCNT on cells from mice that were frozen for a week, and found that brain cells were the best donor nuclei. Surprisingly, the frozen brain cell nuclei actually yielded more healthy clones than living brain cells. This may be because the brain has lots of sugars that can protect cells from freezing damage. Furthermore, the freezing process might partly unravel the tight bundle of DNA in the nucleus, allowing the host egg to access the donor's genetic code more easily.

Inspired by this success, the team tried using SCNT to produce cloned mice from bodies frozen for 16 years. In this case,

Figure 2: The possibility of resurrecting extinct species, such as the woolly mammoth, is now a step closer with the successful cloning of mice from cells that were frozen for 16 years.

Figure 3: The RIKEN team's successful cloning of mice from dead frozen cells could greatly assist the preservation of endangered animals such as the snow leopard, *Panthera unica*.

none of the cloned eggs survived to produce offspring. However, the embryo survived long enough that the researchers were able to establish embryonic stem cells. The stem cell nuclei were injected again into new eggs and transferred into surrogate mothers, who gave birth to four healthy cloned mice (Fig. 1).

Bringing back beasts?

"This serial cloning was my original idea," says Wakayama. "We demonstrated that it can be useful when direct cloning has failed."

One previous study has reported producing clones from frozen cells, but the samples were frozen as a single cell suspension at a much lower temperature of -80 °C, in chemicals that may have had an artificial preservation effect. The work by Wakayama and co-workers is the first successful cloning from cells in bodies stored at conditions very similar to the natural permafrost environment.

Wakayama is hopeful that scientists could eventually produce clones from ancient frozen bodies of extinct species such as mammoths (Fig. 2), but there are other challenges to overcome.

"Cells frozen in permafrost for thousands of years are not only frozen but also dried out, so there is probably more damage than in our 16-year-frozen cells. However, we already published results this year in which we succeeded in using freeze-dried cells for nuclear transfer," he explains[3].

"Ideally we would like to find mammoth brain cells, but our paper demonstrated that even blood cells can be used as nuclear donors. Blood cells are found in any tissue including skin and bones, the tissues most likely to be found in permafrost."

Conservation hopes

As well as raising the prospect of resurrecting extinct species, Wakayama's findings are good news for the conservation of present-day endangered species (Fig. 3). Many researchers work on animals in the field without access to expensive cryopreservation facilities, so they will be glad to hear that animals could be regenerated from damaged samples.

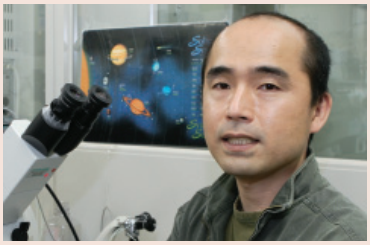
What's more, the research could make life easier and cheaper for scientists wishing to maintain genetic diversity in biological gene banks, such as RIKEN's own BioResource Center in Tsukuba.

"If a mutant mouse was infertile and died suddenly, there used to be no way to produce offspring, even if it had an important phenotype," explains Wakayama. "Now we have shown that dead mice can be cloned and used for further research, even when frozen. Experiments with mammoths are still a long way off, but this 'resurrection' of mice is immediately useful in the lab."

1. Wakayama, S., Ohta, H., Hikichi, T., Mizutani, E., Iwaki, T., Kanagawa, O. & Wakayama, T. Production of healthy cloned mice from bodies frozen at -20° C for 16 years. *Proceedings of the National Academy of Sciences USA* **105,** 17318–17322 (2008).
2. Wakayama, S., Suetsugu, R., Thuan, N.V., Ohta, H., Kishigami, S. & Wakayama, T. Establishment of mouse embryonic stem cell lines from somatic cell nuclei by nuclear transfer into aged, fertilization-failure mouse oocytes. *Current Biology* **17,** 120–121 (2007).
3. Ono, T., Mizutani, E., Li, C. & Wakayama, T. Nuclear transfer preserves the nuclear genome of freeze-dried mouse cells. *The Journal of Reproduction and Development* In press. (2008).

About the researcher

Teruhiko Wakayama was born in Kanagawa in 1967. He received his BSc in animal science in 1990 and MSc in reproduction in 1992, both from Ibaraki University. In 1996, he earned his PhD in reproductive biology from the University of Tokyo. From 1996 to 1998, Wakayama worked as a postdoctoral fellow at the University of Hawaii's Medical School, before being appointed as an assistant professor at that university in 1998. In 1999, he became a research assistant professor at the Rockefeller University, New York, and then moved to California in 2001 to work as a researcher at Advanced Cell Technology. In 2001, Wakayama returned to Japan and was appointed as the head of the Laboratory for Genomic Reprogramming at the RIKEN Center for Developmental Biology. He also teaches at Shiga Medical University as an adjunct professor and at Kyoto University and Kwansei Gakuin University as an adjunct assistant professor.



Superconductivity breaks with convention

Magnetic fluctuations may play an important role in the mechanism for superconductivity in the iron-pnictides

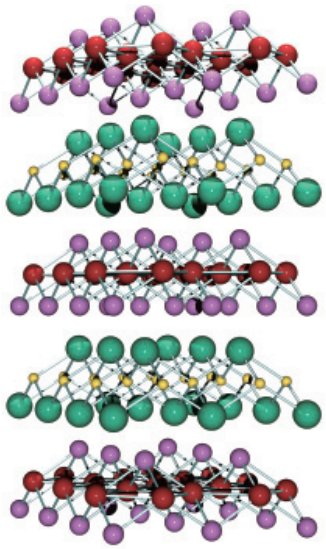


Figure 1: Structure of the iron-based superconductor $SmFeAsO_{1-x}F_x$: Fe (yellow), As (green), Sm (purple), O (red). The excess fluorine (F) substitutes for the oxygen sites.

Two materials, the cuprates, discovered in the mid-1980s, and the iron-based pnictides, discovered in 2008, have been classified as unconventional superconductors. In these materials, the mechanism for superconductivity is believed to be different to that of conventional superconductors, such as aluminum, in which lattice vibrations bind electrons into the so-called Cooper pairs that carry the supercurrent. Rather, in unconventional superconductors, many theorists believe that magnetic fluctuations are needed to pair electrons into Cooper pairs.

Now, a collaborative team of researchers from Switzerland, the UK, China and the RIKEN Nishina Center for Accelerator-Based Science in Wako is using a specialized technique called muon spin rotation (μSR) to better understand the relationship between magnetism and superconductivity in the iron-based pnictides[1].

In a μSR measurement, a beam of muons—charged particles, such as protons, that carry a net spin—impinges on the sample. The muon beam is prepared so that all of the muon spins are pointing in the same direction, but when the muons experience the field associated with any magnetism in the sample, their spins begin to depolarize. Specialized detectors can tell the final spin direction of the muon and from that, determine the local magnetic properties of the sample and how these properties evolve with time.

The researchers, including RIKEN scientist Isao Watanabe, performed μSR experiments at the RIKEN-RAL Muon and the ISIS facilities in the Rutherford Appleton Laboratory, UK. They studied two samples of the pnictide high-temperature superconductor $SmFeAsO_{1-x}F_x$, both of which had the same superconducting transition temperature of approximately 45 K (-228.15 °C), but different fluorine contents. $SmFeAsO_{1-x}F_x$ has a structure similar to that of all of the pnictides: alternating planes of iron arsenide (FeAs) and samarium oxygen (SmO) (Fig. 1).

The team's measurements show that slow, magnetic fluctuations in $SmFeAsO_{1-x}F_x$ increase at—or just above—the superconducting transition temperature in both samples. By ruling out the contribution from the spins on the Sm sites, Watanabe and colleagues believe that the main sources of these magnetic fluctuations are the spins on the Fe ion sites. Moreover, since these fluctuations are largest near the superconducting transtion, the researchers argue that: "The results support the idea that Fe spin fluctuations play an active role in the pairing mechanism for superconductivity. It is already known, for example, that the transition temperature is lower in pnictide compounds where spin fluctuations are weaker."

These experiments will provide important clues to theorists trying to solve the puzzle of superconductivity in unconventional superconductors. ■

1. Drew, A. J., Pratt, F. L., Lancaster, T., Blundell, S. J., Baker, P.J., Liu, R. H., Wu, G., Chen, X. H., Watanabe, I., Malik, V. K., *et al.* Coexistence of magnetic fluctuations and superconductivity in the pnictide high temperature superconductor $SmFeAsO_{1-x}F_x$ measured by muon spin rotation. *Physical Review Letters* **101,** 097010 (2008).

Nuclear condensation

The excited energy state of an oxygen nucleus could consist of a condensate of alpha particles

Our understanding of the electronic structure of atoms has changed very little since the development of quantum mechanics almost three-quarters of a century ago. And with the completion of the Large Hadron Collider at CERN, Switzerland, it is expected that the final elements of the Standard Model of particle physics will soon be confirmed. Yet, surprisingly little is known about the behavior of atomic nuclei at the scale between atoms and subatomic particles.

To better understand the processes that determine the behavior of atomic nuclei, Yasuro Funaki of the RIKEN Nishina Center for Accelerator-Based Science, Wako, and his colleagues in Japan, Germany and France, have performed state-of-the-art calculations to test the theory that the particles within an oxygen nucleus could coalesce together to form an unusual quantum state of low nuclear density, known as a Bose–Einstein condensate[1].

The protons and neutrons of an atomic nucleus in its lowest energy state are believed behave in a manner similar to molecules of a simple liquid with no defined structure (Fig. 1a). At energies above the ground state, however, some order can emerge. The protons and neutrons within a carbon-12 nucleus, for example, can arrange into tightly bound clusters of three alpha-particles, consisting of two protons and two neutrons each. Such clustering plays an important role in the creation of elements in stars, and is believed to be the principle means by which carbon nuclei form.

In previous work, Funaki and colleagues investigated the mechanisms by which the alpha-particles within a carbon-12 nucleus might interact[2]. As well as confirming the formation of an excited state consisting of three alpha particles with a nuclear density four times lower than the ground state, their calculations suggested that these particles might themselves coalesce into a Bose–Einstein condensate.

In this unusual form of matter, the constituent particles of a system all occupy exactly the same quantum state. Bose–Einstein condensates are mostly commonly observed to emerge when a dilute atomic gas of certain elements is cooled to a temperature close to absolute zero. Although this state was predicted early last century, and demonstrated in 1995, it was only suggested recently that it might arise within atomic nuclei and other nuclear systems, such as collapsing stars.

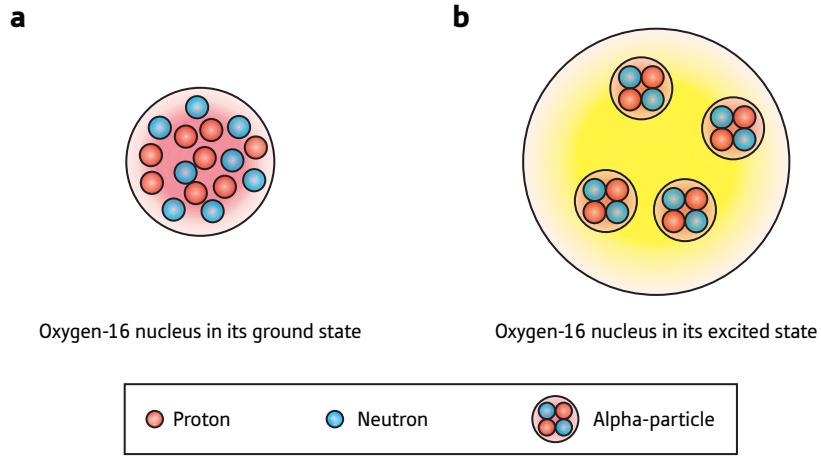


Figure 1: Structure of atomic nuclei. (a) In the ground state, the protons and neutrons of an oxygen-16 nucleus jostle randomly like the molecules of a liquid. (b) In the excited state, the calculations of Funaki and colleagues suggest these protons and neutrons cluster together to form four alpha particles. Moreover, these alpha-particles can themselves condense into a single quantum state, similar to that of a conventional Bose–Einstein condensate.

In their latest work, the researchers turn their attention to oxygen-16, the next nucleus above carbon-12 to support a condensed system of alpha particles (Fig. 1b). Their calculations suggest that such a state forms at energies consistent with experimental observations. ■

1. Funaki, Y., Yamada, T., Horiuchi, H., Röpke, G., Schuck, P. & Tohsaki, A. *α*-particle condensation in ^{16}O studied with a full four-body orthogonality condition model calculation. *Physical Review Letters* **101**, 082502 (2008).
2. Funaki, Y., Tohsaki, A., Horiuchi, H., Schuck, P. & Röpke, G. Analysis of previous microscopic calculations for the second 0$^+$ state in ^{12}C in terms of 3-*α* particle Bose-condensed state. *Physical Review C* **67**, 051306 (2003).

Writing between the lines

Nano-patterning on silicon: a single compound reacts with silicon surface to form perpendicular molecular lines

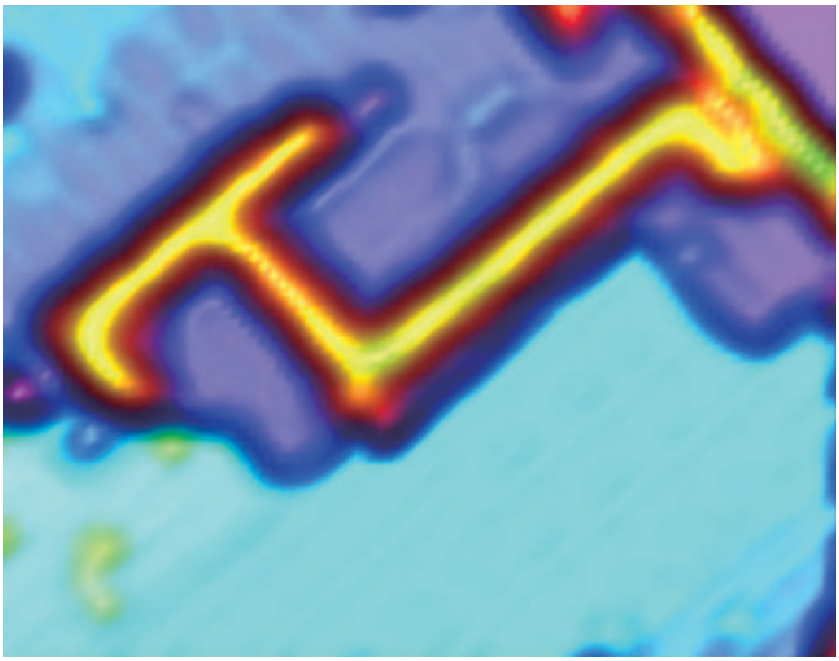


Figure 1: Scanning-tunneling microscopy image of acetophenone lines on a silicon surface. The lines of acetophenone can be seen as a bright orange line against the blue background of the surface.

Miniaturization of microprocessor components can be achieved by two opposing methods: so-called top-down and bottom-up approaches. In top-down approaches, smaller and smaller circuits are produced by optimizing and improving upon larger scale patterning technology. Bottom-up approaches rely on the assembly of single molecule building blocks to produce patterns—ultimately, the pattern size that can be achieved by either method will coincide. One potential bottom-up method creates molecular lines—and ultimately patterns—by reacting molecules with the atoms at the surface of a material. The major challenge for researchers is to control the direction in which these lines grow.

Most microprocessor devices are produced from silicon, so patterning of silicon surfaces is of high importance. Writing in the *Journal of the American Chemical Society*[1], Md. Zakir Hossain and co-workers from the RIKEN Advanced Science Institute in Wako have shown that reaction of a single compound—acetophenone—with a silicon surface can result in the growth of straight lines of molecules (Fig. 1).

The surface of the silicon comprises pairs of silicon atoms, known as dimers, arranged in parallel rows. Various molecules have been previously shown to form straight lines by reaction with the surface of the silicon—either along the dimer rows or perpendicular to them. Importantly, the direction of growth of these lines depended—until now—only on the molecule reacting with the silicon surface.

The silicon surface is prepared by reaction with atomic hydrogen, which results in silicon hydrogen bonds over most of the surface. However a few silicon atoms do not react, forming so called dangling bond sites, which are very reactive. Acetophenone molecules react with the dangling bond and go on to create a new dangling bond site at an adjacent silicon dimer. This sets up a chain reaction and produces a molecular line. The direction in which the lines of molecules grow depends on whether the new dangling bond is formed in a silicon dimer in the same row or a parallel row.

The distance between silicon dimers in the same row or those in adjacent rows is different, and there is a consequent energy difference in the two possible growth directions. "Acetophenone happens to have a geometry that means it can grow lines in either direction," explains Hossain. "Creation of a chiral center upon adsorption also seems to play important role in producing lines in both directions, which we believe will provide opportunities to control the growth direction." ■

1. Hossain, M. Z., Kato, H. S. & Kawai, M. Self-directed chain reaction by small ketones with the dangling bond site on the Si(100)-(2 x 1)-H surface: acetophenone, a unique example. *Journal of the American Chemical Society* **130**, 11518–11523 (2008).

Oxides flex their bonds

Structural and electronic rearrangements discovered in the new oxide $LiRh_2O_4$ provide hints for improving electricity generation from heat

The interplay between the electronic properties and atoms of a crystal is the origin of many fascinating phenomena such as superconductivity. Physicists from the RIKEN Advanced Science Institute, Wako, the University of Tokyo and Osaka University have now discovered another intriguing phenomenon in the newly synthesized oxide compound $LiRh_2O_4$, which could lead to a more efficient generation of electricity from heat.

$LiRh_2O_4$ belongs to one of the most common families of oxides, the spinels. Spinels are an attractive playground for physicists: the unique geometry of their atomic lattice can make a mutually convenient arrangement of electrons and ions difficult. At room temperature, the rhodium ions in $LiRh_2O_4$ are forced to assume a state of mixed valency, $Rh^{3.5+}$, whereas the electrons are distributed indiscriminately between the different orbital electronic states.

Led by Hidenori Takagi, the researchers studied how this uneasy arrangement in $LiRh_2O_4$ plays out at lower temperatures[1], and they have discovered several electronic and structural rearrangements occurring at different temperatures (Fig. 1). At 230 K (-43.15 °C), the crystal suddenly expands in one direction and contracts in another, a structural change attributed to the 'Jahn-Teller effect'. A common occurrence in oxides, this effect explains how the crystal distorts itself to gain energy by lifting the equivalence between the different orbital electronic states, in this case favoring the *yz* and *zx* orbitals over the *xy* ones.

Surprisingly, at the temperature of 170 K (-103.15 °C) the electrical resistivity suddenly increases, and the material changes from a metal to an insulator. This transition indicates that the $Rh^{3.5+}$ ions have separated into $Rh^{3+}$and Rh^{4+} ions (Fig. 1). Intriguingly, it is the Jahn-Teller effect that dictates this transition because it is the electrons from the Rh^{4+} ions occupying the *xy* orbitals that are exposed by the crystal distortion. "The Jahn-Teller effect really is the master of the physics here," notes Takagi.

The implications of this discovery may extend well beyond the interest of physicists because, owing to the indiscriminateness of electrons, the thermoelectric power of $LiRh_2O_4$ is enhanced dramatically when the Jahn-Teller effects are about to occur. A large figure for thermoelectric power is the key to efficient generation of electricity from heat. "Our study provides an important clue for the exploration of high-performance thermoelectrics and therefore bridges basic and applied physics," says Takagi. The restructuring seen in this spinel compound may well prove an important template for more efficient electricity generation. ■

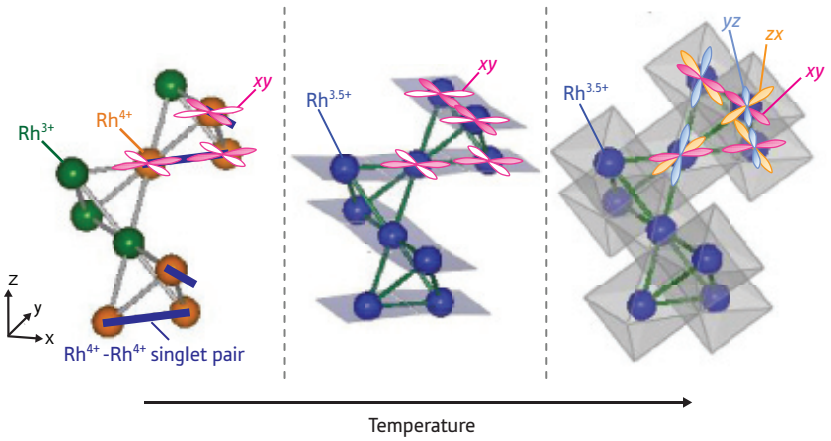


Figure 1: The different states of $LiRh_2O_4$. Below 170 K (left), the Rh ions are separated into ions with integer valency. Below 230 K (center), in contrast to the other orbitals, the *xy* orbitals are only partly occupied. At high temperatures (right), the Rh ions are all in a state of mixed valency and the *yz*, *zx* and *xy* orbitals are energetically equivalent. The octahedra denote the positions of the oxygen ions.

1. Okamoto, Y., Niitaka, S., Uchida, M., Waki, T., Takigawa, M., Nakatsu, Y. , Sekiyama, A., Suga, S., Arita, R. & Takagi, H. Band Jahn-Teller instability and formation of valence bond solid in a mxed-valent spinel oxide $LiRh_2O_4$. *Physical Review Letters* **101**, 086404 (2008).

An attractive match

The search for improved 'multiferroic' materials may benefit from a new discovery in an iron-based oxide compound

Materials known as 'multiferroics' hold great promise as memory storage devices owing to coupling between their magnetic and electric properties. Alas, in the multiferroic materials known to date, this coupling typically is very weak and limited to low temperatures, hampering their uptake in commercial applications. Now, researchers from the RIKEN Advanced Science Institute, Wako, in collaboration with colleagues from the Japan Science and Technology Agency, the University of Tokyo and Tohoku University, have revealed strong multiferroic coupling in the oxide compound $DyFeO_3$.

In multiferroic compounds ferromagnetism is coupled with ferroelectricity, a phenomenon where electric charges are separated in a material, such that an internal electric polarization is created. This coupling can be used for sensing applications, but also has potential in memory devices where data is typically stored as magnetic information and read out electronically.

Recently, some oxides of manganese, iron as well as others have been shown to possess strong coupling, but ferroelectricity in these materials is rather weak and only the electrical polarization can be switched by a magnetic field, and not vice versa—a showstopper for many applications. "Our goal is to find materials that show a full coupling between ferromagnetism and electric polarization, hopefully at room temperature," says Yusuke Tokunaga, outlining the team's research strategy.

As reported in *Physical Review Letters*[1], the researchers have demonstrated that $DyFeO_3$ shows large ferroelectric polarization combined with a strong multiferroic coupling. They found the origin of this behavior is the layered structure alternating between the dysprosium (Dy) and iron (Fe) layers (Fig. 1), where the Fe atoms attract Dy atoms through their antiparallel magnetic orientation. In a zero magnetic field, the antiparallel pairs of Dy and Fe atoms cancel out the overall electric polarization.

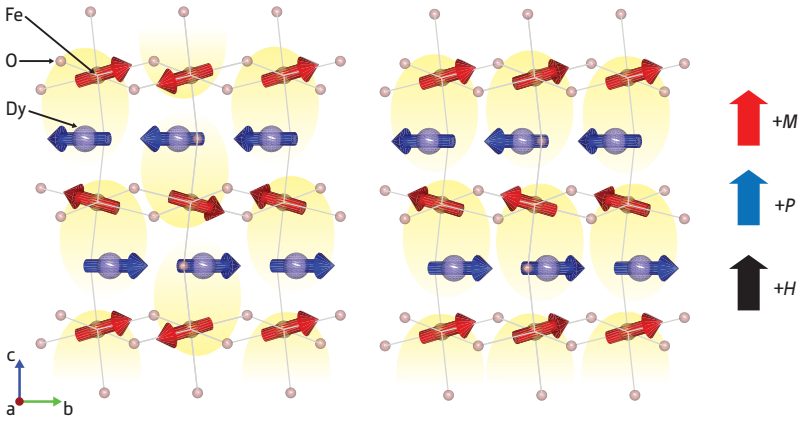


Figure 1: Multiferroic coupling in $DyFeO_3$. In the absence of an external magnetic field, no net electric polarization (yellow areas) occurs: overall the electric polarization averages out. In the presence of a magnetic field *H* the Fe atoms rearrange their magnetic orientation *M*, leading to a net electric polarization *P*.

Under the influence of a sufficiently strong magnetic field, however, the magnetic orientation of the Fe atoms rearranges slightly, which then leads to an electric polarization. As the electric polarization is a direct consequence of the magnetic structure, the multiferroic coupling is very strong—about two orders of magnitude larger than that of most other multiferroic materials.

Unfortunately, temperatures below -269 °C remain a necessity for the observation of this effect. Furthermore, the magnetic field required for the realignment of the magnetic orientation of the Fe atoms is relatively high. Nevertheless, Tokunaga is convinced that $DyFeO_3$ represents a promising blueprint: "We believe $DyFeO_3$ will serve as a template for materials with a large multiferroic coupling, even at higher temperatures." ■

1. Tokunaga, Y., Iguchi, S., Arima, T. & Tokura, Y. Magnetic-field-induced ferroelectric state in $DyFeO_3$. *Physical Review Letters* **101**, 097205 (2008).

A spiral of spins

The complex arrangement of spins in a magnetic oxide gives rise to a magneto-electric effect

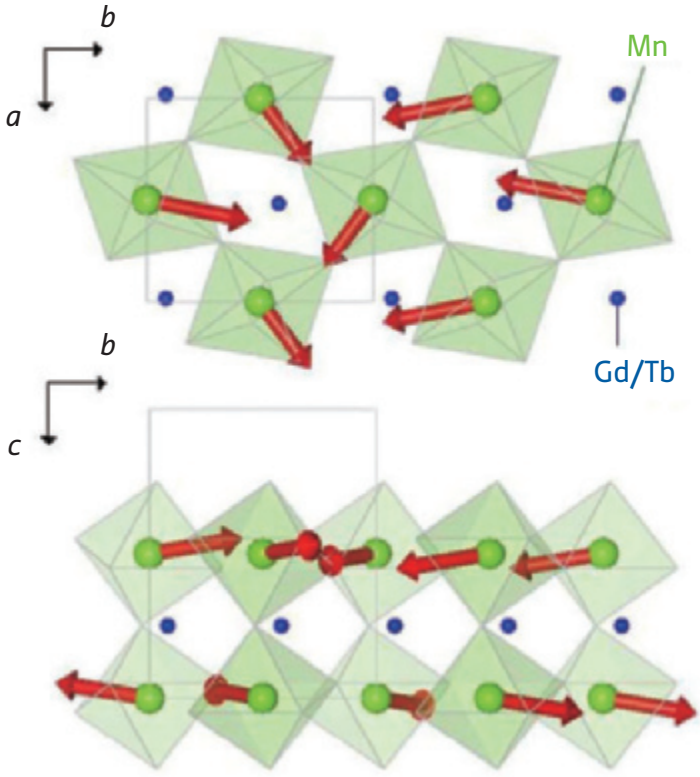


Figure 1: The spiral magnetic structure in $Gd_{1-x}Tb_xMnO_3$ as viewed looking at the *a*-*b* plane and the *b*-*c* plane. The red arrows denote the direction of the spins on the Mn sites. The green octahedra indicate the Mn sites, each of which is surrounded by 6 oxygen sites.

In a magneto-electric material, a magnetic field can induce a ferroelectric moment—a displacement of the ions that creates an electric field. Similarly, an electric field can induce a change in the material's magnetic structure. These materials have caught the attention of technologists who are interested in developing them as future data storage devices: it is much easier to make a compact storage system that can be switched electrically, rather than with the current system of magnetic read/write heads.

Unfortunately, relatively few magneto-electric materials exist, which is why Daisuke Okuyama of the RIKEN Advanced Science Institute, Wako, and Yuichi Yamasaki of the University of Tokyo and colleagues are aiming to better understand the connection between ferroelectricity and magnetic structure at the microscopic level in $TbMnO_3$. $TbMnO_3$ is one of the most well-studied magneto-electric materials.

At low temperatures, a magnetic field can rotate the ferroelectric polarization from pointing along the *c*-axis of this material to pointing along the *a*-axis. To really understand this effect, however, the researchers needed a microscopic picture that explains why magnetism and ferroelectricity are connected. This in turn required knowing what the magnetic structure looked like—both in zero and high magnetic fields.

Okuyama, Yamasaki and colleagues were confronted by the problem that the best experimental technique for resolving a material's magnetic structure—neutron diffraction—cannot be performed at high magnetic fields. They therefore devised a clever alternative[1]: they studied a similarly structured material, $Gd_{1-x}Tb_xMnO_3$, which they believe has the same magnetic structure in zero magnetic field that $TbMnO_3$ has at high magnetic field.

After careful analysis of over 150 neutron diffraction peaks, the team has determined that at temperatures close to ~20 K (~ -253 °C) the manganese (Mn) spins in $Gd_{1-x}Tb_xMnO_3$ spiral in the *a*–*b* crystallographic plane (Fig. 1). The team has also shown that the formation of this spiral-like magnetic phase occurs at the same temperature that the material develops a ferroelectric moment. Based on comparisons of this spin structure with that of $TbMnO_3$ at zero field, in which the spins spiral in the *b*-*c* plane, the team argues that the electric polarization rotates 90 degrees in $TbMnO_3$ in the presence of magnetic field because the field changes the sense of rotation of the spiral spin structure.

Armed with the knowledge of how a magnetic field changes the electric polarization in $TbMnO_3$, Okuyama says that: "The control of the magnetic structure with an electric stimulus is our next target." ■

1. Yamasaki, Y., Sagayama, H., Abe, N., Arima, T., Sasai, K., Matsuura, M., Hirota, K., Okuyama, D., Noda, Y. & Tokura, Y. Cycloidal spin order in the *a*-axis polarized ferroelectric phase of orthorhombic perovskite manganite. *Physical Review Letters* **101**, 097204 (2008).

A balancing act

A common mechanism may explain cognitive dysfunction during both aging and Alzheimer's disease

Cognitive decline may occur during aging, or due to genetic mutations that predispose individuals to develop Alzheimer's disease. Now, a team of scientists, led by Akihiko Takashima at the RIKEN Brain Science Institute in Wako, has found support for their hypothesis that a similar molecular abnormality could account for cognitive dysfunction during both Alzheimer's disease and aging. They report their findings in a recent issue of *PLoS ONE*[1].

The researchers subjected aged mice (19–25 months) and adult mice (9–15 months) harboring genetic mutations associated with Alzheimer's disease to a test of spatial memory—the Morris water maze. Both groups of mice were trained to find a platform submerged in a pool of water based on visual cues around the pool (Fig. 1). When this training period was complete, the researchers could assess how well the mice remembered the platform location by determining how much time the mouse spends near the platform during a 'probe trial'. They found that both the aged mice and the mice with the Alzheimer's disease mutations had spatial memory deficits.

The neurotransmitter GABA (γ-aminobutyric acid) controls inhibitory signaling in the brain, and GABA receptor blockers have previously been shown to improve cognition in aging rats. To see if this was also true in Alzheimer mutant mice, the researchers administered a GABA receptor blocker, and saw restoration of normal spatial memory in the Morris water maze. The treated mice were also better at recognizing a new object placed into their cage, which is a measure of 'declarative memory'.

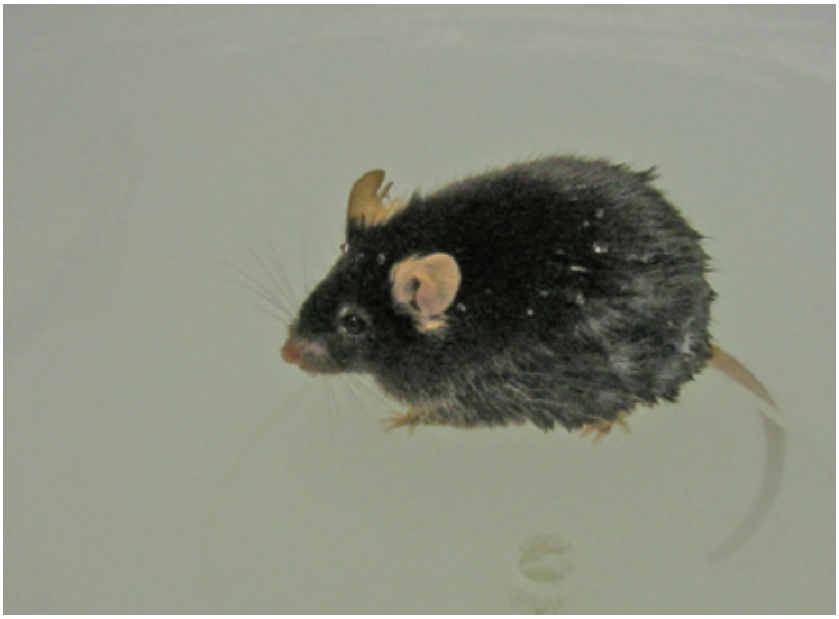


Figure 1: A mouse sitting on a platform in a Morris water maze during a test of its spatial memory.

The researchers then examined synaptic plasticity in a part of the brain that plays a role in spatial memory—the hippocampus. They found deficits in synaptic plasticity in hippocampal slices from both aging and Alzheimer mutant mice. However, normal synaptic plasticity could be restored by adding a GABA receptor blocker. This suggests that both aging and Alzheimer's disease mutations may affect memory by increasing GABA-mediated inhibitory signaling in the hippocampus.

These findings show that GABA receptor blockers may be an effective therapeutic strategy to enhance cognitive function during both aging and Alzheimer's disease. This work also indicates that an imbalance between excitatory and inhibitory signaling in the brain may result in memory dysfunction. Yuji Yoshiike, the study's first author, says the findings suggest that "even when memory declines because of the accumulation of neurotoxic molecules during aging, memory may be improved by restoring the balance between synaptic excitation and inhibition." ■

1. Yoshiike, Y., Kimura, T., Yamashita, S., Furudate, H., Mizoroki, T., Murayama, M. & Takashima, A. $GABA_A$ receptor-mediated acceleration of aging-associated memory decline in APP/PS1 mice and its pharmacological treatment by picrotoxin. *PLoS ONE* **3**, e3029 (2008).

The rewards of courtship

Social interactions with females induce synaptic plasticity in dopamine neurons of male songbirds

Rewarding stimuli and addictive drugs both enhance the activity of dopamine neurons in the ventral tegmental area (VTA) of the brain. Drugs of abuse, such as cocaine and amphetamine, can also produce long-lasting strengthening of synaptic communication between glutamate-secreting neurons and their dopamine neuron targets in the VTA. Such plasticity may play a role in the behavioral process of addiction.

Now, in a recent issue of *PLoS ONE*[1], Ya-Chun Huang and Neal Hessler at the RIKEN Brain Science Institute in Wako have reported that social courtship behavior in male zebra finches increases synaptic plasticity in VTA dopamine neurons in a similar fashion as previous studies have reported for drugs of abuse.

The researchers examined three groups of male birds: those who sang without a female present ('undirected singing'), those who sang with a female present ('directed singing') (Fig. 1), and those who did not sing with a female present. An hour later, they prepared brain slices from these birds and measured glutamate-induced synaptic activity in both dopaminergic and non-dopaminergic VTA neurons.

Male birds that exhibited undirected singing did not show any augmentation of synaptic activity in their VTA. However, the researchers found that both groups of male birds that had a female present—whether they sang or not—demonstrated synaptic potentiation in dopaminergic, but not in non-dopaminergic, neurons in the VTA.

Huang and Hessler determined that the strengthening of synaptic communication they observed after exposure to females was not due to an increase in glutamate release onto the dopaminergic neurons. Rather, the increase in dopamine neuron synaptic plasticity in the VTA reflected changes within the dopaminergic neurons themselves. Further experiments are needed to determine the exact molecular mechanism by which social interactions with females render VTA dopamine neurons in male birds more responsive to glutamate.

Figure 1: A male zebra finch (right) singing to two female finches (left). Such courtship singing increases the strength of synaptic connections to brain dopamine neurons.

The similarities in synaptic plasticity in the VTA in response to both drugs of abuse and to social interactions with females indicate that courtship behavior is rewarding for male zebra finches. In addition, because dopamine neuron activity is required for pair bonding in mammals, these findings suggest that the synaptic plasticity observed in VTA dopamine neurons in response to female exposure may also be involved in formation and maintenance of the monogamous relationships that characterize the zebra finch species.

"As female choice is critical during courtship, we next plan to test whether brain reward systems are also activated when females are courted by a desirable male," says Hessler. ■

1. Huang, Y.-C. & Hessler, N.A. Social modulation during songbird courtship potentiates midbrain dopaminergic neurons. *PLoS ONE* **3**, e3281 (2008).

From genes to plant metabolism

An analytical survey maps out the genetic connections of the flavonols

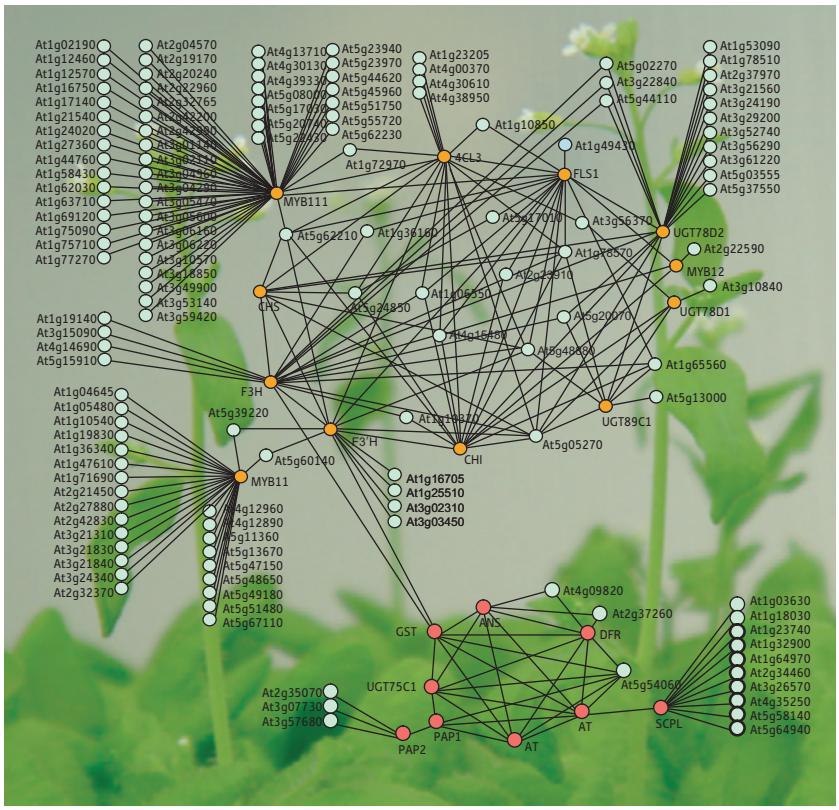


Figure 1: Relationships of the coexpression of genes involved in the flavonoid pathways in *Arabidopsis*. Orange circles represent known flavonoid-related genes, red circles represent the closely linked anthocyanin-related genes, and pale green circles represent the genes that may be involved in flavonoid metabolism.

Plant molecular biologists from RIKEN's Plant Science Center in Yokohama and Chiba University are using a combination of bioinformatics and biochemical analytical techniques to complete a map of all the reactions involving flavonols in the model plant *Arabidopsis thaliana*. Already the researchers have succeeded in identifying a new gene that encodes an important enzyme and in determining the physiological role played by another gene. The information gained in the study should be applicable to other plants.

Flavonols are health-promoting, dietary antioxidants. In plants, they are involved in defense responses, such as reactions to pathogens and UV radiation. The genes and biochemical pathways for flavonol synthesis have been well studied in several plants, but the details of subsequent chemical modification are less well known.

The research team is involved in a major project applying the latest techniques to finding the links between the genes of *Arabidopsis*, for which the entire genome has already been sequenced, and the compounds found in the plants themselves. The flavonol study is aimed at identifying these connections for a whole class of compounds, and results were published recently in *The Plant Cell*[1].

Initially, the researchers catalogued all the flavonol related compounds in *Arabidopsis* by comparing what was present in normal plants with what they found in a mutant form in which no flavonol was produced. They determined the compounds in the flowers, leaves, stems and roots of the plant by preparing extracts and putting them through liquid chromatography–mass spectroscopy (LC–MS) analysis. The study detected 30 flavonol-related products in *Arabidopsis*, some of which were intermediates for making others. Two other compounds have been reported in earlier studies.

Using plants with flavonol-related mutations, the LC–MS results, and data from previous studies, the researchers determined the structures and metabolic relationships of 15 newly identified and eight known flavonols. In a technique known as transcriptome coexpression analysis, they were then able to use program to identify the flavonol-related genes in the published genome, linking enzymes and regulatory factors with the products found in the plants themselves (Fig. 1).

On the basis of their results they undertook detailed analysis of two genes using genetically engineered mutants, uncovering one previously unknown gene and determining the metabolic role of another. "We now wish to complete our map of the flavonoids in *Arabidopsis* and then in other plant species, including the metabolic relationships," says lead author, Keiko Yonekura-Sakakibara. ■

1. Yonekura-Sakakibara, K., Tohge, T., Matsuda, F., Nakabayashi, R., Takayama, H., Niida, R., Watanabe-Takahashi, A., Inoue, E. & Saito, K. Comprehensive flavonol profiling and transcriptome coexpression analysis leading to decoding gene–metabolite correlations in *Arabidopsis*. *The Plant Cell* **20**, 2160–2176 (2008).

Plant immunity: PUBs are bad for the health

Genes known as *pubs* have a negative effect on a plant's immune system

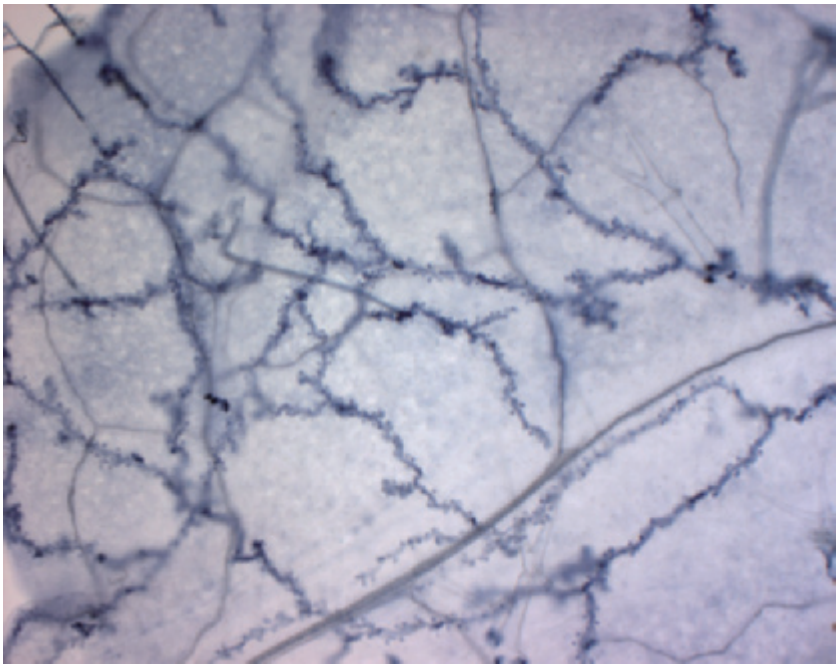


Figure 1: An *Arabidopsis* leaf infected with water mold, or oomycetes, (seen as Y-shaped filaments). Plants lacking the *pub* genes have stronger immune systems than normal plants, which can hinder the growth of pathogens such as oomycetes.

Disease-causing pathogens carry unique molecular motifs that can be recognized by plants. The motifs, known as pathogen-associated molecular patterns or PAMPs, trigger several reactions in the plant which together generate a defensive immune response.

Ken Shirasu and co-workers at the RIKEN Plant Science Center, Yokohama, and the John Innes Centre, Norwich, UK, have discovered a triplet of genes that appear to hinder the PAMP immune response in *Arabidopsis* plants[1]. The genes, called *pub22*, *pub23* and *pub24*, code for enzymes called ubiquitin ligases (PUBs), which help to mark other proteins by attaching them to the universal protein ubiquitin.

The researchers decided to study the *pub* genes because they are similar to a gene known to promote disease resistance in tobacco. To their surprise, they found that when the three genes were deactivated in a mutant strain of *Arabidopsis*, the plant's immune response improved. This means that the genes have a negative effect on immunity.

One of the first immune responses triggered by exposure to PAMPs is the oxidative burst, a rapid production of reactive oxygen compounds. In the mutant *Arabidopsis* plants, the oxidative burst was much stronger, and lasted longer than in wild-type plants. The mutants also showed prolonged activity of signaling molecules and genes known to improve the immune response, and more controlled cell death at infected sites.

These immune system enhancements are not specific to one type of pathogen—they occurred in response to several PAMP stimuli taken from bacterial flagella, the cell walls of fungi, and bacterial translation elongation factor. Even more impressively, the absence of *pub* genes hinders the pathogens themselves. Bacteria and moulds attacking the mutant plants showed up to 30 times less growth than on wild-type plants (Fig. 1).

It is likely that the PUB enzymes break down or block the activity of other molecules that promote immunity, by binding the molecules to ubiquitin. A similar phenomenon has been observed in mammals, where ubiquitination has a detrimental effect on protein signaling.

In the future, the researchers hope to identify the exact molecules that are targeted by the PUB triplet. This could help to solve the biggest mystery—why plants have retained genes that make them more vulnerable to disease.

"Plants face pathogens every day and need appropriate levels of immune responses, so they don't waste energy," explains Shirasu. "Our knowledge of these regulatory genes could improve disease resistance in agriculture, especially when crops are transferred long distances to areas where they encounter completely new pathogens." ■

1. Trujillo, M., Ichimura, K., Casais, C. & Shirasu, K. Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in *Arabidopsis*. *Current Biology* **18**, 1396–1401 (2008).

Making sense of the antisense

Additions to the genome map yield important clues on how plants respond and adapt to adverse environmental conditions

Figure 1: Many previously unidentified genes, transcribed under conditions of environmental stress, are now known for the model plant *Arabidopisis thaliana*.

A team of plant biologists in Japan has mapped uncharted areas of the plant genome by describing the full set of RNA molecules transcribed from genes activated under environmental stress. Importantly, as part of this compendium known as the transcriptome, the researchers have identified many non-protein-coding RNAs. Although abundant in living organisms, the function of non-coding RNAs remains a mystery, but the team has provided evidence that they play regulatory roles in gene regulation under the stress.

Led by Motoaki Seki of the RIKEN Plant Science Center in Yokohama, the researchers have described the transcriptome of the model plant *Arabidopsis thaliana* (Fig. 1) under conditions of drought, high salinity and cold by using the tiling array technique—a popular tool employed to identify all the genes that are activated and transcribed into RNAs under particular conditions—on a whole-genome scale.

The researchers found nearly 8,000 previously unidentified transcribed regions, including many that are induced or suppressed in response to stress. And, about 80% of the RNAs that they identified belonged to so-called sense/antisense pairs. Protein coding RNAs are transcribed from DNA in the 'sense' direction and their complementary molecules are transcribed in the opposite, or 'antisense', direction. Sense/antisense pairs can be transcribed in opposite directions from the same genetic locus.

As published recently in *Plant and Cell Physiology*[1], Seki and his colleagues have found a linear correlation between the expression of sense transcripts and antisense transcripts in plants under environmental stress. The importance of sense/antisense pairs lies in the potential for antisense RNA to regulate translation of sense RNAs into proteins by various mechanisms. The researchers therefore believe that antisense RNAs may help plants to adapt under various stresses by regulating the translation of sense RNA into proteins. Interestingly, they found that the transcription of sense RNA is necessary for the expression of the antisense RNA and thus conclude that sense and antisense RNA co-regulate the stress response.

Improving the stress tolerance of crops by gene manipulation is a goal that requires plant biologists to have an intimate knowledge of the molecular mechanisms behind plant reactions to environmental challenges. The findings that non-coding antisense RNA plays a role in gene expression under environmental stress and sense/antisense pairs may co-regulate stress responses greatly adds to this body of knowledge. The latter finding also expands our understanding of coordinate gene regulation in higher eukaryotes, notes Seki. ■

1. Matsui, A., Ishida, J., Morosawa, T., Mochizuki, Y., Kaminuma, E., Endo, T.A., Okamoto, M., Nambara, E., Nakajima, M., Kawashima, M. *et al. Arabidopsis* transcriptome analysis under drought, cold, high-salinity and ABA treatment conditions using a tiling array. *Plant and Cell Physiology* **49**, 1135–1149 (2008).

The molecular machinery of drought response

Regulating the response to dehydration in plants is a dynamic process that occurs at the molecular level

New work by researchers from RIKEN in Japan has demonstrated the dynamic process of controlling stress responses in plants. To survive, plants must react quickly to environmental hazards such as drought, cold and salt. Stress responses are genetically controlled and require complex molecular machinery to regulate their timeliness and intensity.

The machinery is composed, in part, of protein spools around which DNA winds to keep the double strands of genetic information stable and compact. These spools are formed from histone proteins and their close association with genes on the DNA strand enables them to influence and regulate genetic activity. Histones can be targeted by functional molecules that attach to their tail-ends and this modification process is a key step in the genetic regulation of abiotic stress responses. Several genes that are switched on by drought stress have been identified in previous studies of the model plant *Arabidopsis thaliana*.

Motoaki Seki from the RIKEN Plant Science Center in Yokohama and his team have now revealed how some of these drought-inducible genes are regulated by histone modification under conditions of drought stress by use of chromatin immunoprecipitation (ChIP), a method that allows purification of protein-DNA complexes[1].

Having focused their research on four drought-inducible genes, Seki and colleagues determined that the activation of some of these genes in *Arabidopsis* was associated with modifications to histones H3 that are associated with these particular genes. They noted increases in the attachment of two types of functional molecule to target sites on the H3 proteins. In particular, one type of histone modification occurred after an increase in gene transcription, the process of copying DNA to RNA by the enzyme RNA polymerase II (Fig. 1). This indicates that the process of histone modification is dynamic and changes throughout the period during which the molecular machinery is active.

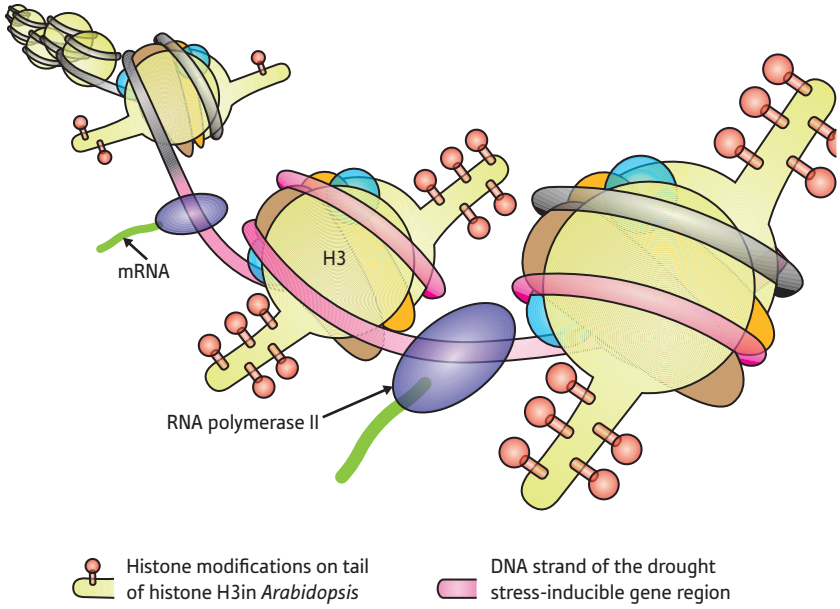


Figure 1: Histone H3 modifications change dynamically throughout the transcription process on drought stress-inducible gene regions in *Arabidopsis*. As the molecular machinery of gene transcription, including RNA polymerase II, moves downstream along the DNA strand, the modified histone H3 tails influence gene transcription—even after the process has already begun.

This research boosts the understanding of the transcriptional regulatory gene network under drought stress in plants. It will also help to build our knowledge of plan responses to other environmental stressors. The findings are, says Seki, vital not only to the understanding of molecular response mechanisms but also to allow genetic engineering to improve the stress tolerance of crop plants.

The researchers "intend to identify the key sites of histone modification and key histone modification enzymes involved in the drought stress response," says Seki. They will also explore the role of histone modification in the memory of stress perception in plants. ■

1. Kim, J.-M., To, T. K., Ishida, J., Morosawa, T., Kawashima, M., Matsui, A., Toyoda, T., Kimura, H., Shinozaki, K. & Seki, M. Alterations of lysine modifications on histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. *Plant and Cell Physiology* **49**, 1580–1588 (2008)

The immune cell march

A color-shifting fluorescent protein allows researchers to observe immune cell migration that occurs in living animals

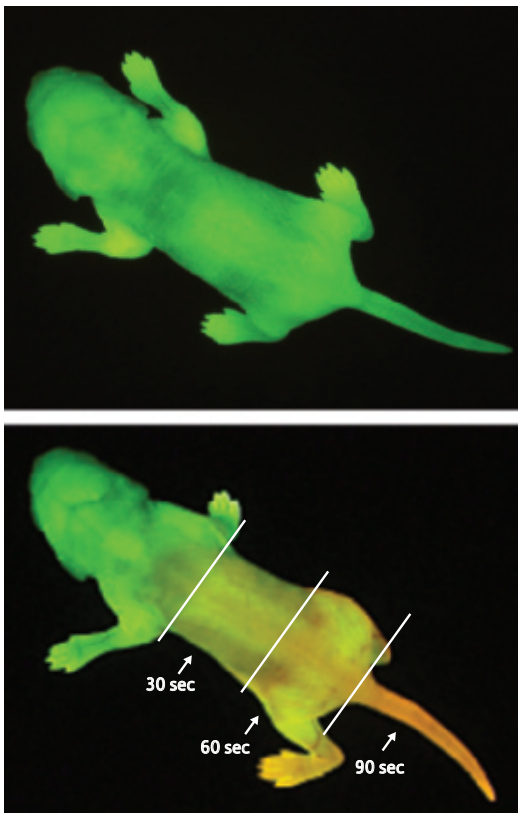


Figure 1: A newborn mouse (top) expressing the color-swapping Kaede protein in its cells. When the mouse is exposed to violet light for the indicated times (bottom), its green cells turn red, and can be tracked as they move through the body.

Immune cells migrate throughout the body to monitor different organs and rapidly respond to invading pathogens. Now it is possible to monitor immune cell transport in a line of genetically engineered mice that was created by a team of researchers led by Osami Kanagawa at the RIKEN Research Center for Allergy and Immunology in Yokohama, and including Yoshihiro Miwa at the University of Tsukuba, and Atsushi Miyawaki at the RIKEN Brain Science Institute in Wako.[1]

The mice were modified to express a new color-shifting fluorescent protein called 'Kaede' in all their cells. Normally, the Kaede protein glows green. But when cells expressing the Kaede protein are exposed to violet light, it glows red (Fig. 1), without any effect on cellular function.

To track the cells, the researchers made a small incision in the skin near the groin of the mice to expose the inguinal lymph node to violet light, which caused all of the Kaede protein in the cells to become red. This effectively flagged the origin of the red cells.

Using this technique, the researchers determined the speed of transport of different types of lymph node immune cells—T cells, B cells, and dendritic cells—to different tissues and organs in the body. They also located where the cells would migrate. The different types of immune cells migrated from place to place at different speeds, and migrated to different locations, including the blood, other lymph nodes, the spleen, bone marrow, liver and lung.

Many of the inguinal lymph node cells migrated to the axillary lymph node in the armpit, suggesting that these two lymph nodes may be directly connected to each other through a lymphatic vessel. Kanagawa and colleagues confirmed this by injecting blue dye into the inguinal lymph node, which was rapidly detected in the axillary lymph node.

The researchers also were able to observe the migration of immune cells from the skin, an organ that is not part of the immune system. When they exposed the skin to violet light, the immune cells found there migrated into a nearby lymph node.

Future experiments could use the mice expressing the Kaede protein to monitor cell movement during autoimmune disease induction, and during immune responses to pathogens. "We recently made another mouse line, in which the Kaede protein can be expressed in a tissue-specific manner, and we would like to use these mice to study when and where the initial immune response starts," says Kanagawa. ■

1. Tomura, M., Yoshida, N., Tanaka, J., Karasawa, S., Miwa, Y., Miyawaki, A. & Kanagawa O. Monitoring cellular movement *in vivo* with photoconvertible fluorescence protein "Kaede" transgenic mice. *Proceedings of the National Academy of Sciences USA* **105**, 10870–10875 (2008).

Looping-the-loop

A unique model may describe the genetic switch that controls whether a T cell becomes a helper or a killer

Immunologists have come a step closer to understanding the genetic controls that direct the fate of the lynchpins of the immune system—T lymphocytes.

As T lymphocytes mature, they develop into different types, or lineages, which are characterized by different cell surface markers and have different roles in the immune response.

Helper T cells, which coordinate the immune response and promote antibody production, carry the CD4 surface marker protein, and require interaction with immune proteins called MHC class II molecules during development and differentiation. Killer T cells, which are cytotoxic and capable of directly killing infected or cancerous cells, carry the CD8 surface marker protein and must interact with MHC class I molecules during development and differentiation.

But the molecular mechanisms governing lineage decision of an immature T cell, or thymocyte, expressing both the CD4 and the CD8 markers to differentiate into CD4-positive, CD8-negative T helper or CD4-negative, CD8-positive T cytotoxic lineages are largely unknown.

Now, a team led by Ichiro Taniuchi at the RIKEN Center for Allergy and Immunology in Yokohama has started to unravel some of the genetic controls directing the expression of lineage-specific genes.

A transcription factor called ThPOK has been identified in previous studies as being essential for the differentiation of MHC class II-selected thymocytes into helper T cells.

Taniuchi and colleagues used a fluorescent marker protein to visualize the expression of the *ThPOK* gene during the process of commitment to a specific T cell lineage[1]. The gene has several regulatory elements, known as enhancers and silencers, which were modified in turn to assess the impact on lineage determination.

From these results, the researchers have developed a model that suggests stimulation of the T cell receptor during the initial stage of thymocyte differentiation induces ThPOK expression by reversing the activity of the ThPOK silencer. ThPOK then antagonizes the CD4 silencer element, allowing expression of CD4, which in turn allows continued MHC class II-stimulation of the cell.

This has the effect of increasing ThPOK expression, generating a second auto-regulatory loop through the action of ThPOK to antagonize the silencer element within its own gene. The positive auto-feedforward mechanism amplifies and then stabilizes ThPOK expression in fully committed helper T cells (Fig. 1).

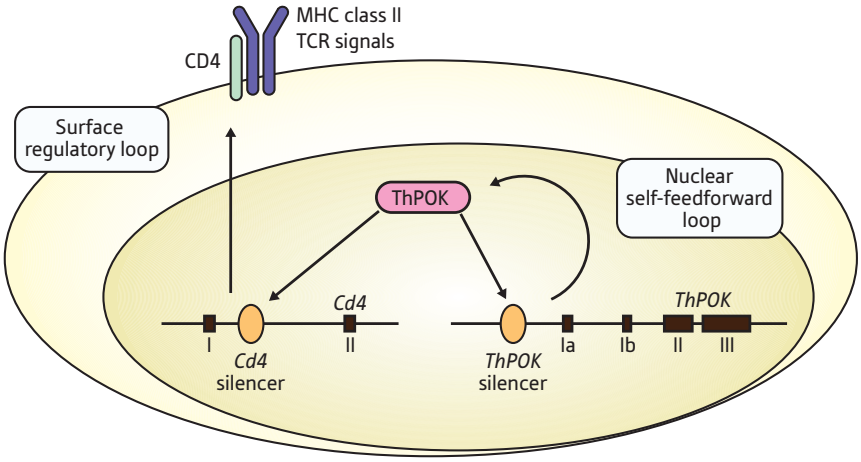


Figure 1: Regulation of ThPOK expression in helper T cells. ThPOK (magenta) reverses the silencing of the *Cd4* gene as well as its own gene in a positive auto-feedforward mechanism. (TCR, T Cell Receptor; roman numerals denote segments of the *Cd4* and *ThPOK* genes).

"Our model is unique and could be the first one that describes an auto-regulatory loop by antagonizing a transcriptional silencer within its own locus," says Taniuchi. "I do expect that a similar mechanism may function in other lineage decision processes." ■

1. Muroi, S., Naoe, Y., Miyamoto, C., Akiyama, K., Ikawa, T., Masuda, K., Kawamoto, H. & Taniuchi, I. Cascading suppression of transcriptional silencers by ThPOK seals helper T cell fate. *Nature Immunology* **9**, 1113–1121 (2008).

How the body senses tissue damage

A receptor on macrophages can detect excessive cell death and recruit help

A receptor, induced on the surface of macrophages under stressful conditions, can detect tissue injury, stimulating inflammation and possibly repair, a RIKEN-led team of molecular biologists has discovered. Their work could provide new leads for anti-inflammatory drugs and healing.

Stress, age and body maintenance generate a continuous supply of dead cells, which normally are cleaned up by the macrophages that engulf pathogens and cellular debris. This mechanism, however, becomes overwhelmed at times of large-scale tissue damage, such as that caused by radiation or injury. To deal with such emergencies, the body needs a sensor which not only can detect the scale of the problem, but also that the dead tissue is not foreign.

Earlier research by another group had suggested that cellular stress leads to an upsurge in the activity of a gene, *Mincle*, which codes for a surface receptor in macrophages. So the RIKEN-led research team investigated the function of this receptor further. Their findings were published recently in *Nature Immunology*[1].

Initially, the researchers found that the Mincle receptor is associated with another signaling receptor chain, FcRγ and triggers macrophage activation through a specific sequence known as the immunoreceptor tyrosine-based activation motif (ITAM). This stimulates the release of cellular hormones—cytokines and chemokines—that summon neutrophils to take part in inflammation and possibly tissue repair. Using a system involving green fluorescent protein to detect ITAM-mediated cell activation, the researchers found that Mincle responds to the presence of dead cells.

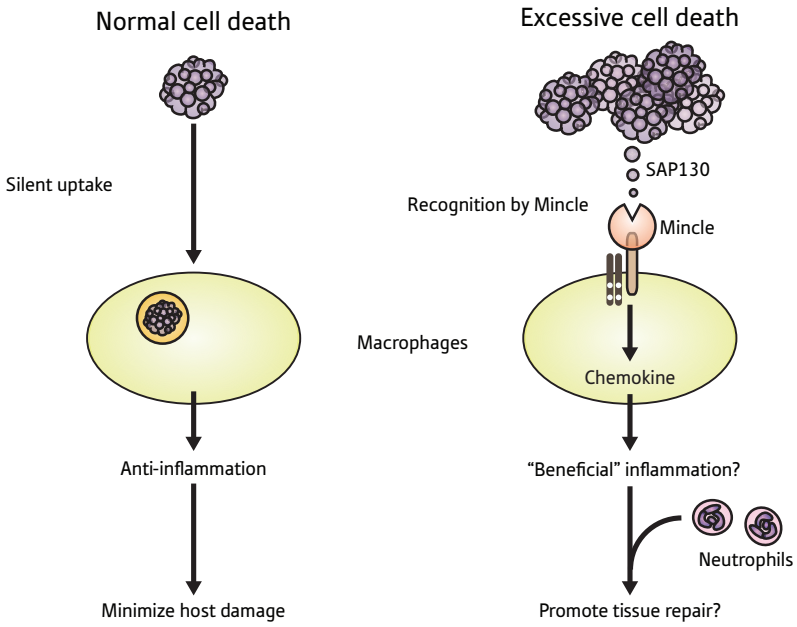


Figure 1: How Mincle senses tissue damage and induces inflammation.

They then purified protein material from dead cells bound to the Mincle receptor, and discovered it was SAP130, a protein found in cell nuclei. SAP130 is released from cells where it can come into contact with the Mincle receptor only after they die and break down (Fig. 1). In further experiments, the researchers determined the Mincle alert system works in mammals by showing that in living mice in which thymus cells had been killed by irradiation, the recruitment of neutrophils to the site of the damage was prevented by Mincle-specific antibody.

According to the group director, Takashi Saito of the RIKEN Center for Allergy and Immunology in Yokohama, the research group now wants to determine the role of the alert system in diseases involving tissue damage; how activation of Mincle is related to the induction of autoimmune diseases such as rheumatoid arthritis; and whether it is possible to inhibit or cure inflammation and/or autoimmune diseases by blocking Mincle. ■

1. Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K. & Saito, T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nature Immunology* **9**, 1179–1188 (2008).

The right mix for neuron culture

Eliminating signaling molecules from tissue culture results in the generation of hypothalamic neurons from embryonic stem cells

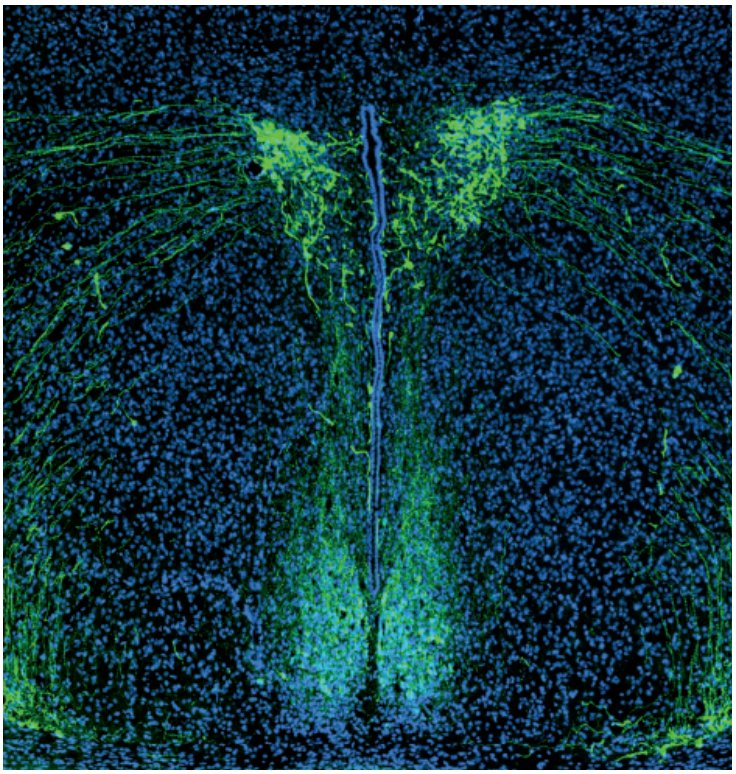


Figure 1: Vasopressin-producing neurons (green) in the embryonic hypothalamus.

The hypothalamus is a part of the brain containing groups of neurons called nuclei, which regulate many body functions including blood pressure, stress, hunger, and sexual development and function. Understanding how the different populations of hypothalamic neurons develop from stem cells may enable scientists to cure diseases that are associated with hypothalamic dysfunction.

A team of researchers, led by Yoshiki Sasai at the RIKEN Center for Developmental Biology in Kobe, has now discovered that embryonic stem (ES) cells can mature into hypothalamic neurons only when as many exogenous signaling factors as possible are removed from the liquid medium in which the cells are grown[1].

Mouse and human embryonic stem cells are often grown in liquid medium containing multiple growth factors such as those in 'knockout serum replacement' (KSR). ES cells grown in KSR often express markers characteristic of the telencephalon, which gives rise to brain structures such as the cerebrum and striatum. The KSR still contains factors, such as insulin, that may affect the maturation of the ES cells. So, when the researchers cultured the ES cells without KSR, they observed maturation of the cells into hypothalamic progenitor cells.

Insulin was the factor that inhibited hypothalamic maturation of the ES cells, since very few ES cells cultured in insulin expressed hypothalamic progenitor cell markers, such as the protein Rax. In addition, the researchers determined that ES cells were most sensitive to insulin during days 4 and 5 in culture.

Sonic hedgehog (Shh) is a signaling factor known to affect the maturation of progenitor cells throughout the central nervous system. Sasai and colleagues found that treatment of Rax-expressing hypothalamic progenitor cells with Shh induces development of neurons from areas of the hypothalamus that are involved in controlling appetite. On the other hand, Rax-expressing hypothalamic progenitor cells that are not treated with Shh go on to mature into hypothalamic neurons that express arginine-vasopressin, a hormone known to be involved in regulating water retention in the kidneys, and blood pressure (Fig. 1).

Because these findings were all derived from work on mouse ES cells in tissue culture, additional experiments are needed to determine whether the mechanisms for hypothalamic development identified in the work would also apply in the setting of normal brain development and in humans.

In future experiments, Sasai says, "we will continue to study the molecular and cellular mechanism for the generation of other parts of the brain." ■

1. Wataya, N., Ando, S., Muguruma, K., Ikeda, H., Watanabe, K., Eiraku, M., Kawada, M., Takahashi, J., Hashimoto, N. & Sasai, Y. Minimization of exogenous signals in ES cell culture induces rostral hypothalamic differentiation. *Proceedings of the National Academy of Sciences USA* **105**, 11796–11801 (2008).

Many tracers make light work

A new type of biological camera can trace several different molecules at once in a live animal

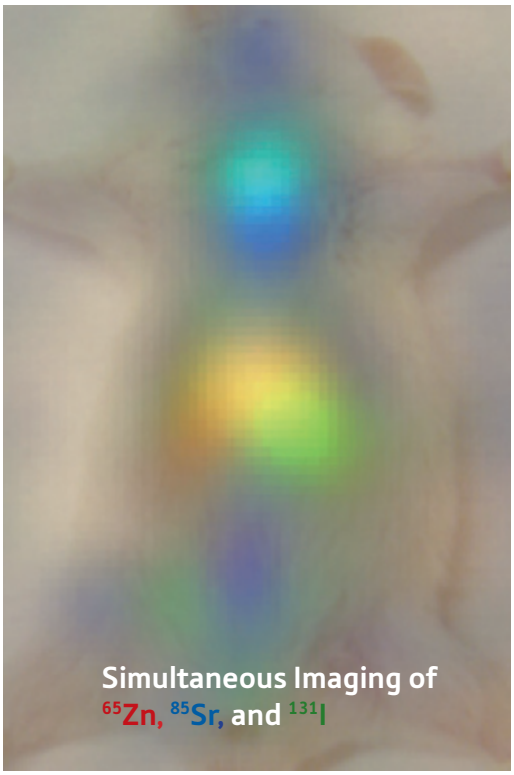


Figure 1: Compound image from a Compton camera showing the positions of three different radioisotopes, zinc (red), strontium (blue) and iodine (green), in a live mouse. (This work was completed in compliance with Japan's ethical standards for experiments on live animals.)

Doctors and scientists can visualize specific biological processes in living creatures by monitoring radioactive tracer molecules. So far, imaging techniques have largely been limited to seeing one tracer molecule at a time, which is unlikely to provide the full picture of complex functions or diseases.

Now Shuichi Enomoto, Shinji Motomura and co-workers at the RIKEN Center for Molecular Imaging Science (formerly the RIKEN Molecular Imaging Research Program) in Kobe and Wako have produced images of three radioactive isotopes at the same time in a live mouse[1]. The researchers adapted a gamma-ray imaging device called a semiconductor Compton camera, which was originally developed for gamma-ray astrophysics.

"We had been working on research and development of 'multitracer' technology," explains Motomura. "A multitracer contains radioisotopes of various chemical elements, so that many elements and their interactions can be observed by one experiment. Later we proposed realizing multiple molecular imaging with a semiconductor Compton camera."

The Compton camera consists of two detectors made from intermeshed strips of germanium, and can probe a wide range of gamma ray energies. "An extremely pure crystal of germanium can work as a radiation detector with high energy resolution," explains Motomura. "Two sets of germanium electrodes are arranged in strips at right angles, so that the gamma-ray energy and hit positions can be detected."

To test their modified Compton camera for biological imaging, the researchers chose three common radioactive tracers—isotopes of iodine, strontium and zinc—and injected them into an eight-week-old male mouse. The mouse was anaesthetized and scanned for 12 hours, producing both 2D and 3D images. The three tracers were distinguished by identifying their different emission energy peaks, and could be represented together in images by allocating three different colors: red, green and blue (Fig. 1).

All the tracers collected in areas where they would normally be expected: zinc tends to accumulate in the liver or in tumors, while strontium collects in the bones and iodine is taken up into the adrenal and thyroid glands. The researchers observed similar concentrations and distributions of the tracers every 3 hours over the 12-hour scanning period, implying a fast and long-lasting imaging capability.

The researchers believe their results show great promise for the Compton camera in biological imaging. At present these germanium-based detectors are very expensive, but there could be strong demand in future, once the researchers improve their equipment to provide higher resolution images in a shorter time. ■

1. Motomura, S., Kanayama, Y., Haba, H., Watanabe, Y. & Enomoto, S. Multiple molecular simultaneous imaging in a live mouse using semiconductor Compton camera. *Journal of Analytical Atomic Spectrometry* **23**, 1089–1092 (2008).

The recession gene

A master switch allows bacteria, already living in extreme environments, to survive times of crisis

Figure 1: Izu Mine Hot Springs in Kawazu, south east of Tokyo, where *Thermus thermophilus* HB8 is found.

Japanese researchers have identified a molecular mechanism by which bacteria found in hot springs can survive hard times. Interestingly, about half the genes involved are similar to those found in humans, but the key regulator gene is not.

The researchers—all associated with RIKEN's Harima Institute and the SPring-8 synchrotron—form one of three teams involved in a major project to document the molecular processes of the HB8 strain of *Thermus thermophilus*, an extremophile bacterium that occurs naturally at temperatures of up to 85 ˚C (Fig. 1). This bacterium was selected because it is relatively simple, built around 2,200 genes, about half the number of the model bacterium, *Escherichia coli*. The research team is particularly interested in the regulation of transcription, whereby DNA is translated into functional proteins.

In order to survive, organisms have to respond to changes in environmental conditions. At times of nutrient depletion, for instance, bacterial metabolism switches from activities to do with growth and replication to those concerned with adaptation and survival. This involves decreasing the activity of one set of genes and increasing the activity of another, and is often regulated by transcription factors. One group of proteins known to be involved is from the cyclic AMP receptor protein/fumarate and nitrate reduction regulator (CRP/FNR) family, of which there are four representatives in *T. thermophilus*.

The researchers investigated one of these regulators which they named stationary phase-dependent regulatory protein (SdrP), because they found its activity increased at times of nutrient depletion when the bacterium entered what is known as the stationary phase. They published details of their work recently in *Molecular Microbiology*[1].

Using an *sdrP*-deficient strain, they found the gene was non-essential, although the strain showed growth defects and increased sensitivity to certain chemical stresses. Loss of functional SdrP was manifested in decreased activity of eight dependent gene promoters. Based on the amino acid sequences and three dimensional structures of the protein products of the genes regulated by these promoters, the researchers speculate they are involved in activities such as securing nutrient and energy supply, and protecting against oxidation damage to DNA—in short, preparing the bacterium to survive times of crisis.

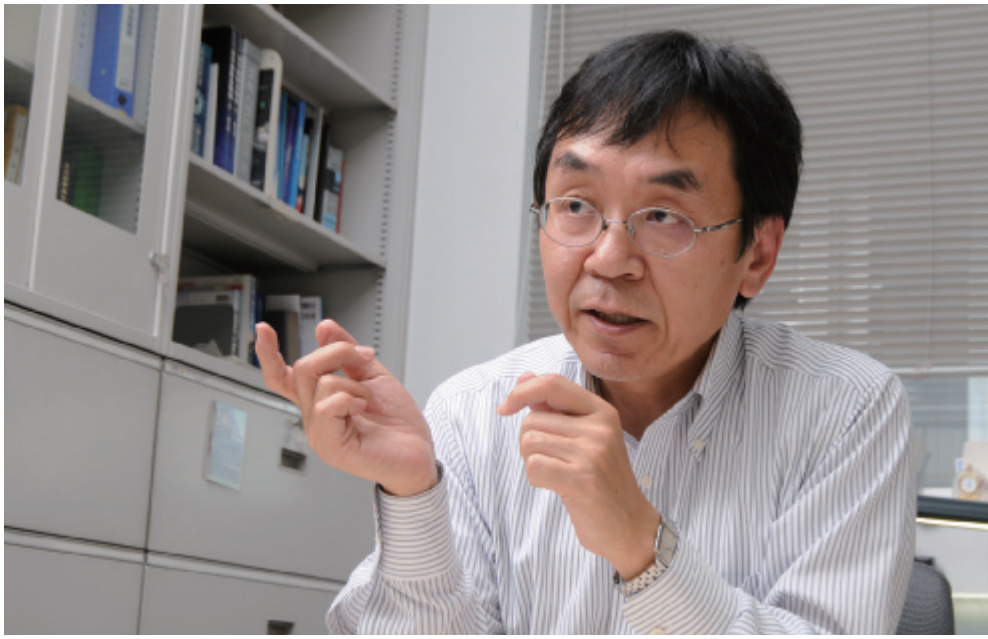
The researchers are continuing their work to understand the essential transcription mechanism of *T. thermophilus*, a bacterium thought to be close to early life forms. "We hope this research will help us understand more complicated biological phenomena, including those of human cells," says team leader, Akeo Shinkai of the RIKEN SPring-8 Center. ■

1. Agari, Y., Kashihara, A., Yokoyama, S., Kuramitsu, S. & Shinkai, A. Global gene expression mediated by *Thermus thermophilus* SdrP, a CPR/FNR family transcriptional regulator. *Molecular Microbiology* **70**, 60–75 (2008).

Chemical synthesis of sugar chains to unravel the mysteries of their roles in biological phenomena

Yukishige Ito

Chief Scientist, Director of Synthetic Cellular Chemistry Laboratory
Advanced Science Institute



In the field of life sciences, compounds known as glycan chains are attracting considerable attention. This is because sugar chains are known to be mediators of the complex biological phenomena of higher organisms, and they are associated with immune responses and various diseases. Yukishige Ito, Chief Scientist of the Synthetic Cellular Chemistry Laboratory, which is involved in the development of original techniques to synthesize various sugar chains, thereby seeking to accelerate the research on sugar chains, states, "compared with the research conducted on DNA and proteins, the results of the research conducted on sugar chains are quite often less clearcut because it is difficult to synthesize and understand the complex structures of sugar chains."

Sugar chains that serve as the key to biological phenomena and the mechanism of diseases

Sugar chains consist of various components called monosaccharides. For example, starch is a chain of glucose units, which are constituents of the food we eat. In a more general sense, sugar chains are chains of various monosaccharides arranged in different orders. They are classified into various categories depending on the types and numbers of sugar units, the order of connection of the units, their three-dimensional structure, and the manner in which their branches are formed.

The majority of the proteins that constitute higher organisms such as human beings exist in the form of glycoproteins, which are proteins into which sugar chains are incorporated. Glycoproteins can interact with other proteins by virtue of the ability of sugar chains to act as markers, which are involved in biological phenomena. For example, sugar chains serve as labels when the activities of immune cells are controlled or when cells recognize each other (Fig. 1). Regarding the importance of sugar chains, Ito explains, "various functions of proteins based on a large variety of sugar chains support complex vital activities of higher organisms."

Many viruses initially combine with sugar chains on the surface of a cell before breaking into it. For example, hemagglutinin (HA) proteins on the surface of the influenza virus bind with sugar chains that contain special sugars, called sialic acids, on the surface of a cell. Then the influenza virus breaks into the cell and multiplies inside it before bursting out to break into another cell. This infectious process is repeated continuously.

However, the influenza virus cannot burst out of the cell when the cell membrane contains sugar chains that contain sialic acids. The influenza virus therefore uses neuraminidase (NA) proteins on its surface to break off the sialic acids from the cell membrane so

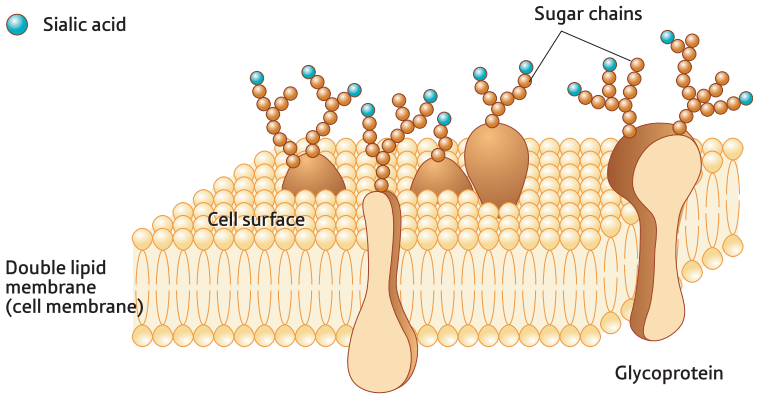


Figure 1 : Sugar chains on cell surfaces.
Cell surfaces are covered with sugar chains, which are also called 'the face of cells.' They act as markers when cells encounter each other.

it can escape from the cell. Tamiflu, a therapeutic agent for influenza, can interfere with the this function of NA proteins and prevent the influenza virus from leaving the cell, thereby preventing the virus from infecting one cell after another. It is not well known that Tamiflu is a result of research into sugar chains (glycobiology).

The structures of sugar chains on the surface of a cancer cell are known to be significantly altered. The studies conducted in glycobiology are attracting more attention for cancer diagnosis and treatment-oriented research studies.

Importance of experts in chemical synthesis

In comparison with the research conducted into DNA and proteins, research on sugar chains has been less developed, although it is important for elucidating biological phenomena and in fighting diseases. In the research field of molecular biology, chemically synthesized DNA or parts of proteins (peptides) are used in many experiments. The development of synthetic techniques is facilitated by advances in organic chemistry, although biologists are not fully aware of this. The development of synthetic techniques has a very long history; this has enabled the automated synthesis of samples, which are being used in current experiments. Research in biology has witnessed rapid progress as a result of advances in organic chemistry.

In comparison with DNA and peptides, sugar chains cannot easily be synthesized because they have complex structures, with many branched chains and various stereoisomers (Fig. 2). In the present circumstances, not everybody can synthesize sugar chains, and therefore the expertise of professionals is required.

Quality control system of glycoproteins

The Synthetic Cellular Chemistry Laboratory at RIKEN has synthesized almost all the sugar chains in a cellular organelle, the endoplasmic reticulum, that are involved in protein folding and has conducted advanced research to elucidate their roles in various biological phenomena.

Proteins are chains of amino acids arranged as defined by genetic information. However, proteins cannot function normally unless they are folded into proper structures. It is coming to be understood that sugar chains have an important role in the process of protein folding.

In a cell, a high-mannose-type sugar chain, containing many mannose sugars, binds to a chain of amino acids in the endoplasmic reticulum, thereby forming a glycoprotein (Fig. 3). Some glucose units, which are different from mannose, are attached to the end of the high-mannose-type sugar chain. The process is thought to continue with the removal of all glucose units except one from the high-mannose-type sugar chain; this is then combined with a protein (chaperone) that facilitates folding. Once folding is complete, the remaining single glucose unit is removed. The folding process does not always succeed. However, it is wasteful to discard misfolded glycoproteins, so a mechanism exists for recycling misfolded glycoproteins.

UGGT (a glucose transfer enzyme) verifies whether or not the folding has been performed properly, and it attaches a single glucose unit to the original sugar chain of the misfolded glycoprotein. A chaperone protein then binds with the glycoprotein, activating the folding process again. UGGT attempts to attach a single glucose unit exclusively to misfolded proteins or 'loser' proteins, and ignores successfully folded 'winner' proteins. Thus, UGGT serves as a guard to check whether folding has been properly performed.

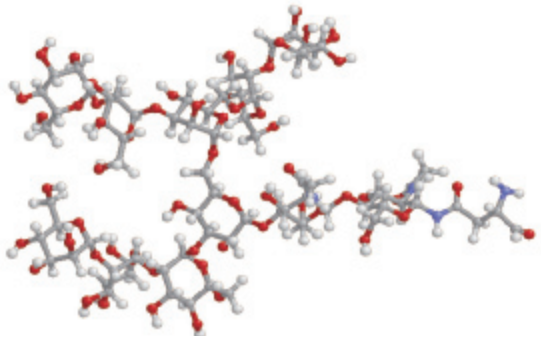


Figure 2: Example of sugar chain structure.
Sugar chains have various complex structures, including those with many branch chains that are combinations of various sugars in different orders and those with various stereoisomers that are slightly different from each other in their three-dimensional structures.

The mechanism of the 'quality control system of glycoproteins' has not yet been studied in detail. This is because of the large variety of sugar chains, and it is almost impossible to select individual sugar chains with slightly different structures for detailed investigation from natural sources. Even if some sugar chains required for investigation are selected, their amounts will be too small to be used in experiments.

Ito says, "we have focused on high-mannose-type sugar chains, and we have extended our research to synthesize these sugar chains chemically." Ito and his team have devised a method for dealing with different shapes of raw materials for the synthesis of sugar chains and for determining the order in which the chemical reactions must take place. They have successfully developed an efficient technique to synthesize only the sugar chains that have the required structures. In other words, they have succeeded in chemically synthesizing all the high-mannose-type sugar chains that are related to the quality control system of glycoproteins.

The number of chemical reactions available for the synthesis of sugar chains is limited. The most interesting part of this study is the development, by Ito and his team, of a new synthetic method based on their own unique ideas that can be applied under severe constraints.

How to distinguish between 'winner' and 'loser' proteins

One of the mysteries of the quality control system of glycoproteins is the manner in which UGGT, a guardian, can distinguish between the winner and loser proteins. Amino acids, which are components of proteins, are divided into two groups—hydrophilic amino acids, which are very soluble in water, and hydrophobic amino acids, which are insoluble in water, just as oil is. Since proteins are surrounded by water, they become stable when hydrophilic amino acids are exposed on the exterior and hydrophobic amino acids are hidden in the interior. However, in some proteins, hydrophobic amino acids may be exposed to the exterior. A hypothesis proposes that UGGT distinguishes between the winner and the loser proteins according to whether or not a cluster of hydrophobic amino acids is exposed on the outside of a glycoprotein. However, it has been difficult to prove the hypothesis experimentally.

Ito and his team have artificially combined chemically synthesized sugars with hydrophobic molecules, and they have investigated the reactivity of UGGT to this compound. The investigation has shown that UGGT causes a glucose sugar to be attached rapidly to the compound. The compound is considered to mimic misfolded proteins, or loser proteins. Thus, it is assumed that the experiment supports the hypothesis that UGGT distinguishes between the winner and the loser glycoproteins depending on whether or not hydrophobic amino acids are exposed. The investigation has also shown that the reactivity of UGGT depends significantly on a slight difference in the sugar chain structure (Fig. 4). A complex mechanism seems to be involved in the process of distinguishing between winner and loser proteins. In this manner, the mechanism of biological phenomena can be elucidated in detail by synthesizing artificial compounds.

Defective proteins are degraded

In fact, some loser proteins cannot have glucose units reattached after repeated attempts. UGGT ignores such defective proteins.

It remains unknown how UGGT distinguishes between loser and defective proteins. This is one of the very important points that remains to be investigated.

Defective proteins are attached to proteins called ubiquitins with the assistance of an enzyme, and they are finally decomposed inside an intracellular organelle called proteasome. A variety of enzymes cause ubiquitins to be attached to defective proteins. In 2004, a group at the Tokyo Metropolitan Institute of Medical Science discovered for the first time that some enzymes use sugar chains as a marker to cause ubiquitins to be attached to defective proteins. Ito's team used various sugar chains and identified the types of chains that serve as markers in this process.

Sugar chains have been known to be involved in the decomposition of proteins in proteasomes. A proteasome is a type of narrow tunnel, and the protein molecules are disentangled and broken down when they enter it. However, proteins with sugar chains cannot enter the tunnel. Thus, the sugar

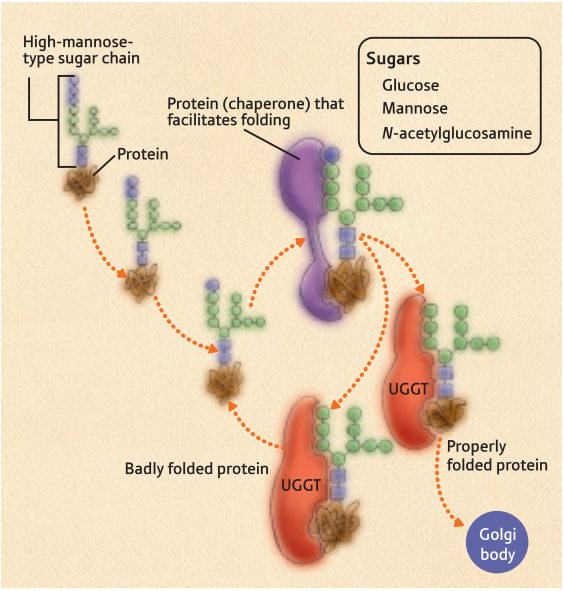


Figure 3: Quality control system of glycoproteins.
A protein having a single glucose unit binding to the end of a high-mannose-type sugar chain is combined with a protein (chaperone) that facilitates folding. When the folding is completed, the last single glucose unit at the end of the sugar chain is removed, and UGGT (a glucose transfer enzyme) combines with the protein to check that the folding has been properly performed. Properly folded proteins migrate to subcellular organelles called Golgi bodies, in which the structure of sugar chains is further transformed. Misfolded proteins are replenished with a single glucose, and the combined proteins are returned for another attempt. These functions of high-mannose-type sugar chains remained a mystery until recently.

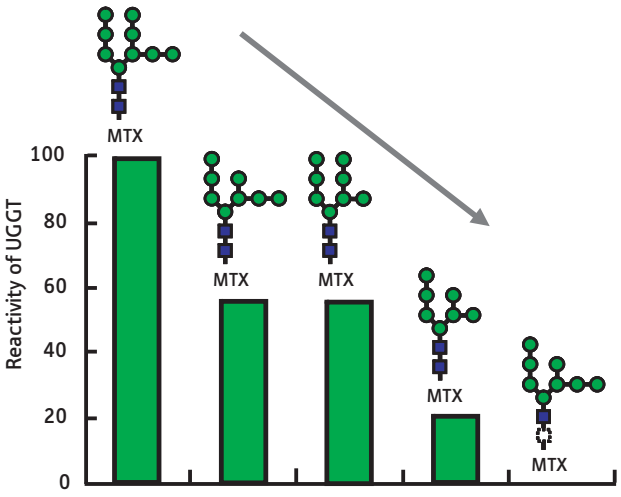


Figure 4: Effects of the difference in sugar chain structure on the reactivity of UGGT. Compounds composed of a hydrophobic molecule (MTX) and various types of high-mannose-type sugar chains are used to investigate the reactivity of UGGT. The investigation results show that its reactivity depends significantly on slight differences in sugar-chain structure.

chains are though to be chopped off before the protein enters the proteasome. In fact, experiments conducted in Ito's laboratory confirmed that proteins with sugar chains cannot be decomposed in proteasomes. Tadashi Suzuki and the members of the Glycometabolome Team at the Advanced Science Institute are attempting to elucidate the decomposition processes related to such sugar chains. Ito continues his study in cooperation with the Glycometabolome Team. Suzuki and his team are using the sugar chains that Ito and his team synthesized in order to analyze the key mechanism of enzymes.

Most glycoproteins pass through the above-mentioned quality control system of glycoproteins. If the system is not working, the number of defective proteins increases. These proteins may remain in cells without being decomposed, thereby causing serious diseases. For example, Alzheimer's disease and Parkinson's disease are considered to be caused by the accumulation of defective proteins, although the involvement of sugar chains remains unclear. Disorders of glycosylation, which means that defective sugar chains are produced, is thought to cause various symptoms. Progress in the study of sugar chains may establish that disorders of glycosylation are at the root of various diseases. In the future, therapeutic agents aimed at correcting disorders in the biosynthesis of sugar chains will be developed.

Establishing efficient methods for synthesizing specified sugar chains

Many genes that produce enzymes for assembling sugar chains have been discovered by Japanese researchers. Japan is now the world leader in the study of sugar chains. However, the number of chemists engaged in the study of the chemical synthesis of sugar chains is small. Ito's laboratory is one of the few laboratories in the world in which complex sugar chains can be synthesized.

Ito and his team are developing simpler synthetic techniques for sugar chains. Theoretically, any high-mannose-type sugar chains can be synthesized by chemically synthesizing long sugar chains in large quantities and using them as raw materials to obtain shorter lengths of sugar chains, either by chopping up the longer chains with the assistance of enzymes or by combining the chopped small pieces with each other. Such techniques can help biologists synthesize the required chains by themselves. In fact, cells use enzymes to create various sugar chains from long chains.

It is difficult to precisely recreate in a test-tube the phenomena that occur in cells. Furthermore, some enzymes are difficult to synthesize. Ito's team has devised a method of using raw materials with different structures in developing special techniques for creating any high-mannose-type sugar chain by using a combination of enzymes that are relatively easy to use in synthesis. They have already succeeded in synthesizing about 70% of all high-mannose-type sugar chains.

In April 2008, the Chemical Biology Department, which is a new organization within the Advance Science Institute, was opened. In this newly formed organization, researchers in biology and chemistry work jointly to elucidate the mechanisms of biological phenomena and to facilitate drug discovery. The above-mentioned Glycometabolome Team belongs to the Systems Glycobiology Research Group in the Chemical Biology Department. "We intend to conduct further joint researches with the Chemical Biology Department," says Ito.

Research at the Synthetic Cellular Chemistry Laboratory will bring about a significant breakthrough in the study of sugar chains.

About the researcher

Yukishige Ito was born in Hyogo, Japan, in 1954. He graduated from the Faculty of Pharmaceutical Sciences, University of Tokyo, in 1977, and obtained his PhD in 1982 from the same university. After two years of postdoctoral training at the Department of Chemistry, Massachusetts Institute of Technology in Cambridge, Massachusetts, USA, he returned to Japan as a research scientist at RIKEN, where he started his career in carbohydrate chemistry. He spent two years from 1991 as a visiting scientist at Cytel Corp. and Scripps Research Institute in San Diego, California, USA. He was promoted to senior scientist in 1996 and to chief scientist in 1998. Since then he has been the director of his own research group. His research focuses on the synthesis and functional analysis of glycoprotein-related compounds and the development of methodologies for the efficient and selective synthesis of glycoconjugates.

RIKEN president Noyori visits Malaysia

RIKEN President Ryoji Noyori went to Malaysia from Nov. 30 to Dec. 3 on a trip to sign an agreement on a joint graduate school program between RIKEN and the University of Science, Malaysia (Universiti Sains Malaysia). He also delivered two lectures at the invitation of the Academy of Sciences Malaysia (Academi Sains Malaysia).

The trip was aimed at strengthening cooperation with the Southeast Asian nation. He concluded a coordinated graduate school agreement that expands the current program, which has been in place since 2001, from the life sciences to include physics and chemistry. Every year three or four grad students (for a total of 10) in this expanded International Research Associate program will receive research guidance for up to three years.

President Noyori discussed wider cooperation between RIKEN and USM in the future, and met four doctorate students who will study at RIKEN from next year for three years under the coordinated graduate school agreement.

On Dec. 2, President Noyori delivered two lectures at the University of Malaya (Universiti Malaya). In the morning, he gave a Nobel Laureate Lecture, 'Chemistry: The Key to Our future' and in the afternoon, a Dialogue with Researchers, 'Asymmetric Catalysis: Science and Opportunities.' A good-size audience of young researchers and students gathered for the afternoon lecture, and afterward participated in a discussion that President Noyori enjoyed very much. ■

Former RIKEN researcher 'honored' with Ig Nobel

A group of Japanese scientists including former RIKEN researcher Toshiyuki Nakagaki were among this year's winners of the Ig Nobel Prizes, recognized for their discovery that a unicellular amoeboid organism can figure out the shortest distance in a maze.

Nakagaki, now an associate professor at Hokkaido University, who worked previously at the RIKEN Frontier Research System (now the Advanced Science Institute), along with Atsushi Tero, a researcher at the Japan Science and Technology Agency, and Ryo Kobayashi of Hiroshima University, received the prize for cognitive neuroscience at Harvard University on October 4.

The scientists' research revealed that despite having no brain or nerves, *Physarum polycephalum* can compute the shortest route in a maze. Placed in a maze 3 cm square, the organism ordinarily clogs every path by extending itself across them. But if food is placed at the maze's entrance and exit, it will connect the two points in the shortest possible distance.

The three Japanese scientists sang their acceptance speech at the ceremony, which was hosted by the tongue-in-cheek journal Annals of Improbable Research and was attended by real Nobel prizewinners and an audience of over 1,000 people. Nakagaki expressed his thanks for the award and noted that, despite conventional wisdom, *P. polycephalum* "is actually smarter than we thought."

The Ig Nobel prizes are awarded in 10 categories every year by the journal as a humorous version of the real Nobel Prizes.

Japanese scientists have won the award in the past. In 2004, the peace prize went to Daisuke Inoue for inventing karaoke. The following year, Yoshiro Nakamatsu won the nutritional science award for taking photos of every meal he ate for 35 years and analyzing the effects of the food on his brain activity. In 2007, Mayu Yamamoto won the chemistry award for discovering how to extract vanilla flavor from cow dung. ■

Cheiron2008 — The second AOFSRR summer school

The Asia-Oceania Forum for Synchrotron Radiation Research (AOFSRR) held its second summer school from September 29 to October 8 at SPring-8, the largest synchrotron radiation facility of the RIKEN Harima Institute. Fifty young scientists and engineers from eleven countries attended the summer school, which provided 10 days of significant talent-nurturing interchange.

The school was cosponsored by RIKEN, JASRI (Japan Synchrotron Radiation Research Institute, which operates SPring-8), KEK (Japan's High Energy Accelerator Research Organization), and AOFSRR. The curriculum included lectures on synchrotron radiation science and technology, covering everything

from synchrotron operation to industrial applications. The sessions concluded with a talk on new scientific directions and possibilities in synchrotron science.

The participants also had the opportunity to conduct real experiments using SPring-8 beamlines, during two one-day practical courses.

The 'Meet the Expert' sessions, round-table discussions for 5 to10 students, were a good opportunity for students to meet experienced scientists and ask practical, specific questions about their research.

The summer school's name, Cherion, is derived from ancient Greek mythology. Cheiron was an immortal god, intelligent, civilized and kind, as well as a teacher who would impart his knowledge only to those mortals most worthy of it. This matches the purpose of the summer school, which seeks to nurture the best and brightest young minds in science from the Asia-Oceania region. Tetsuya Ishikawa, the director of the SPring-8 center, says: "We believe they will become kingpins in synchrotron radiation science for [the] Asia-Oceania region." ■

Dr Yuji Kamiya
Growth Regulation Research Group
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230-0045, Japan

Dear Dr. Kamiya,

It´s some years back, but the happy memories of my time at RIKEN are still very fresh indeed—as if it was just yesterday. My first visit to RIKEN was in summer 1998, when I was a PhD student and had never dreamt of visiting Japan. As part of a collaboration between my former laboratory in México and your group, you invited me to spend two months at the Frontier Research Program in a beautiful and amazing place, the Wako institute.

Two years later, you visited us at my former laboratory. At that time I was in the last stage of my PhD research, and you offered me the opportunity to work as a postdoc in your excellent laboratory at the Plant Science Center (PSC). In 2001, I had the good fortune to receive a letter of invitation for a fellowship from JSPS. I was really happy to have the chance to revisit Japan.

In April 2002, I started my postdoctoral stay. My work research at PSC was focused on isoprenoid biosynthesis in plants. Isoprenoids represent the largest group of biologically active metabolites and are essential for the numerous physiological and developmental processes in plants. Of these, various metabolites have potential applications. Although my experience on this topic started during my PhD course, my colleagues gave me the opportunity to learn and increase my experience about isoprenoids and other exciting fields in plant science. It was also great working in a center dedicated to plant research, spending my first year of my stay at the Wako institute and the second year at the Yokohama institute.

For me, as a researcher, it was exciting to work at PSC; and it was a very good opportunity to meet many excellent scientists. Many became very good friends including Shinjiro Yamaguchi, Hiroyuki Kasahara, Mitsunori Seo, Eiji Nambara and Tetsuo Kushiro. However, the most rewarding privilege was to meet you. Kamiya-sensei (as everybody calls you) you have impressed me the most in my professional life. You are an outstanding scientist, a great mentor and a wonderful person. I am deeply moved when I think of my time as postdoc in your group. The working environment was most congenial. Everybody was helpful to me in conducting my research. I really appreciate that. You and your colleagues also made sure that I was part of your social group outside the laboratory. I remember with nostalgia the nice parties, delicious barbeques, and the exciting ski trip. In addition, I liked going bicycle riding. I traveled in my free time to see new places (temples, bonsai village, horse racing track, and more).

I must not fail to mention that it was not only the scientific knowledge and experience that I received at RIKEN that was useful, but the lessons that I learnt on the human aspects my co-workers were even more inspiring and helpful. Their sincerity, punctuality, dedication, their work ethic, their eagerness to learn and to work overtime, are human qualities that have been a source of abiding inspiration for me and I often share them with friends and students.

When my fellowship ended in March 2004, I returned sadly to my country and joined my former institute in Cuernavaca city. It is known in México as the city of eternal spring. I am glad to remain in close contact with you and other Japanese friends and colleagues. In fact, two colleagues from PSC, Shinjiro-san and Kasahara-san, visited me last year. Their stay here was not only pleasant but also particularly useful for me to advance research in collaboration with leading experts in the plant science field.

In my opinion, RIKEN has a high level of research, driven by excellent people. I am very grateful for the scientific experience I acquired at RIKEN, which stimulated and helped me in my further research activities. I want to thank JSPS for its financial support. I hope many other Méxican investigators will have the opportunities that I have had. Then, we can to establish a solid scientific collaboration between México and Japan in the near future.

With my best regards,

Juan Manuel Estévez Palmas
Research Associate
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