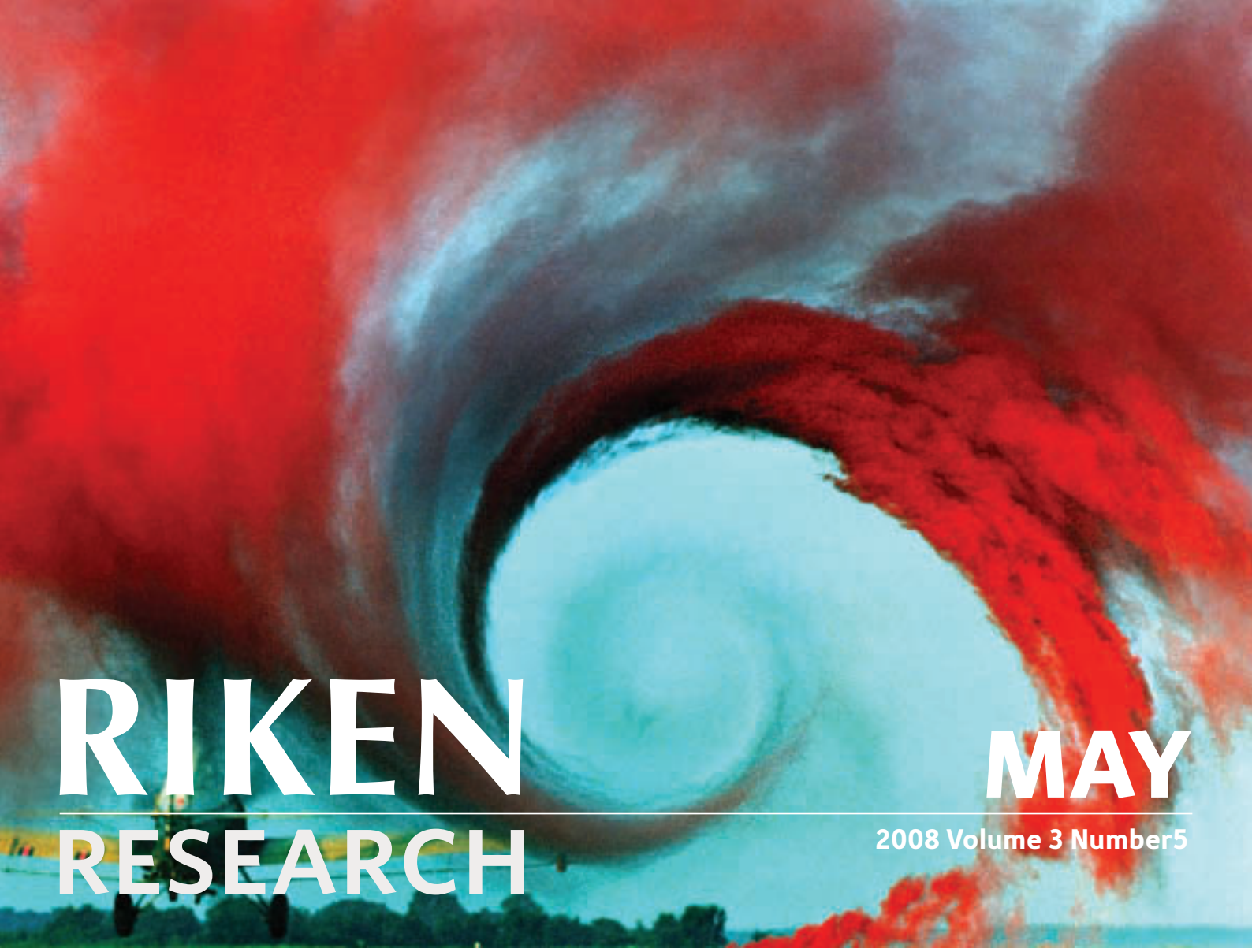

RIKEN RESEARCH

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Wave-particle whirling vortices

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Understanding cancer in mice and men

A new mouse model of human leukemia may provide fresh insights on the genesis of the disease

Japanese and American researchers have used a mouse model engrafted with human cells to characterize a rare population of cancerous stem cells that gives rise to acute myelogenous leukemia (AML)[1].

The cells are hematopoietic stem cells, a subset of bone marrow-derived cells that gives rise to essentially all of the cell types in the blood and immune systems. When isolated from patients with AML, the cells are called leukemic stem (LS) cells, and produce only the immature leukemic cells characteristic of this disease, rather than the range of cells from the blood and immune system produced by healthy hematopoietic stem cells.

"LS cells are the cells that can develop leukemia by self-renewing and generating non-stem leukemic cells. They are not normal hematopoietic stem cells," says principal investigator Fumihiko Ishikawa. "It is believed that normal stem cells acquire some mutations to become LS cells. But nobody has demonstrated that so far."

The team, based at the RIKEN Research Center for Allergy and Immunology in Yokohama, developed the mouse model to better study the pathogenic mechanisms leading to the development of human leukemia. The model uses as recipients a strain of mice with a severe immunodeficiency that have been further compromised by a mutation that inactivates a major subset of the immune system's signaling molecules. These mice cannot recognize human cells as 'non-self'; and by using newborn mice for the engraftment of the human cells, a more robust and longer-lived model is created.

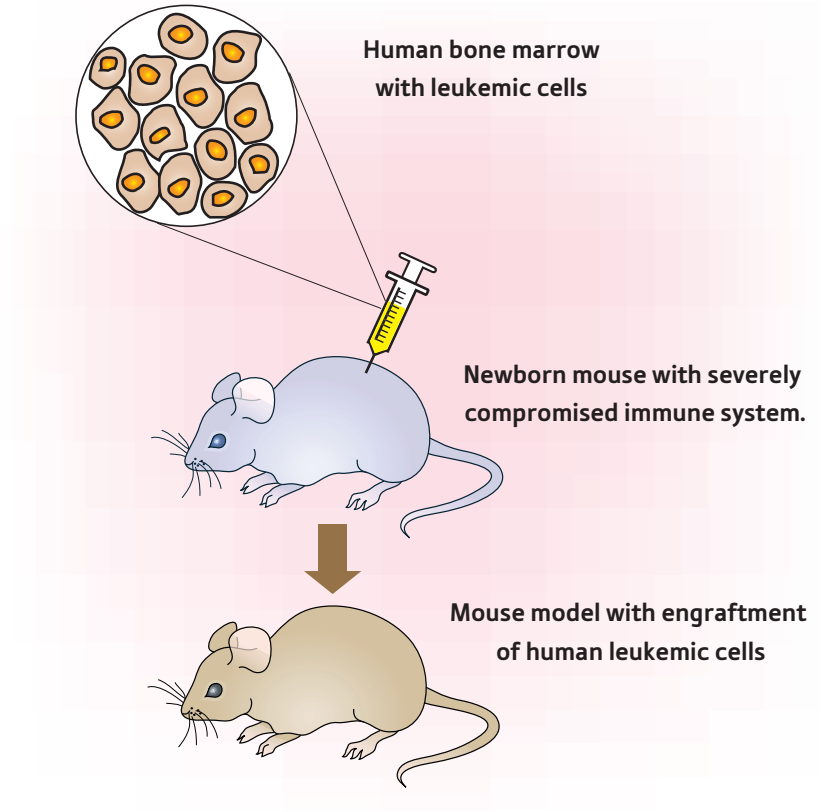

Figure 1: Creation of a mouse model of human AML from a severely immunocompromised mouse, which has been injected with human leukemic cells.

Successes with LS cell engraftment

Initially, the researchers demonstrated that human AML cells could be engrafted efficiently into the immunocompromised mice. To do this, human bone marrow cells enriched to contain 80–90% leukemic cells were intravenously injected into mice whose immune systems had been destroyed by sublethal irradiation (Fig. 1). Engraftment of the human cells occurred in nearly 40% of the newborn mice compared with only 13% of adult mice.

Next the researchers showed that the LS cells resided in the subset of the leukemic bone marrow cells expressing the so-called CD34 cell-surface protein but not CD38; as such, the cell-surface protein profile is characteristic of undifferentiated hematopoietic stem cells. When these cells were engrafted into the mouse model, they homed to the bone marrow and were also detectable in the peripheral blood. Injection of other subsets of human leukemic cells, either expressing both CD34 and CD38, or neither of them, did not result in detectable engraftment.

Engraftment was dose dependent, with as few as 1,000 of the LS cells required. Furthermore, these cells were capable of both self-renewal and differentiation into non-stem leukemic cells.

The LS cells could also be serially transferred from mouse to mouse. Serial transplantation over a cumulative period of more than one year successfully demonstrated the long-term self-renewal characteristics of the LS cells.

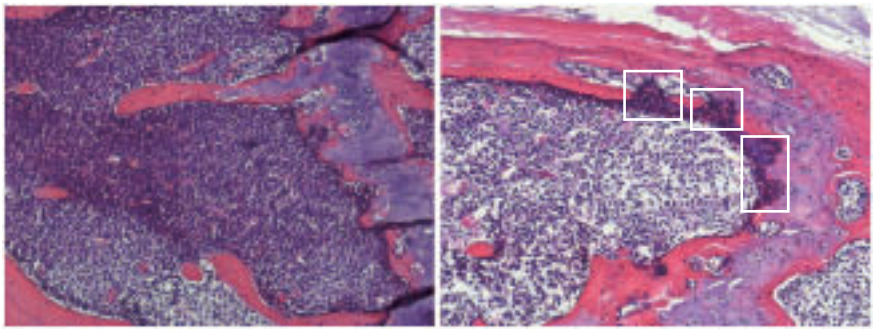

Figure 2: Bone sections derived from AML-engrafted mice before (left) and after (right) chemotherapy. Without chemotherapy, vigorous proliferation of AML cells was seen throughout the bone marrow. After chemotherapy, AML cells at the central region were efficiently eradicated, while LS cells at the endosteal region (white squares) survived.

Specifics on bone-marrow homing

Antibody staining of bone sections showed that the LS cells homed specifically to and engrafted within the micro-environmental niche at the endosteum, a layer of connective tissue on the inner surface of the bone cavity (Fig. 2). This site is where hematopoietic stem cells would normally reside and the process of hematopoiesis, or blood and immune cell differentiation, would take place. The researchers showed that within three days of the LS cells being injected into the mice, the endosteal region, which was essentially empty of cells due to the irradiation required to prepare the mice for engraftment, was lined with LS cells. By 4 months post-engraftment, the bone marrow was packed with AML cells.

The presence of the LS cells suppressed the normal formation of new murine blood cells, a phenomenon also observed in AML patients. The evidence suggests that competition between increasing numbers of LS cells and normal hematopoietic stem cells may be responsible for the eventual suppression of normal hematopoiesis in AML patients.

In addition, the LS cells were relatively resistant to the cytotoxic agent Ara-C, due to the majority of them being in the quiescent (G_0) phase of the cell cycle. The researchers believe this explains why AML relapse after chemotherapy is common—non-stem leukemic cells, but not LS cells, are eliminated by cell cycle-dependent cytotoxic agents used to treat the disease. Thus the cell cycle quiescence of the LS cells may protect them from the cytotoxic agents.

Retention of original characteristics

Finally, genetic analysis of the engrafted LS cells showed they retained characteristic gene expression patterns, even after serial transplantation. Analysis of the genes expressed suggests that various genetic targets found in the LS cells may be potential targets for LS cell-specific therapies.

According to Ishikawa, the retention of phenotype, function and gene expression means the model will be a useful tool for the study of the pathogenic mechanisms underlying AML. Using the model, the rare LS cells can be isolated from recipient mice in numbers adequate for large-scale genetic analysis, which may lead to the identification of new therapeutic targets specific to AML. The model may also be useful for testing new therapeutics and treatment modalities for the disease.

"This xenotransplant model will be helpful for developing a cell-bank for human primary AML stem cells and for testing safety and efficacy of various treatment modalities for AML," Ishikawa notes. "It has already enabled us to identify the major reason and mechanism for AML relapse." ■

1. Ishikawa, F., Yoshida, S., Saito, Y., Hijikata, A., Kitamura, H., Tanaka, S., Nakamura, R., Tanaka, T., Tomiyama, H., Saito, N., *et al.* Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nature Biotechnology* **25,** 1315–1321 (2007).

About the researcher

Fumihiko Ishikawa received an M.D./Ph.D. degree from the Kyushu University School of Medicine for his work on human hematopoiesis using xenotransplantation models under the mentorship of Mine Harada, Takeshi Watanabe and Makio Ogawa. His contributions in the laboratory has been directly related to his experiences in the clinic as a hematologist/oncologist, including *in vivo* examination of normal human hematopoetic and immune systems, modeling of human hematopoietic malignancies such as AML, ALL and acute T-cell leukemia and the study of human hematopoietic stem cell plasticity. He currently leads the Research Unit for Human Disease Models at the RIKEN Research Center for Allergy and Immunology, where he is continuing his work in hopes of developing new therapeutic approaches to hematopoietic malignancies and other disorders.

No loose ends

Tying short RNA molecules into loops gives them a stability boost, which could lead to more effective therapeutic strategies for modulating gene expression

RNA interference, in which short interfering RNA (siRNA) molecules are used to target specific genes for downregulation *in vivo*, is among the most powerful molecular biology techniques to emerge in recent years. RNAi was developed as a research tool following the discovery of naturally occurring siRNAs, which are processed from larger RNA transcripts by the cellular enzyme Dicer to yield very small (typically 21-nucleotide) RNAs containing specific sequences complementary to their endogenous target genes.

Synthetic siRNAs can be expressed directly from transgenes as small, hairpin-forming RNAs, which are subsequently converted into linear molecules by the action of Dicer; alternatively, they can also be introduced directly into cells in a pre-linearized form. In the laboratory, siRNAs have delivered considerable precision and efficiency in modulating gene expression in cultured cells and model organisms. Many scientists also see considerable promise in clinical applications of this technology, although there are a number of serious technical roadblocks that remain to be overcome.

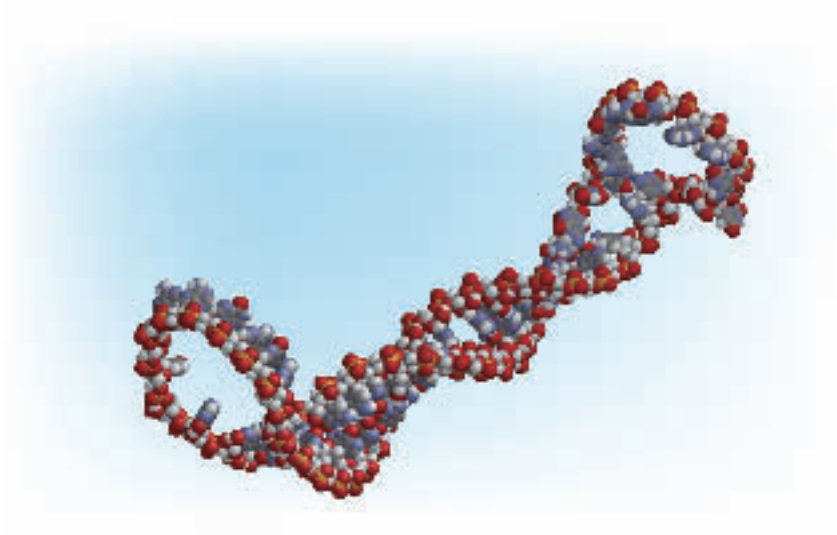

Figure 1: Three-dimensional structure of one of the Ito group's 'dumbbell siRNA' constructs.

Delivering the message

Chief among these impediments is finding a safe and effective means for siRNA delivery. Simple injection of siRNAs is not an option, as unprotected RNA molecules are rapidly degraded in the body, and so more complicated strategies are required—each with its own issues.

The introduction of fully functional siRNA genes into patients is also problematic, due largely to the same complications that plague gene therapy research. "Virus vectors that proliferate *in vivo* are potentially risky … vector systems can not be controlled in cells, and the dose of RNA is very important for clinical RNA interference," explains Hiroshi Abe, a research scientist in Yoshihiro Ito's laboratory at the RIKEN Advanced Science Institute in Wako. "If excess RNA is administered, cells will respond to these as foreign bodies, using the immune system."

Another alternative involves the use of RNAs composed of unnatural nucleotides containing various chemical modifications. These synthetic RNAs are able to resist enzymatic degradation, and can therefore survive longer within the body, but Abe points out that this stability comes at the cost of reduced efficacy at gene silencing. "This remains a problem for unnatural molecules in RNAi technology," he says.

Ito's group has taken an innovative approach to deliver natural siRNAs effectively, based on the understanding that RNA-degrading enzymes typically start by chewing at loose RNA ends—they circularized their siRNAs, designing molecules that self-assemble into stable 'dumbbell' shapes[1], with a base-paired central stem connecting two loops at either end (Fig. 1). By closing off the ends, the exonuclease should be left with no means of attack and the siRNA should acquire enhanced stability (Fig. 2).

Experimentally, these constructs behaved as expected, with the modified structure providing a considerable stability boost. Dumbbell and linear siRNA constructs were each incubated in the presence of exonucleases; after two hours, the percentage of intact linear siRNA was one-sixth that of the dumbbell construct. Similar results were obtained with RNAs incubated in human serum, which more closely mirrors the conditions likely to be experienced by siRNAs in clinical applications.

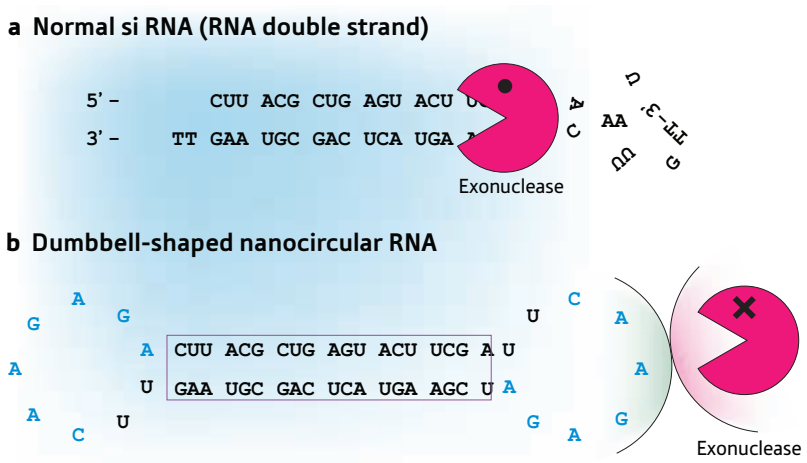

Figure 2: Linear siRNA molecules (a) are vulnerable to rapid degradation by exonuclease enzymes, which target the loose ends. Circularizing those ends (b) provides protection against exonuclease digestion, resulting in increased efficiency of inhibition of target genes.

However, since naturally occurring siRNAs must also be processed by the enzyme Dicer before they can be effective, a key concern of Ito's team was ensuring that their dumbbell constructs were not just stable, but also capable of being cleaved by Dicer and successfully inhibiting target genes.

The researchers treated a variety of linear and dumbbell constructs of different lengths with recombinant Dicer in order to examine processing efficiency, and found that the dumbbell constructs were generally capable of being processed, but typically at a far slower rate than the linear constructs.

This delayed processing did not impede their performance as gene inhibitors, however, and the dumbbell constructs generally surpassed their linear counterparts in triggering specific inhibition of target genes when injected into cultured human fibroblast cells. For some constructs, the improvement of suppression was as high as three-fold for the dumbbell siRNAs. The duration of effective gene suppression was also extended for the dumbbell constructs, perhaps as a result of their delayed processing by Dicer.

Building smarter dumbbells

Encouraged by these initial findings, Ito, Abe and colleagues have pursued strategies to further enhance the effectiveness of their constructs. Abe indicates that optimizing the dumbbell's loop structure has become a major focus since the publication of this work, in order to develop optimized constructs that strike an effective balance between greater stabilization, efficient enzymatic processing, and potent inhibition of target genes.

"We have tried modification of the loop position and found that this offers improved stability," says Abe. "We have also found that this loop is very important for controlling the editing position used by Dicer—in other words, we can decide the cleavage site of RNA by designing the loop position to produce appropriate siRNA species."

With this progress in optimizing the biological activity of their dumbbell constructs, improving the means of delivery is now an important goal, and Abe adds that he and his colleagues are currently exploring the use of a variety of promising nanomaterials that could help deliver their dumbbell constructs into cells safely and efficiently. ■

1. Abe, N., Abe, H. & Ito, Y. Dumbbell-shaped nanocircular RNAs for RNA interference. *Journal of the American Chemical Society* **129**, 15108–15109 (2007).

About the researcher

Yoshihiro Ito was born in Gifu, January 12, 1959. He received his Bachelor's (1981) and Master's (1983) degrees in polymer chemistry at Kyoto University and was awarded a doctorate in engineering from the same university in 1987. Since then he has held a number of posts at various institutions including Research Fellow of the Japan Society for the Promotion of Science (1987), assistant (1988) and associate (1998) professor at Kyoto University, research fellow at the University of California, Irvine (1992–1993), professor of the University of Tokushima (1999), and Project Leader at the Kanagawa Academy of Science and Technology (2002–2007). He has also been a visiting professor at Zhengzhou University, the Nagasaki Institute of Applied Sciences, Tokyo Metropolitan University and Tokyo Institute of Technology. Now, he is Chief Scientist and Director of the Nano Medical Engineering Laboratory at the RIKEN Institute of Physical and Chemical Research. His research focuses on biomaterial science, regenerative medical engineering, combinatorial bioengineering for the creation of functional polymers, and soft nanotechnology.

Magnetic flux quanta get a dance lesson

Alternating electric current can be used to precisely control tiny vortices of magnetism

Swirling cyclones of magnetism at the sub-micron scale that can trouble superconducting devices have been tamed by RIKEN scientists. Their technique could help to minimize magnetic noise in sensitive superconducting detectors, and could even help to build a new generation of devices for supercomputers.

When cooled below a critical temperature, superconductors carry electricity with no resistance. But magnetic fields can disrupt this behavior by introducing magnetic flux quanta into the material. These quanta, also known as vortices, are the basic units of magnetism, just as the charge of an electron is the fundamental unit of electricity.

Scientists can control how these vortices move by introducing tiny asymmetric traps, or nano-holes, into the structure of the superconducting material. But since the pattern of these tiny traps is fixed once the device is made, it's a relatively inflexible approach that restricts the way the vortices can be moved around.

Now, a team including Franco Nori and Sergey Savel'ev of RIKEN's Frontier Research System in Wako, have shown how to precisely control the movement of magnetic flux quanta with an alternating electric current (AC) with two frequencies[1].

The scientists tested the method on a high-temperature superconductor made from bismuth, strontium, calcium and copper ($Bi_2Sr_2CaCu_2O_{8+\delta}$). When the electrical current oscillates back and forth, the vortices obediently follow their rhythm.

Nori, also based at University of Michigan, US, says that the technique is like leading the magnetic flux quanta through a series of dance steps (Fig. 1). "The applied current acts as the leading dance partner and the vortices follow the steps imposed by the current," he says.

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Figure 1: Controlling the movement of tiny vortices of magnetism in superconducting devices is similar to leading a dance partner through steps.

More complicated rhythms are created by adding more overlapping alternating currents, allowing the scientists to steer their magnetic flux quanta through the material. "The two 'control knobs' we use are the ratio of the AC frequencies, and the relative phase difference between them," explains Nori.

Savel'ev, also at Loughborough University, UK, adds: "By slowly varying either one of these two control knobs, vortices are pushed either in one direction or the opposite."

Nori says that the technique could also be used to manipulate trapped ions, moving electrons around in certain types of crystal, or even separating different types of very tiny particles.

In the longer term, the scientists hope that the technique could contribute to the burgeoning field of 'fluxtronics'—moving magnetic quanta around to manipulate computer data. This would potentially be much faster that conventional methods relying on shuttling electrons between transistors. ■

1. Ooi, S., Savel'ev, S., Gaifullin, M. B., Mochiku, T., Hirata, K. & Nori, F. Nonlinear nanodevices using magnetic flux quanta. ***Physical Review Letters* 99,** 207003 (2007).

Quantum corkscrews from twisting electron waves

RIKEN researchers have shown that electron beams, like light, can be twisted into vortices that have useful functions

NASA

Figure 1: A classical aerodynamics vortex with twisting red smoke produced by a rotating airplane. Quantum analogs have been made with light, and are now proposed for electron waves.

Recently scientists discovered that light can be twisted like a corkscrew around its direction of travel. This unusual quantum feature allows photons to whirl around in a vortex, even when no external force is applied to the beam. Now researchers from the RIKEN Frontier Research System in Wako have shown that the same kind of vortices can be produced in beams of electrons[1], promising novel applications.

"When a light or electron beam is twisted, waves at the central axis cancel each other out forming a dark core, like at the eye of a storm (Fig. 1)," says RIKEN scientist Franco Nori, also with the University of Michigan in the USA. His RIKEN collaborator Sergey Savel'ev, also at Loughborough University in the UK, adds: "As the photons or electrons spin around the axis, they carry orbital angular momentum that can rotate an electric dipole."

To explain these properties, the researchers solved the Schrödinger equation of quantum mechanics for a twisting beam of electrons. This produced new dynamical equations that are highly analogous to those found for light. The similarities arise because the twisting angular momentum of the electrons interacts with their forward motion in the same way that intrinsic angular momentum (spin) interacts with the motion of photons, which is known as spin-orbit coupling.

The theory implies that vortices in electron beams have all the features of optical vortices. This reinforces the famous concept of wave-particle duality, which states that all particles have a wave associated with them. More importantly, it means that the useful applications of optical vortices could be replicated at much shorter wavelengths.

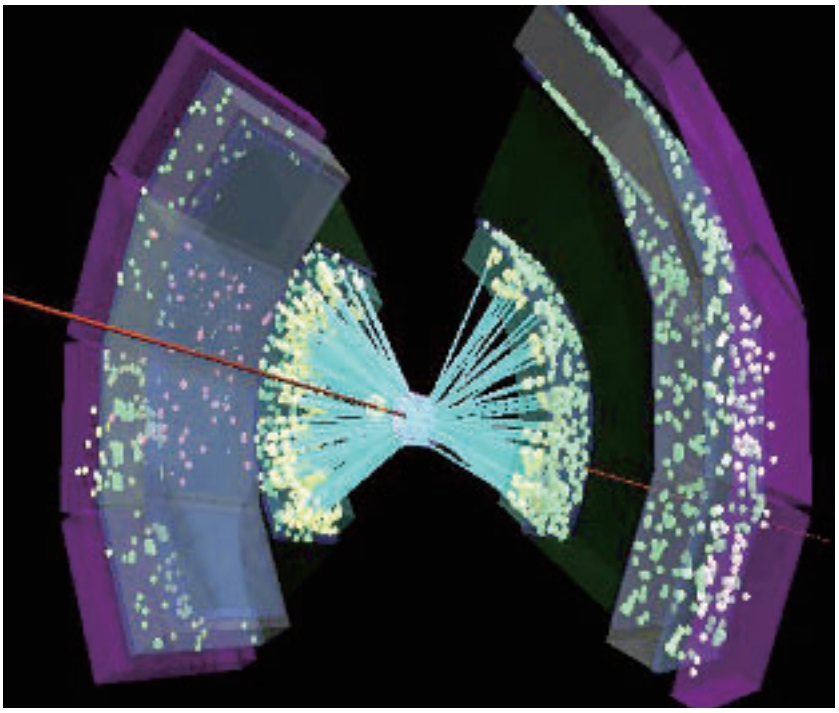
In practice, optical vortices can be made by passing a laser beam through a fork-shaped computer generated hologram. Electron-beam vortices could be produced in a similar fashion, using a thin crystal plate with a dislocation. Such vortices could power tiny nanomotors and nano-engines, or could be used in telecommunications by storing information in the optical vorticity, or the intensity of twisting. The vorticity is robust against perturbations, so this potential future technology could reduce the loss of information during optical communications.

Furthermore, electron vortices are predicted to cause a shift of the electron beam at right angles to an electric field. "The unique electron microscope developed by Akira Tonomura's group, also at RIKEN, could observe this unusual effect," says Nori. "Such work would considerably expand the textbook analogy between matter and waves which Tonomura helped to establish in pioneering experiments." ■

1. Bliokh, K. Y., Bliokh, Y. P., Savel' ev, S. & Nori, F. Semiclassical dynamics of electron wave packet states with phase vortices. *Physical Review Letters* **99,** 190404 (2007).

Bound quarks loosen up

Theorists propose a new estimate for the temperature at which heavy, bound quarks dissociate in the quark–gluon plasma



Courtesy of Brookhaven National Laboratory

Figure 1: Special detectors (grey areas) at BNL's Relativistic Heavy Ion Collider are designed to measure the particles that emerge from the collision between gold nuclei. The center of the image is the location of the collision and the blue lines indicate the particle trajectories.

In the first 10 millionths of a second after the Big Bang, scientists believe that matter existed in a hot and dense state known as the quark–gluon plasma. Thirteen billion years later, physicists at Brookhaven National Laboratory's (BNL) Relativistic Heavy Ion Collider (RHIC) are recreating the conditions necessary to observe this unusual state of matter by colliding high-energy gold nuclei.

Writing in *Physical Review Letters*[1], Ágnes Mócsy, a theoretical physicist at the RIKEN-BNL Research Center, and Péter Petreczky of the BNL nuclear theory group, make predictions with important implications for these experiments. They show that particles found in the quark–gluon plasma, known as 'quarkonium states', break apart at much lower temperatures than many scientists have recently believed. Since this suppression in the quarkonium dissociation temperature is a direct result of 'free' quarks in the quark–gluon plasma, scientists view the effect as further evidence that the plasma has formed in a high-energy collision.

Quarks, and the gluons that mediate the interactions between them, are fundamental particles so strongly attracted to one another that they are never found in isolation under normal conditions. Rather, quarks bind in pairs or triplets to form protons, neutrons and other more exotic particles and only extremely high temperatures and pressures can pull them apart.

To create such temperatures (>10^{12} °C), gold nuclei at the RHIC are accelerated toward each other at very high energies (Fig. 1). The energy density generated at the collision of two nuclei is sufficiently great to free the quarks that are bound in the protons and neutrons.

Quarkonium states, however, contain such tightly bound quarks that they can survive above the theoretical temperature, Tc, at which the quark–gluon plasma forms. Unbound quarks in the plasma can shield—or 'screen'—the interaction between these tightly bound quarks and lower the temperature at which quarkonium states dissociate.

Underlining the importance of screening, Mócsy explains: "The effect is an unambiguous signature of the quark–gluon plasma."

Mócsy and Petreczky calculated the dissociation temperatures of several quarkonium states, including the so-called J/ψ particle. Members of the high-energy nuclear physics community have held that this particle survives to around $2Tc$, but Mócsy and Petreczky have shown that screening suppresses its dissociation temperature to around $1.2Tc$. "These results are therefore against the trend," says Mócsy.

Detectors can measure the products of the J/ψ particle decay and determine its presence in the evolution of the quark–gluon plasma. ■

1. Mócsy, Á. & Petreczky, P. Color screening melts quarkonium. ***Physical Review Letters* 99,** 211602 (2007).

Heavy out of frustration

Constraints on the arrangement of electrons and ions in a metal compound lead to electrons with a very heavy mass

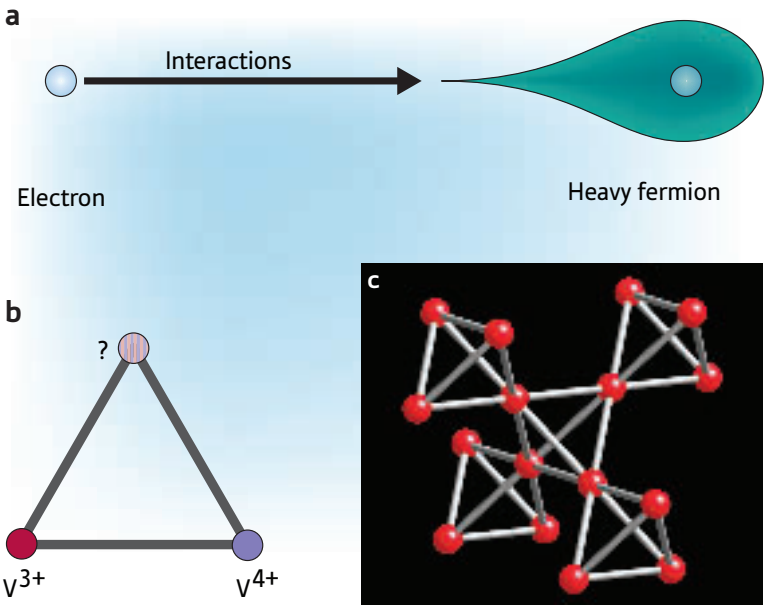


Figure 1: Heavy fermions in LiV_2O_4. (a) The interaction between electrons and the atoms of the crystal lattice leads to heavy fermions that have a large effective mass. (b) The attempt to distribute two different ions on a triangular structure inevitably fails owing to geometric constraints. (c) Geometric frustration in LiV_2O_4 is shown here for the crystal lattice of the V ions, which is composed of triangular units.

Researchers at RIKEN's Discovery Research Institute in Wako, in collaboration with colleagues at Nagoya University, have unraveled the mechanism that leads to an unusual electronic state in the metal compound LiV_2O_4.

At low temperatures, certain metallic materials containing magnetic elements sometimes show a peculiar behavior, where the propagating electrons have an extraordinarily heavy mass: about 100 to 1000 times larger than that of 'real' electrons. Known as heavy fermions, these heavy electrons are believed to originate from an interaction between the propagating electrons and the magnetic elements.

Dubbed 'Kondo coupling', this intriguing interaction was first described in 1964 by the Japanese physicist Jun Kondo. In Kondo coupling, the heavy fermions are comprised of electrons that are slowed down by the surrounding magnetic elements (Fig. 1a). Recently, however, a few materials—including LiV_2O_4—have been discovered to show heavy fermion properties, but none of the tell-tale signs of Kondo coupling.

Led by Hidenori Takagi from RIKEN, the researchers have investigated the origin of the heavy fermions in LiV_2O_4. The existence of heavy fermions in the absence of Kondo coupling suggests an interesting interaction between electrons and the magnetic elements. Indeed, "based on optical experiments, we are proposing a new mechanism for the heavy fermion mass in LiV_2O_4," explains Takagi. Their results are published in *Physical Review Letters*[1].

The researchers studied the optical response of LiV_2O_4 at different wavelengths. The measurements reveal large differences between the material's reflectivity at room temperature and at low temperatures, where the heavy fermions are formed.

The observed changes in optical response are symptomatic of major changes in the energy states of the material that are not typically a sign of heavy fermions. Rather, they suggest a periodic arrangement on the crystal lattice of the two types of vanadium ions, V^{3+} and V^{4+}, in LiV_2O_4. Such a periodic arrangement would prevent the formation of heavy fermions. However, the crystal structure of LiV_2O_4 makes any periodic arrangement impossible owing to geometric constraints—it has a 'geometrically frustrated' configuration (Fig. 1b,c). The only remaining explanation, then, is that the changes in energy states lead to the formation of heavy fermions.

These results are an important advance, as "this is a new route to heavy fermion formation, and we identified a new electronic state produced by geometrical frustration," comments Takagi. Further studies might therefore uncover a number of related exotic effects associated with this unusual and complex electronic state. ■

1. Jönsson, P. E., Takenaka, K., Niitaka, S., Sasagawa, T., Sugai, S. & Takagi, H. Correlation-driven heavy-fermion formation in LiV_2O_4. *Physical Review Letters* **99**, 167402 (2007).

Calculating chemistry

Computational models offer a better understanding of how metal-containing reagents participate in chemical reactions

Although nature provides us with a supply of substances that we have adapted for a number of uses, the importance of being able to create 'designer' molecules should not be underestimated. Since the birth of the chemical industry in the late 1800s, the benefit to society of laboratory-made pharmaceuticals and materials has been vast.

The evolution of synthetic chemistry has typically occurred through a trial-and-error process—albeit based upon chemists' observations and intuition. A reaction is optimized by changing the conditions, such as temperature or solvent, or by using different reagents. As pointed out by Masanobu Uchiyama from RIKEN's Discovery Research Institute in Wako, however, "these processes can be time-consuming and expensive."

One solution to this problem is to produce theoretical models of how a chemical reaction proceeds so that a deeper understanding of the process can be realized—and the factors that can be changed to improve it. Uchiyama and co-workers have used computational methods to look at how metal-containing compounds react with organic molecules known as enones[1].

Enones contain a carbon–carbon double bond that is one bond away from a carbon–oxygen double bond. These compounds undergo reactions (Fig. 1) in which a carbon-based group (R^1) can be added in one of two locations. When so-called 'soft' metals—such as copper (Cu) or zinc (Zn)—are used, a 1,4-addition reaction puts the R^1 group at the '4' position. In contrast, 'hard' lithium (Li) reagents cause the R^1 group to bond to the '2' position in a 1,2-addition process.

Uchiyama and co-workers investigated the reaction between enones and organozinc reagents by modeling the transition states—the highest energy points along the reaction pathway—for both 1,2- and 1,4-additions. They found that the 1,4-addition proceeds through an 'open' transition-state structure that is lower in energy than the more compact one found for 1,2-addition. This result explains the experimentally observed preference for 1,4-addition with organozincates.

Simulations also revealed that while Cu and Zn reagents both lead to 1,4-addition, the reaction mechanism is totally different in each case. Whereas the charge on the Cu atom changes during the reaction, no oxidation/reduction processes are seen in the case of Zn. Thus, an important and long unsolved issue in organic chemistry has been settled by computational chemistry.

"Computational models of these reactions will lead to further modification of organometallic reagents, including zincates," suggests Uchiyama, "and may lead to improvements in reactivity and selectivity—as well as entirely new reactions." ■

1. Uchiyama, M., Nakamura, S., Furuyama, T., Nakamura, E. & Morokuma, K. Reaction pathway of conjugate addition of lithium organozincates to s-trans-enones. *Journal of the American Chemical Society* **129,** 13360–13361 (2007).

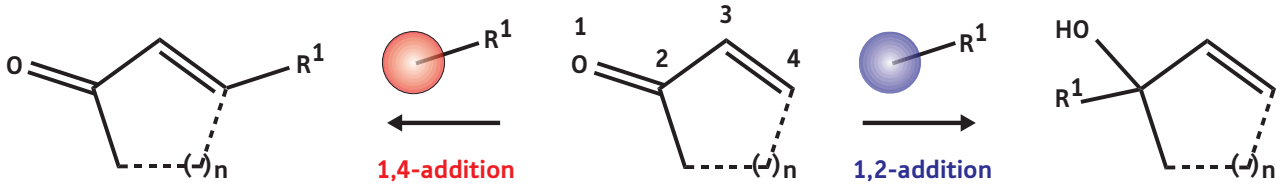


Figure 1: Enones undergo addition reactions with organometallic reagents in which a new carbon chain is added at either the '2' or '4' position. Reagents based on so-called 'hard' metals, such as lithium (Li), lead to 1,2-addition (blue), whereas those incorporating 'soft' metals, such as copper (Cu) or zinc (Zn), lead to 1,4-addition (red)—also known as 'conjugate' addition.

Seeking schizophrenia genes

Researchers map genetic alterations associated with human schizophrenia

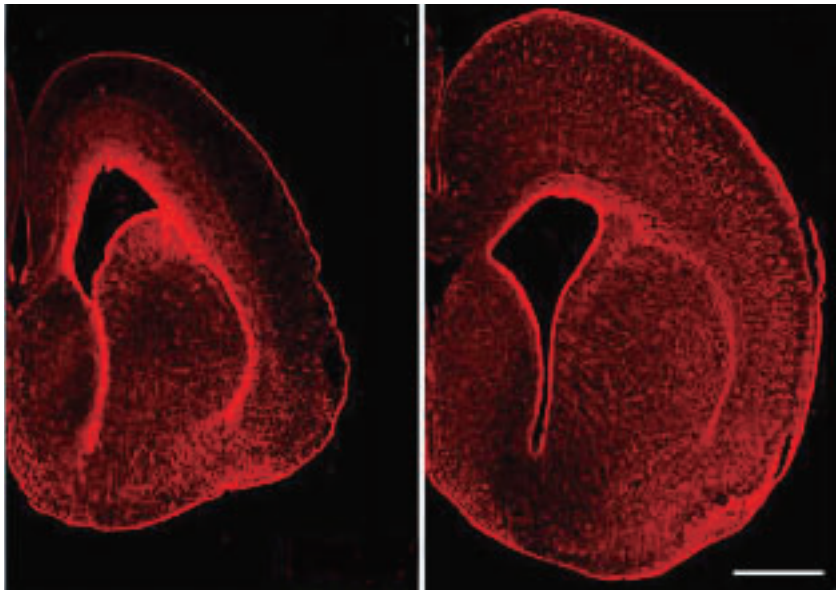


Figure 1: Expression of Fabp7 protein in embryonic and neonatal mouse brains. Fabp7 (red) shows abundant expression in (a) embryonic day 16 and (b) neonatal mouse brains.

Japanese scientists have linked atypical expression patterns of the gene *FABP7*, which encodes the brain fatty acid binding protein 7, with human schizophrenia. Although initially attributed to environmental abnormalities, this debilitating disease is now accepted as being influenced by a strong, yet likely multifactorial, genetic component.

The phenotypic, or behavioral, outcomes of schizophrenia are perhaps just as complicated as the genotypic alterations underlying the disease. Fortunately, suppression of a particular startle response—known as prepulse inhibition (PPI)—provides an easily measurable biological readout of the sensory motor gating mechanisms that are often impaired in schizophrenia.

In an effort to identify genes associated with schizophrenia, a team led by Takeo Yoshikawa at the RIKEN Brain Science Institute in Wako, mapped genetic alterations associated with PPI in mice[1].

After tracking the PPI responses of a panel of distinct inbred mouse strains for over one year, the researchers intercrossed the strains having the lowest and highest PPI scores. Next, the team scanned the genomes of the progeny for sets of microsatellite markers, or genetic 'tags', and compared the presence of these tags with the PPI scores.

Using progressively rigorous sets of tags, the researchers linked impaired PPI to a region of chromosome 10 containing approximately 30 genes. The team honed in on *Fabp7* (Fig. 1), one gene within this region, because of its influence over the metabolism of the polyunsaturated fatty acid DHA (docosahexaenoic acid), a process often impaired in schizophrenia.

Encouragingly, although stronger in males than in females, human schizophrenia patients exhibit abnormally high expression of *FABP7* similar to mice exhibiting defective PPI responses. Notably, mice rendered genetically deficient in *Fabp7* also score low in PPI measurements and display stronger behavioral responses to chronic NMDA receptor antagonist treatment, another feature of schizophrenia.

Although the team detected defects in the maintenance of neural progenitor cells in *Fabp7*-deficient mice, future work is needed to elucidate the precise molecular mechanism through which alterations in *Fabp7* expression promote schizophrenia-like behavior in mice and humans.

Similarly, why males seem to be more strongly affected by *Fabp7* over-expression remains unclear. However, sex hormone-responsive elements in the DNA regions controlling *Fabp7* expression might play a role.

"It is well known that malnutrition *in utero* increases the probability of future schizophrenia. Our results raise the importance of cohort studies to examine whether replenishment of DHA in pregnant mothers can be beneficial in reducing the chance of schizophrenia development in offspring," says Yoshikawa. ■

1. Watanabe, A., Toyota, T., Owada, Y., Hayashi, T., Iwayama, Y., Matsumata, M., Ishitsuka, Y., Nakaya, A., Maekawa, M., Ohnishi, T., ***et al.*** ***Fabp7*** maps to a quantitative trait locus for a schizophrenia endophenotype. ***PLoS Biology*** **5,** 2469–2483 (2007).

Brain repairs

Healing from spinal cord injury requires use of different regions of the brain

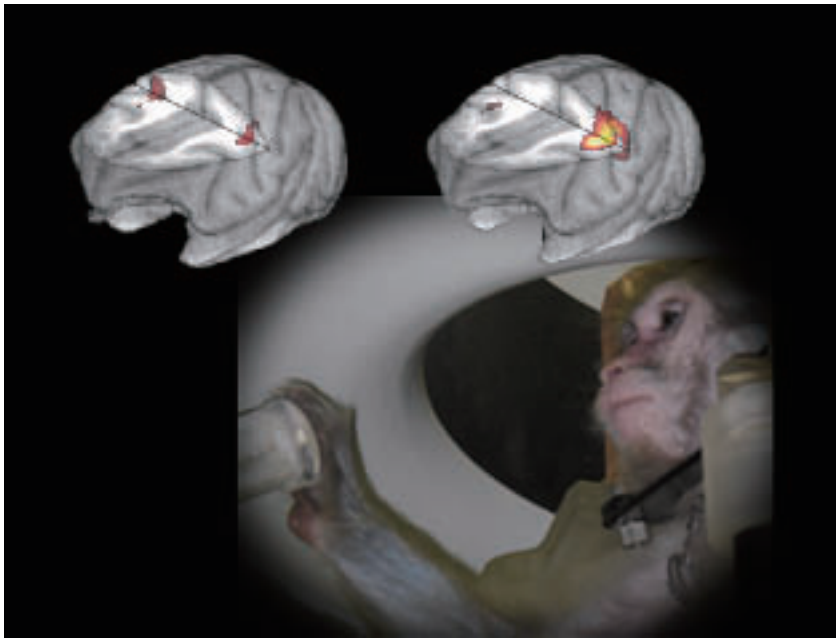


Figure 1: A test monkey in a PET scanner and two representative images during the early and late phases of recovery. After a spinal cord injury affecting the dexterous finger movement of the right forepaw, the early phase of retraining results in both the ipsolateral and contralateral sides of the brain being active (left image). During the later phase of recovery, the contralateral side of the brain is again the predominately active region when the right forepaw is in use, whereas activity in the ipsolateral brain regions has ceased (right image).

New research from Japan using non-human primates and a brain imaging technique called Positron Emission Tomography (PET) has shown that compensatory mechanisms in the brain contribute to recovery from spinal cord injury.

After partial injury to the spinal cord or brain, retraining to complete physical tasks, or neurorehabilitation, often relies on recruiting remaining intact brain regions to compensate for the injured neurons. But exactly which regions the brain uses to rewire itself, and when, has puzzled scientists.

Now, Hirotaka Onoe at the RIKEN Molecular Imaging Program of the Kobe Institute, in collaboration with Tadashi Isa at the Japanese National Institute for Physiological Sciences and colleagues, has used PET scanning to follow the recovery of injured monkeys to address this question[1].

The researchers transected a neural pathway of three monkeys such that they could no longer use the fine motor skills of their right forepaws to carefully grasp a small piece of food. Finger dexterity, however, could recover with rehabilitation within three months.

PET scans showed that pre-injury, as occurs normally, only the opposite—or contralateral—side of the brain to the right forepaw is active when the limb is in use. However, after injury, the same—or ipsolateral—side of the brain as the right forepaw also becomes active to help compensate for the injured neuronal pathway.

Eventually, though, the ipsolateral activity declines as the injured neuronal pathway heals and the contralateral brain regions again become the predominant areas of activity (Fig. 1). Indeed, this compensation by the ipsolateral brain region is only needed in the initial stages of recovery as early inactivation of these ipsolateral regions impedes recovery, whereas inactivation at later times results in no impediment.

From these results the team has concluded that normally the activity of the ipsolateral side of the brain is inhibited when an appendage is in use, but after injury this inhibition is relaxed in order to allow some use of that limb and simultaneously allow the injured contralateral neuronal pathway to recover. However, once sufficient recovery of the injured neuronal pathway has occurred—either by restoration of the original wiring or recruitment of other nearby wiring as a workaround solution—the inhibition of the ipsolateral brain region returns.

Onoe and colleagues hope that by better understanding this recovery process it will be possible to use PET scanning to follow the progress of spinal cord injury patients during their rehabilitation and predict which ones might be good candidates for recovery. ■

1. Nishimura, Y., Onoe, H., Morichika, Y., Perfiliev, S., Tsukada, H. & Isa, T. Time-dependent central compensatory mechanisms of finger dexterity after spinal cord injury. *Science* **318,** 1150–1155 (2007).

Helping pollen out of its coat

New insights into how pollen matures could also hold the key to strategies for improving agricultural biotechnology

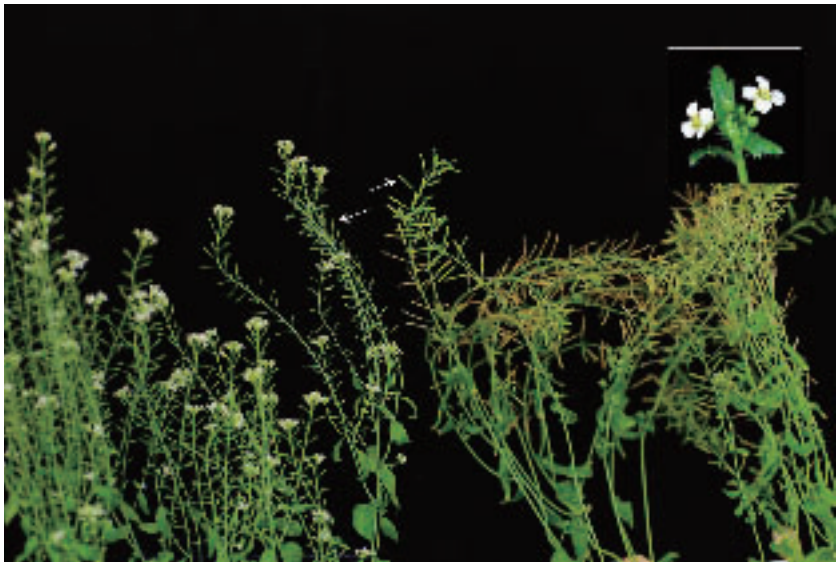


Figure 1: *Arabidopsis thaliana* plants at fruit ripening stage. Plants lacking MS1 (left) do not produce seeds because of their defects in pollen development, while wild-type plants (right) have fruitful seedpods (indicated by arrows). Inset shows open flowers of wild-type plants.

For many plant species, reproduction depends on distribution of pollen grains from 'male' plants to 'female' plants. The outside world can be a hostile place, however, and these grains need protection; this comes in the form of exine, an external matrix that provides the foundation for the outer pollen coat. "Exine is extremely resistant against chemical and microbial degradation," explains Takuya Ito, an investigator at the RIKEN Discovery Research Institute in Tsukuba.

Identifying genes involved in exine formation could prove valuable for biotechnology applications; for example, genetically altered crop strains with built-in male sterility could prevent cross-pollination with naturally occurring strains. One candidate gene encodes the protein Male Sterility1 (MS1). MS1 is believed to act as a transcription factor, a protein that binds directly to genes and activates them—in this case, genes regulating pollen development and exine formation. To confirm this, Ito and colleagues created a 'dominant-negative' version of MS1, in which a protein domain known to act as a repressor of gene activity was tacked onto the end, changing MS1 from an 'on' switch to an 'off' switch. They then tested the effects of this mutant protein in *Arabidopsis thaliana,* a well-characterized plant research model[1].

Plants completely lacking MS1 are male-sterile (Fig. 1), with profound defects in exine formation. Similarly, plants expressing dominant-negative MS1 produced pollen with clear structural defects and exhibited impaired fertility—in some cases, complete male sterility—suggesting that the capacity to activate gene expression is essential to MS1 function.

Ito and colleagues then tried to identify genes regulated by MS1, examining changes in the expression patterns of more than 22,000 genes between wild-type and MS1-deficient plants. They found nearly 100 genes with altered activity, including several potentially involved in the production of a molecule called sporopollenin, a core component of exine. According to Ito, this may represent important progress in understanding the process of pollen development. "The sporopollenin biosynthetic pathway is utterly unknown," he says, "and therefore we are excited about isolating candidate genes."

Ito also points out that with further research, their 'dominant negative' MS1 could offer a useful tool for engineering male sterility in various crops, as his team has identified versions of this protein in a wide range of flowering plants. "We think this method could be applied for agriculture," he concludes, "but our current construct showed weaker effects in petunia, suggesting we need to improve the gene construct for use in other plant species." ■

1. Ito, T., Nagata, N., Yoshiba, Y., Ohme-Takagi, M., Ma, H. & Shinozaki, K. ***Arabidopsis MALE STERILITY1*** encodes a PHD-type transcription factor and regulates pollen and tapetum development. ***Plant Cell*** **19,** 3549–3562 (2007).

How are differences in cell fates generated?

Hitoshi Sawa

Team Leader of the Laboratory for Cell Fate Decision
RIKEN Center for Developmental Biology

The human body comprises about 200 types of cell, all originating from a single fertilized egg. The fertilized egg repeatedly undergoes cell division and produces a variety of cell types, such as nerve cells, epidermal cells, and blood cells. Even though they originate from the same mother cell, different types of daughter cells are fated to have distinct locations and functions in the body. "It is a wonder that this great variety comes from a single fertilized egg. How are differences in cell fates generated? This is what we want to find out," says Hitoshi Sawa, Team Leader of the Laboratory for Cell Fate Decision at the RIKEN Center for Developmental Biology (CDB). At the Laboratory, investigations using nematodes are ongoing to answer the question of cell-fate decision. Their findings will lead to a better understanding of other organisms, and even of the human body.

Why are nematodes used?

Cell-fate decision—an impressive term for the name of a laboratory. "All organisms begin life as a single fertilized egg, from which a variety of cell types are formed by the process of cell division, and each type is destined to have a distinct fate," says Sawa. "Why are such a variety of cell types produced from a single cell? How are the differences in cell fates generated? We want to elucidate this mechanism."

At the Laboratory, investigations using the nematode *Caenorhabditis elegans* are ongoing to explore the mechanism of cell-fate decision. *C. elegans* has a slender body about 1 mm in length (Fig. 1). It takes only three days to develop from birth to the stage where it can lay eggs, and it can be reared at room temperature, feeding on *Escherichia coli*. Because its body is transparent, continuous observations of the process of cell division can be obtained. Additionally, mutants of *C. elegans* artificially produced by gene manipulation can be stored below freezing, and the nematode's total genome has been completely decoded. With these advantages, *C. elegans* is commonly used in life science fields, for example, for research into apoptosis (cell death).

Sawa has another reason for using *C. elegans*. "I use this nematode because its body comprises a small number of cells." He continues to explain that there are only 959 somatic cells in the whole organism. "In addition, its cell lineage is completely known, showing how a single fertilized egg undergoes cell division to form the 959 cells."

It was the UK's Sydney Brenner (2002 Nobel Laureate for Medicine) who clarified the cell lineage of *C. elegans* for the first time in the 1970s. Even now, this nematode remains the only organism whose cell lineage is evident throughout its life, from fertilized egg to adult. "What is the mechanism that determines the fates of individual cells?" asks Sawa. "To this end, *C. elegans* is suitable research material."

Wnt signaling regulates polarity formation

"A variety of cell types are produced from a single fertilized egg. This is wonderful," says Sawa. A cell prior to division is called a mother cell, and the two cells resulting from the first division are called daughter cells. If the mother cell simply undergoes cell division, it only results in an increased number of daughter cells of the same type. "The key resides in the

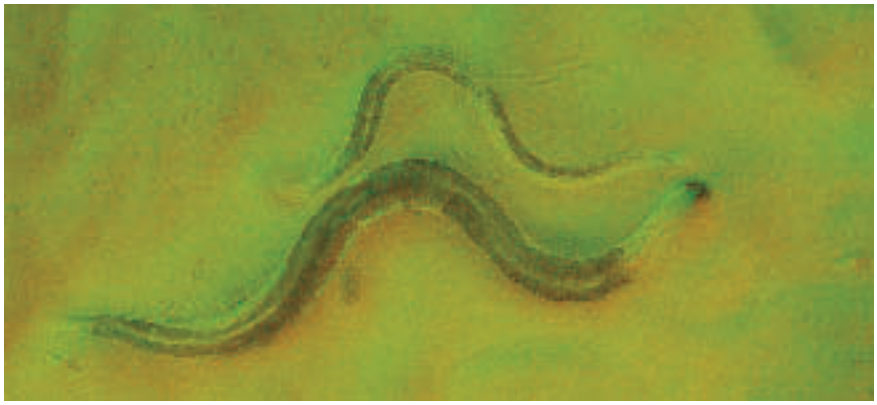


Figure 1 : Nematode *C. elegans*.
A nematode with the scientific name of *Caenorhabditis elegans* is commonly used in life science studies. This organism has a transparent body measuring about 1 mm.

fact that cell division produces distinct cells. In fact, division of a certain cell produces epidermal cells and nervous system cells," Sawa adds. This mode of cell division, which produces two distinct types of daughter cells, is known as asymmetric division. Then why does asymmetric division occur? "We are working to clarify the mechanism underlying this unique process. We have demonstrated that asymmetric division is regulated by Wnt signals produced by the protein Wnt when binding to its receptor on the cell surface."

What is important to the onset of asymmetric division is polarity. Polarity refers to a relative deviation in the material or structure throughout the cell. Division of a homogeneous cell does not produce cells other than those of the same type. By contrast, division of a heterogeneous cell, such as one having an unevenly localized substance, produces distinct types of daughter cells.

"Wnt signaling has long been studied by many authors," says Sawa. "It is known that upon the arrival of Wnt signals, the β catenin protein migrates into the nucleus to activate gene transcription. We found, however, that Wnt signals had a function significantly different from those that have been proposed to date."

Extensive investigations by Sawa and others showed that the arrival of Wnt signals causes β catenin to be localized in the vicinity of the cell membrane (Fig. 2). In addition to β catenin, the protein APC, known as a cancer suppressor gene, is also localized in the vicinity of the cell membrane. "Why does the localization of β catenin and APC occur?" asks Sawa. The details remain unknown. However, he adds that in mutants with abnormal Wnt signaling, neither β catenin nor APC exhibits normal localization. "Because no polarity is formed, cell division produces no more than the same type of daughter cells. This provides strong evidence for the regulation of cell polarity formation by Wnt signals."

"I encountered a big surprise when examining β catenin localization," says Sawa. "When a Wnt signal comes from the posterior side, or the caudal side, β catenin and APC become localized in the vicinity of the cell membrane on the anterior side, or the cephalic side, of the cell." In this case as well, some β catenin migrates to the nucleus when the cell division enters the telophase, because this protein is essentially responsible for regulating gene transcription in the nucleus. Surprisingly, however, nuclear accumulation of β catenin occurs only in one of the two resulting daughter cells. Hence, this accumulation is one-sided. "Then, on which side do you think the nuclear migration of β catenin occurs, the anterior daughter cell or the posterior daughter cell?"

Because of its localization in the vicinity of the cell membrane on the anterior side in the mother cell, β catenin is supposed to migrate to the nucleus of the nearer daughter cell, that is, on the anterior side. "This is a reasonable inference. But the reality is the reverse," says Sawa. "β catenin goes to the nucleus of the posterior daughter cell." (See lowermost image in Fig. 2.)

Sawa says that he was initially puzzled as to how to explain this fact, but he has hypothesized as follows (right panel in Fig. 2). In the anterior daughter cell, the β catenin near the cell membrane seems to prevent other β catenin from going into the nucleus. In the posterior daughter cell, on the other hand, β catenin is able to go into the nucleus without undergoing such suppression, because there is no β catenin already present in the vicinity of the cell membrane. "It is likely that the β catenin near the cell membrane has a different function from that of β catenin at other locations," he adds. Sawa's next goal is to elucidate detailed mechanisms for the

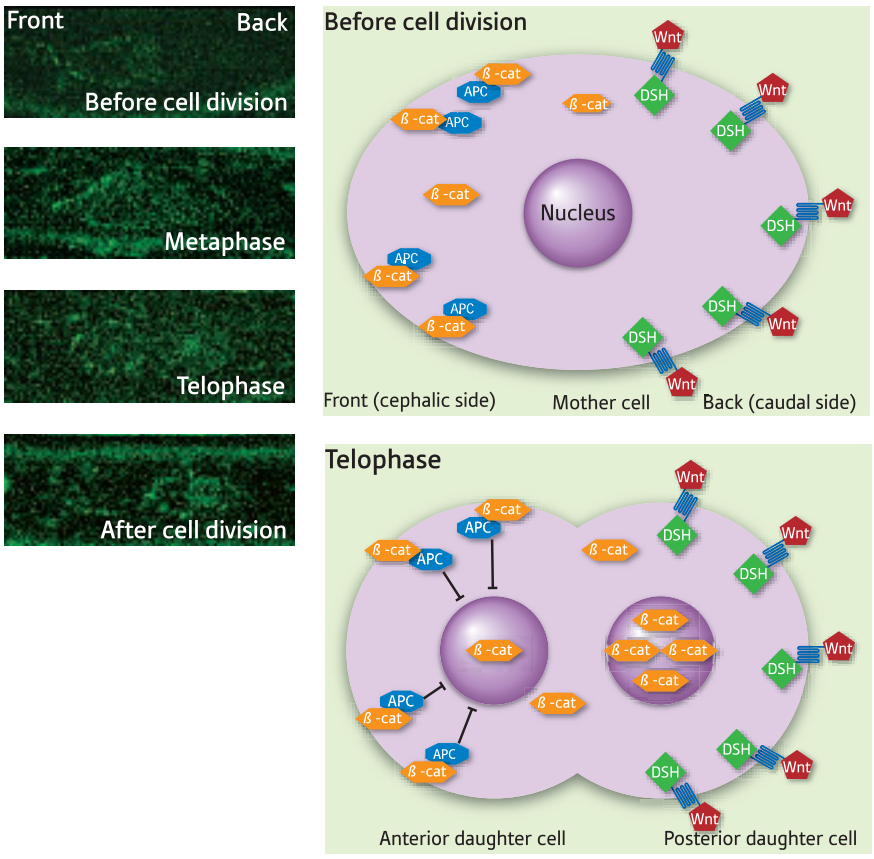


Figure 2 : Photographs and schematic diagrams of β catenin localization by Wnt signaling.
Upon entry of Wnt signals, β catenin (bright green dots in the images on the left) becomes localized in the vicinity of the cell membrane on the anterior side (cephalic side of the individual) of the mother cell. From the telophase to post-division, β catenin accumulates only in the nucleus of the posterior daughter cell (caudal side of the individual). On the other hand, β catenin does not accumulate in the nucleus of the anterior daughter cell. This is probably because the β catenin located in the vicinity of the cell membrane suppresses the nuclear migration of more β catenin.

suppression of the nuclear migration of β catenin by itself.

Complexity in asymmetric cell division

Positional cues to determine the front and back are critical in body formation in organisms. Cell types formed by cell division vary depending on the location of cells in the body. Suspecting the involvement of Wnt signaling in the determination of positional cues, members of the Laboratory for Cell Fate Decision conducted an explicit experiment.

In the tail of *C. elegans* there are cells of a type known as epidermal T cells. These cells receive Wnt signals from the caudal side; the anterior daughter cell becomes an epidermal cell and the posterior daughter cell becomes a nervous system cell (upper panel in Fig. 3). If epidermal T cells are forced to receive Wnt signals from the cephalic side, however, the anterior daughter cell becomes a nervous system cell and the posterior daughter cell becomes an epidermal cell. Hence, the daughter cell types swap between the anterior and posterior sides (lower panel in Fig. 3). "Based on this finding, we demonstrated that Wnt signaling not only regulates the establishment of polarity, but also plays a key role in the determination of positional cues."

In *C. elegans*, almost all events of cell division are accompanied by β catenin localization and polarity establishment owing to Wnt signaling. However, different events of cell division produce distinct cell types. Why is the mother cell capable of producing different cell types with the same signal? The Laboratory for Cell Fated Decision recently showed the involvement of the Hox gene. "Hox, a critical gene for the morphogenesis of organisms, is ubiquitously found in all kinds of organisms, from nematodes to mammals," says Sawa. "We are working on this gene in the belief that it may also be important in determining the mechanism for asymmetric cell division in organisms other than *C. elegans*."

"The mechanism for asymmetric cell division seems to be more complicated than expected. I have been optimistically hoping for a simpler mechanism," Sawa says with a wry smile. His laboratory will proceed by creating or discovering mutants with abnormal asymmetric cell division, exploring genes involved in abnormalities, and elucidating the mechanism for asymmetric cell division. How cells recognize time is another problem to be solved.

Sawa recently became interested in the 'maintenance of cell fates'. "I discovered a mutant whose cells change type during the process of differentiation," he explains. Cell fates once determined must not be left to take their own course. A mechanism for maintaining cell fates must exist. "We are now extensively investigating this mutant for a suspected abnormality in the gene involved in the maintenance of cell fates."

Exploring the human body through *C. elegans*

A researcher specializing in mammalian cells has joined the Laboratory for Cell Fate Decision. Asymmetric cell division is found not only in *C. elegans*, but also in all other organisms. "I cannot believe that *C. elegans* is the only organism showing asymmetric cell division with this complicated system," says Sawa. "I suspect that a similar mechanism may exist in asymmetric cell division in mammals." He expresses concern that although it may be good for a researcher of mammals to undertake this research, such a scientist pays no attention to asymmetric cell division in mammals, thinking 'that's an issue for researchers of nematodes'. "We must take the first step by ourselves," he continues. Both apoptosis and micro-RNA that does not encode any protein were first discovered in *C. elegans*, and

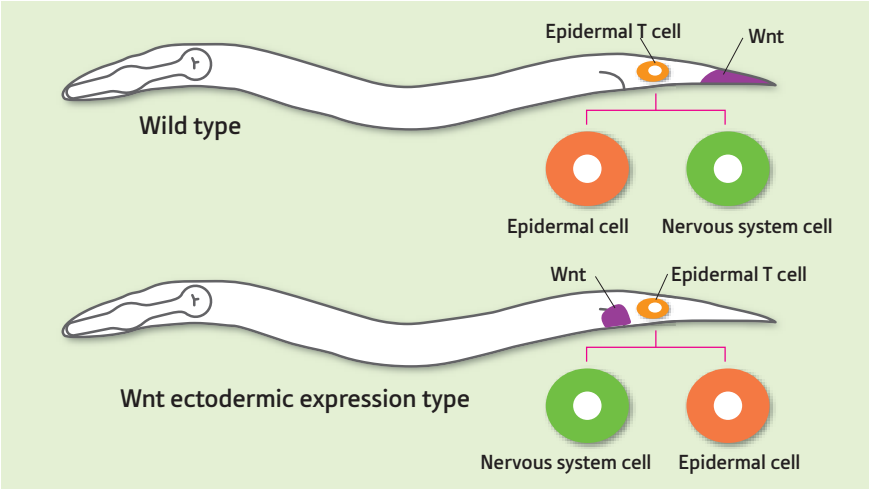


Figure 3 : Positional cue determination by Wnt signaling.
A kind of Wnt is expressed on the posterior side of the epidermal T cell located in the tail of *C. elegans*. If this Wnt signal is forcibly expressed on the anterior side of the epidermal T cell, the resulting daughter cell types would swap between the anterior and posterior sides.

soon the same were found in mammals, and are now being vigorously studied, because Wnt signaling occurs in mammals as well. "I hope that major advances will be achieved in related fields."

"I always bear in mind that investigating nematodes leads to a better understanding of the human body," says Sawa. "A vast amount of information relevant to humans is contained in the tiny body of *C. elegans*, which measures only 1 mm."

For example, Wnt signals are thought to mediate stem-cell division and differentiation. Stem cells produce stem cells per se and differentiated cells by the process of cell division. Although there is the expectation that controlled growth and differentiation of stem cells will lead to applications in regenerative medicine, research achievements remain unsatisfactory. If the mechanism for the control of asymmetric cell division by Wnt signaling is revealed through nematode research, it will be possible to freely control stem-cell growth and differentiation.

Sawa is also expecting his work to find applications in cancer treatment. "A certain cancer develops as a result of Wnt signal activation, which stimulates β catenin to accelerate gene expression, resulting in abnormal growth of cells. Our study has shown that β catenin located in the vicinity of the cell membrane inhibits its own migration to the nucleus. Artificial assembly of β catenin near the cell membrane may provide a new cancer therapy."

Sawa is also focusing on APC, a cancer suppressor gene localized in the vicinity of the cell membrane by Wnt signaling. In nematodes with abnormal APC, epidermal-system stem cells exclusively produce stem cells by cell division (Fig. 4). This process is like carcinogenesis due to the lack of APC. He concludes, "As a researcher of *C. elegans*, I believe that my research into Wnt signaling should lead to regenerative medicine and cancer treatment, and I want to do it."

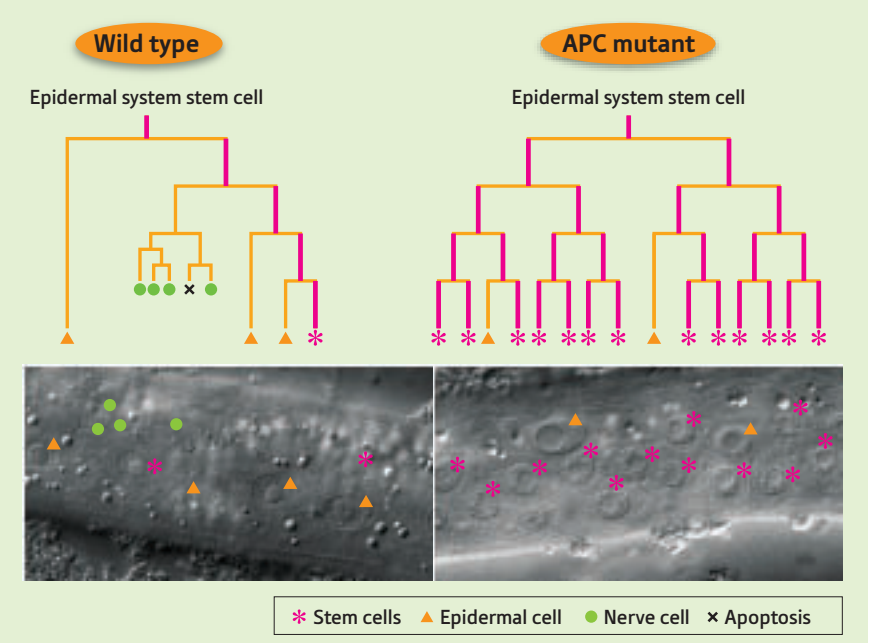


Figure 4 : Abnormal growth of epidermal system stem cells in the APC mutant.
In the normal type of *C. elegans*, the epidermal system cell exhibits asymmetric cell division, with the anterior daughter cell becoming a differentiated cell and the posterior daughter cell becoming a stem cell per se. In *C. elegans* with abnormal APC, both daughter cells become stem cells, which in turn exhibit abnormal proliferation.

1. Novel function of β catenin in asymmetric cell division. *RIKEN CDB Science News* (February 23, 2007).
2. Mechanisms for cell fate determination after asymmetric cell division – Wnt signaling and hox protein regulate the transcription factor PSA-3. *RIKEN CDB Science News* (July 10, 2006).
3. Wnt signals establish cell polarity. *RIKEN CDB Science News* (March 8, 2006).
4. Wnt signals as positional cues for cell polarity: Protein, nucleic acid and enzyme. *Protein, Nucleic acid and Enzyme* (December, 2006).
5. Mizumoto, K. & Sawa, H. Two βs or not two βs: Regulation of asymmetric division by β-catenin. *Trends in Cell Biology* 17, 465-473 (2007).

About the researcher

Hitoshi Sawa was born in Osaka, Japan, in 1962. He graduated from the Faculty of Science, Kyoto University, in 1986, and obtained his PhD in 1991 from the same university. He started his work on *C. elegans* genetics as a postdoc at the Department of Biology, Massachusetts Institute of Technology, in Cambridge, USA. He returned to Japan first as a postdoc at Osaka University, then was appointed as a team leader of the Laboratory for Cell Fate Decision at RIKEN in 2001. His research focuses on the regulation of asymmetric cell division in *C. elegans*.

Sakura Workshop hears about nuclear probes from high-energy

Researchers from around the world gathered on April 1–3 at the main RIKEN campus in Wako to discuss their work using exotic nuclear probes produced by high-energy heavy-ion accelerators.

About 100 people participated in the three-day workshop, including 50 registered participants and about 30–40 other researchers. They heard presentations on the current state of experiments and utilization of the various accelerators. Activities such as collaborations between the research groups were also discussed.

The meeting was held under the auspices of the RIKEN Nishina Accelerator-Based Research Center, and the presentations covered the status of research projects using beams of exotic nuclei produced at high-energy heavy-ion accelerators such as ISOLDE (the On-Line Isotope Mass Separator, located at CERN, in Switzerland), GANIL (Grand Accelerateur National d'Ions Lourds, in France) and RIKEN for materials and life science studies. Special emphasis was placed on recent developments in the production of new probes and the creation of new experimental methodologies.

In particular, Yoshio Kobayashi presented results of experiments using radioactive-ion beams from the RIKEN Radioactive-Ion Beam Factory to investigate the high-purity conversion of special silicon semiconducting materials, and on Mossbaum spectroscopy, a cutting-edge measurement technology. Guido Langouche, from the University of Leuven in Belgium, gave a presentation on 'Hyperfine Interactions: Historical reflections'.

The Sakura Workshop's organizers considered the event to be a great success, and a first step toward the creation of a forum for the next generation of researchers working on materials and life science studies using accelerator facilities around the world. Its success inspired them to try to make the workshop an annual event.

Cress in space! Researchers study plant growth under zero gravity

A unique experiment from RIKEN and Osaka City University flew into orbit aboard a space shuttle in March—a study to elucidate the effects of weightlessness on the growth of cell walls in plants.

Researchers from the RIKEN Plant Science Center put together the experiment, in which the mutant strain *hmg1* of *Arabidopsis thaliana,* or thale cress, a plant commonly used in genetic research, was taken to the International Space Station aboard the space shuttle Endeavour.

It is well known that when animals, including humans, spend extended periods in weightlessness they develop various health problems such as brittle bones. Instead of bones, plant cells are covered by a hard cell wall that counters gravity, allowing the plant to keep growing. Under excess gravity this wall becomes harder, and the cell wall of the *hmg1* mutant, even under normal Earth gravity, becomes harder than that of the wild-type plant.

The *hmg1* mutant is lacking *HMG1,* a gene related to sterol biosynthesis. This mutant shows pleiotropic phenotypes, such as dwarfing, early aging, and male sterility. The researchers had already studied the influence of gravity on growth of the plant, and discovered that in gravity greater than the Earth's, expression of the *HMG1* gene is induced. These findings inspired the zero-gravity research.

The wild type and the *hmg1* mutant were taken into space and raised under both zero-gravity and artificial gravity equal to that on Earth. Their growth is being monitored by video and, when they reach about 10 cm in height, samples will be brought back to Earth and their chemical structure and physical properties, such as cell-wall hardness and elasticity, analyzed.

In the future, growing plants in space may become desirable, and this experiment could be the first step in the development of 'space agriculture'. If the cell wall of the *hmg1* mutant maintains its hardness without the influence of gravity, this may help develop crops that maintain their shape in the weightless zero gravity of outer space.

RIKEN and Hanyang University form tie-up toward Asian Research Network

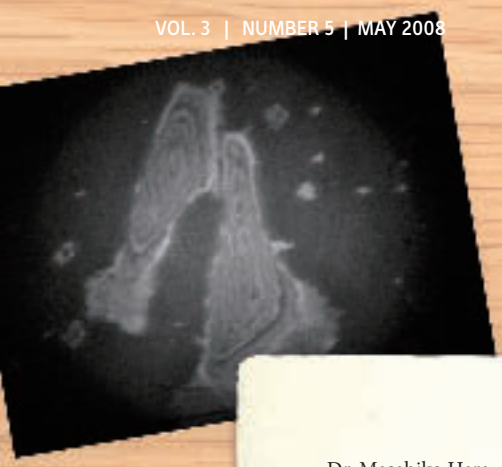
RIKEN and Hanyang University have formed a tie-up to promote research collaboration. Under the agreement, signed April 10, RIKEN will help establish a research base in South Korea, the 'RIKEN-Hanyang University Partnership and Fusion Tech Center (FTC)', at the Hanyang University campus in Seoul. The move is one step forward in a plan to build an Asian Research Network (ARN), which promotes collaborative studies and personnel exchanges to help resolve common problems in Asia.

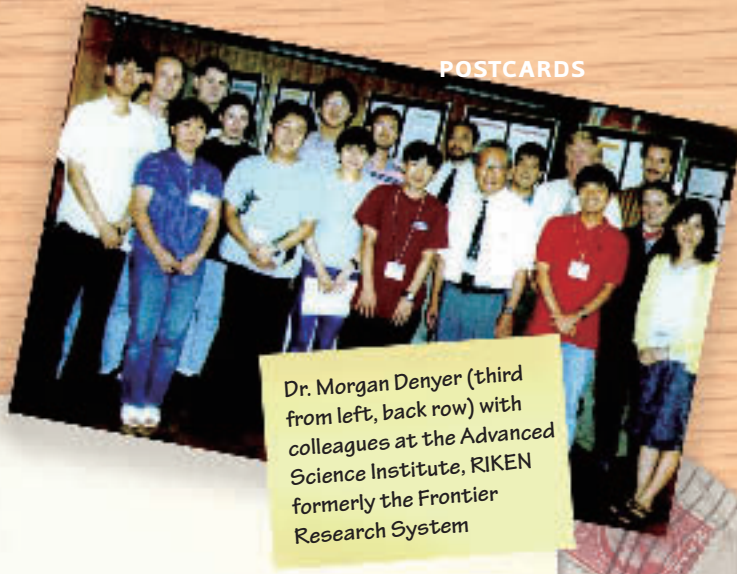
This is not the first collaboration between the two institutions. At RIKEN, Masahiko Hara and others from global collaboration research group, who have been working with Haiwon Lee of Hanyang University, have done research on materials important to the development of nano-devices for the last 20 years. They have also undertaken collaborative studies aimed at 'post-nanotechnology', including the development of new information processing methods using functional materials.

The FTC will be built in South Korea at Hanyang University as a joint base for research in the fields of nano- and biotechnology, and cooperation with Hanyang University is being strongly promoted. The FTC in Seoul will become one of the main research laboratories for ARN.

Hara and Lee have been undertaking a feasibility study for the project, including holding symposiums and talent exchanges, which they began in April 2005.

The agreement also promotes a new system of personnel and information interchange, in addition to research collaboration, between the two institutions. This will help nurture the next generation of Asian researchers and provide an alternative to the usual practice of young scientists going to Europe or America for international experience. This has resulted in a kind of 'brain drain', which it is hoped the new program will help alleviate.





Dr. Morgan Denyer (third from left, back row) with colleagues at the Advanced Science Institute, RIKEN formerly the Frontier Research System

Dr. Masahiko Hara
Laboratory Head
Emergent Functions Asian Collaboration Laboratory
Advanced Science Institute, RIKEN
Wako-city, Saitama, Japan.

Dear Dr Hara,

It is some 11 years since I was fortunate enough to acquire an STA Research Fellowship to join you and Dr Wolfgang Knoll, the present director at Max-Planck-Institut für Polymerforschung, at the Exotic Nano Materials group at RIKEN in Wako and begin the journey to successfully image the cell surface interface. The group, consisting of engineers, chemists and physicists, was focused on surface chemistry, its analyses and imaging. I felt I was an odd addition as I was an electrophysiologist and cell biologist.

My work in 1997 involved collaborating with Andreas Offenhaeusser, Christoph Sprössler and Martin Scholl at the Max Planck Institute for Polymer Research (MPIP) in Mainz, Germany, and led to the development of the so-called point contact model, which describes the electrophysiological interface between electrogenic cells and extracellular recording devices. This model led us to understand that cell surface separation is one of the most important parameters at the cell electrode interface. Therefore, it was important to gain a better understanding of how cells attach to a surface.

Ken Nakajima and Ruggero Micheletto from RIKEN and I began examining novel means of imaging the cell surface interface and became the first to use a scanning near-field optical microscope (SNOM) to image live cells in a fluidic environment. However, my time at RIKEN expired in 1999 so I returned to the UK as a lecturer at Bradford University.

Fortunately, we were able to continue the work as an international collaboration between RIKEN and Bradford and Kyoto Universities with funding assistance from the Royal Society. We moved on from SNOM to surface plasmon microscope systems, which Dr Knoll first developed, as they allow imaging of binding events between molecular species. To overcome poor lateral resolution that prevented interfacial biological imaging, a group headed by Mike Somekh of Nottingham University and I developed an entirely new microscope—the widefield surface plasmon microscope.

We have already demonstrated that this system can be used to image antibody/antigen interactions in real time at lateral resolutions of a micron. Now, we are imaging changes at the cell surface interface induced by known cytokines.

While the journey has been long, reaching our goal of imaging the cell surface interface was made possible because a young biologist joined a team of young physical scientists. Gaining an understanding of other technical disciplines enriched my colleagues and me and allowed us to appreciate that advances in science require coordinated inputs from the physical, chemical and biological sciences. This has informed and shaped the rest of our scientific careers.

With best regards,

Morgan Denyer
School of Pharmacy
University of Bradford
Bradford
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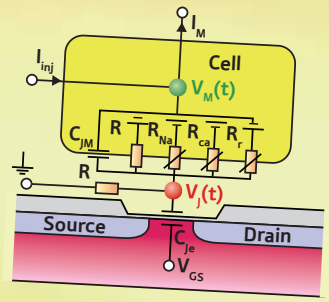
Point contact model



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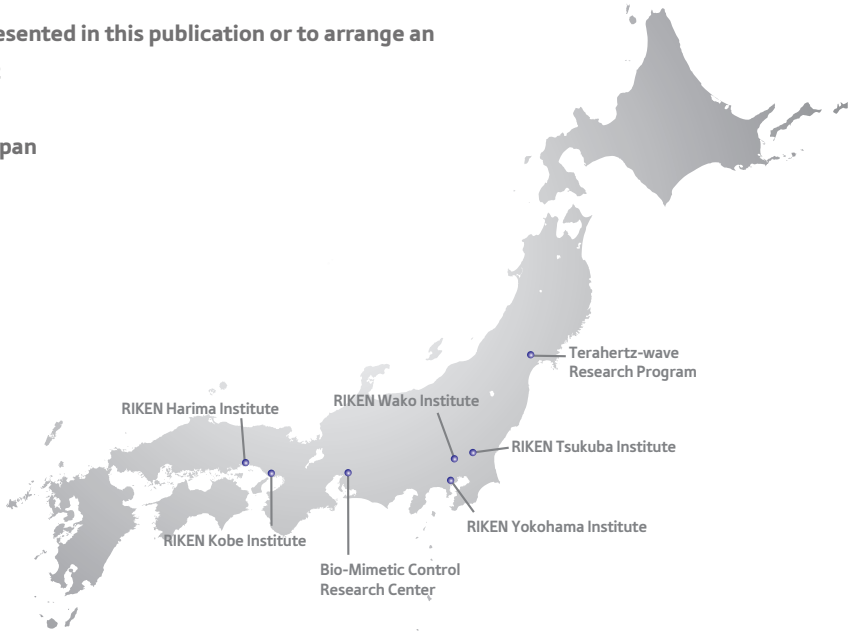

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