RIKEN RESEARCH

MAY
2010 Volume 5 Number 5

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Moving beyond the genome to fight cancer

The first total synthesis of the complex natural product chaetocin expands the tools to reverse lethal gene expressions

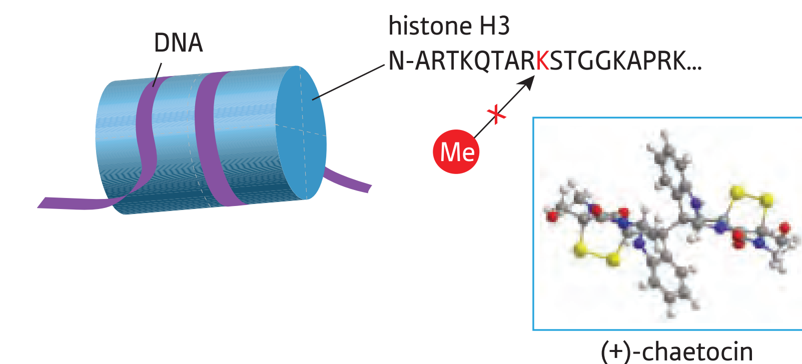

Figure 1: In the epigenome, strands of DNA are wrapped around spool-like histone proteins. The natural product chaetocin inhibits the methylation (Me) of histones in the floppy tail of the histone (shown as a string of letters), allowing this small molecule to control gene expression.

Unlocking our genetic blueprint is well underway with the sequencing of the human genome, but a secondary layer of structure on the genome that affects gene expression, the 'epigenome,' remains largely unmapped. The packing structure of the epigenome can be altered by chemically modifying histones, spool-like proteins around which DNA strands are wrapped within our cells. Histones physically control access to genes, and adding small functional groups such as acetyl or methyl units to them can selectively switch certain genes on and off.

Recent developments in methods that can controllably influence these DNA architectures have focused on the methylation of histone proteins. Now, a research team led by Mikiko Sodeoka from the RIKEN Advanced Science Institute in Wako, Japan, has produced the first total synthesis of chaetocin[1], a natural product that inhibits the activity of histone methyltransferases—enzymes that play critical roles in gene expression (Fig. 1). The results of this work could enable new therapeutics for destructive diseases such as cancer.

A 'tail' of influence

Histones contain floppy 'tail' regions, terminated by an amino acid with a free amine group, that extend from the body of the protein. These tails can influence the epigenome structure and serve as extremely active sites for chemical modification. Histone methyltransferase enzymes catalyze the addition of methyl units to lysine and arginine amino acid side chains in this tail, forming strong bonds in the process. This reaction does not change the genetic code of the protein, but radically influences transcription processes—giving histone methylation an influential role in inherited gene expression patterns.

Normally, the levels of histone methylation are delicately balanced within our cells. However, dysfunction of histone methyltransferases can alter the epigenome and lead to abnormalities—notably, the loss of expression of tumor-suppressing genes. Therapies that can selectively control the activity of these enzymes hold great potential for new cancer therapeutics without the dangerous side effects of chemotherapy.

Natural guides

The number of chemicals that can modulate histone methyltransferase enzymes is limited. According to team-member Yoshitaka Hamashima, also from RIKEN, only a few compounds that can selectively inhibit these enzymes have been reported to date. He says, "it is only chaetocin that comes from natural sources."

Chaetocin is a natural alkaloid produced by *Chaetomium minutum*, a form of wood mold. The complex and elegantly symmetric structure of this molecule features eight rings and several functional groups, most notably a pair of disulfide bridges attached to two terminal rings. Chaetocin has been extensively investigated for its antibacterial behavior and ability to suppress cell growth, and has the potential to play an important role in modifying the epigenome.

Several research groups have produced related analogues of chaetocin, but the total synthesis of this molecule has eluded organic chemists since its discovery forty years ago—setting up a significant test to the synthetic skills of Sodeoka and her team. "The fact that no one had succeeded in the total synthesis after its isolation in 1970 drove us to embark on this formidable challenge," says Hamashima.

Risk and reward

The road toward a total synthesis of chaetocin, however, hit a few dead ends. The team's first attempt, based on a proposed biosynthetic route, failed when a reaction involving radical bromine atoms caused the cyclic structure to decompose. Hamashima says that this unsuccessful result drove development of an unexplored synthetic strategy: coupling of two simple amino acids, serine and tryptophan, into a four-ringed

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Mikiko Sodeoka

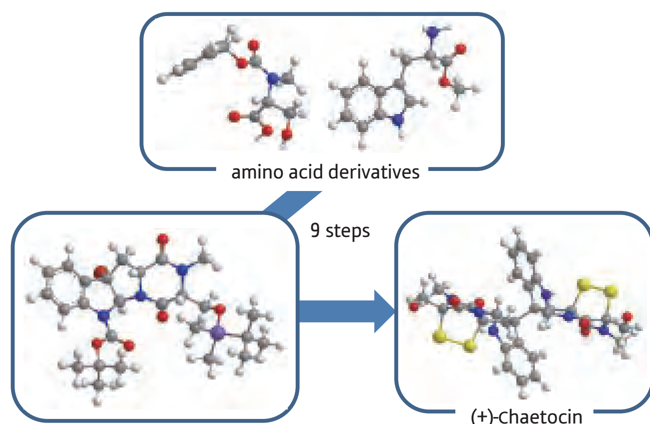


Figure 2: Beginning with the amino acid derivatives, it is now possible to totally synthesize the natural product chaetocin through a highly efficient nine-step reaction.

intermediate complex. Thanks to several mechanistic studies, the team generated, in good yields, an octacyclic precursor with the correct three-dimensional structure by successfully completing a radical bromine reaction (Fig. 2).

The final part of the reaction—construction of the disulfide bridges—involved some risky chemistry, Hamashima notes. "In our initial plan, we expected that the double-decker structure of chaetocin might control the approach of hydrogen sulfide from the outer side. But nobody was convinced that it would work well." The team was extremely gratified when the final step in the reaction, which involved ten bond-forming and -cleaving events, generated chaetocin with the correct geometrical structure.

Overall, the team's method demonstrates a highly efficient way to produce chaetocin, because the total synthesis required only nine chemical transformations.

Bridging differences

With the chemical synthesis of chaetocin complete, Sodeoka and colleagues prepared various analogues of the molecule—two optical isomers of chaetocin, and a version missing the disulfide bridges. The latter allowed them to examine the structure–activity relationship between this natural product and a particular histone methyltransferase enzyme called G9a, in collaboration with Minoru Yoshida's group also from RIKEN Advanced Science Institute. Although both chaetocin isomers showed strong inhibitory activity, the molecule without the sulfur bridges was inactive—demonstrating the critical role of this functionality.

Hamashima says that the target enzyme has a domain, close to chaetocin's binding site, which is full of amino acids called cysteines. Cysteines have a thiol (-SH) side chain that may be able to form transitory bonds with the critical disulfide bridges of chaetocin. "While the exact mechanism is still unclear," he says, "we speculate that such chemical bond formations are responsible for the inhibition of G9a."

The researchers believe that further studies into the molecular mechanisms of chaetocin should deliver a new generation of enzyme-specific pharmaceuticals that can control gene expression patterns—an important step in the treatment of cancerous diseases. "Contributing to human health by creating new drugs is our goal," says Hamashima. "In the future, the day will come when we can wake up silent genes in cells at will by simply adding chemical modulators." ■

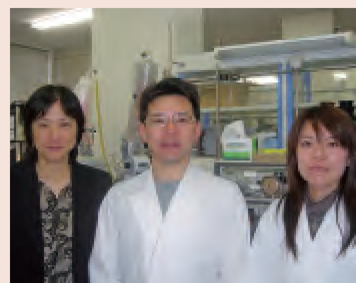
1. Iwasa, E., Hamashima, Y., Fujishiro, S., Higuchi, E., Ito, A., Yoshida, M. & Sodeoka, M. Total synthesis of (+)-chaetocin and its analogues: Their histone methyltransferase G9a inhibitory activity. *Journal of the American Chemical Society* **132**, 4078–4079 (2010).

About the researchers

Mikiko Sodeoka received her BS and MS degrees from Chiba University and was awarded a PhD in pharmaceutical sciences from the same institution in 1989. She worked at the Sagami Chemical Research Center from 1983 to 1986, after which she joined the Faculty of Pharmaceutical Sciences at Hokkaido University as a research associate. She spent time as a postdoctoral fellow at Harvard University, USA, and then joined The University of Tokyo in 1992. In 1996, she became a group leader at the Sagami Chemical Research Center, and later became an associate professor at the University of Tokyo in 1999. In 2000, she joined Tohoku University as a full professor, and since 2004, she has been chief scientist of the Synthetic Organic Chemistry Laboratory at RIKEN. In 2008, she was also appointed as research director of the Sodeoka Live Cell Chemistry ERATO project.

Yoshitaka Hamashima received his BS and MS degrees from The University of Tokyo and was awarded his PhD in 2003 from the same institution. In 2001, he joined Tohoku University as an assistant professor, and was promoted to lecturer in 2005. He is now a senior researcher at RIKEN. He received the Meiji Seika Award in Synthetic Organic Chemistry in 2003, the Thieme Journal Award in 2006, and the Pharmaceutical Society of Japan Award for Young Scientists in 2006. His current research interests include catalytic reactions, organometallic chemistry and bioactive compound synthesis.

Eriko Iwasa graduated from the Faculty of Engineering, Seikei University, and obtained her master degree at Tokyo Gakugei University. In 2007, she entered a doctoral course at Saitama University and joined RIKEN's Junior Research Associate Program. In 2010, she became a research assistant of the Sodeoka Live Cell Chemistry ERATO project.



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Electrons spiral to a new future

Electrons that carry orbital angular momentum are generated for the first time

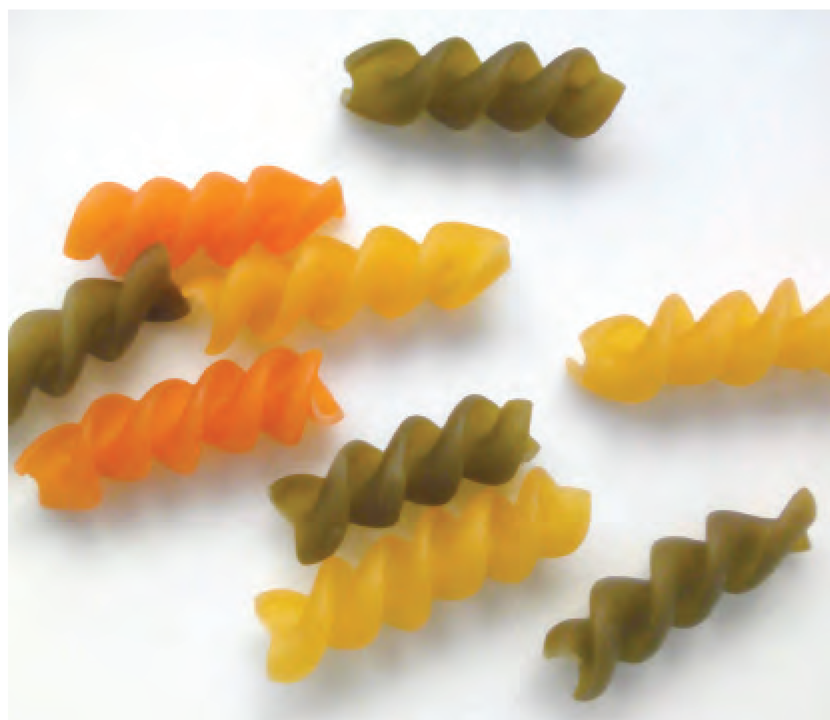

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Figure 1: Electron waves that spiral like fusilli pasta are a sign of electrons with non-zero orbital angular momentum.

A new physical quantity of electrons—orbital angular momentum—has been generated by Masaya Uchida and Akira Tonomura at the RIKEN Advanced Science Institute in Wako, Japan. The work, published in *Nature*[1], could establish novel fields of research and lead to new electron microscopes.

"The ability of optical waves to spiral about their axis as they propagate, which can be described as corkscrew wavefronts, has already found a wide range of applications" explains Uchida.

A wave can be characterized by the shape of its wavefronts: imaginary surfaces that connect all points where the wave is at the same stage in its oscillatory cycle. In a conventional plane wave, these fronts are a series of flat surfaces oriented perpendicular to the direction of propagation.

Some optical wavefronts are shaped like fusilli pasta (Fig. 1); the wavefronts rotate around a central axis and therefore have momentum, orbital angular momentum to be specific, which is associated with the wavefront's shape and pitch.

Since electron waves act like optical waves, Uchida thought that spiraling electron waves were possible. The researchers had to resolve a daunting technological challenge to generate the electrons with orbital angular momentum. A corkscrew wavefront is imprinted on an electron plane wave when it passes through a three-dimensional (3D) structure shaped into a single twist of the desired spiral. But since the height of the twist—determined by the wavelength of electron wave—is less than 100 nanometers, creating such a spiraling nanostructure is difficult.

The researchers simplified this problem by approximating the spiraling structure to several linear steps like a spiral 'staircase'. They crushed the graphite from a pencil into thin films and placed them onto a carbon-coated copper grid. These fragments formed stacked layers resembling a spiral staircase.

To prove that the electrons gained orbital angular momentum as they passed through this simple 3D nanostructure, Uchida and Tonomura mixed the output wave with a second plane wave. They observed the characteristic 'Y'-shaped defect to the parallel-lines pattern that is expected when two plane waves interfere. Measuring the transfer of momentum from the electrons to matter, however, could be a more direct way of identifying spiraling electron waves in the future, the researchers note.

"The next stage of the research is to produce wavefronts with various structure types," says Uchida. "Just as there are many types of pasta, so there are many shapes of electron wave." ■

1. Uchida, M. & Tonomura, A. Generation of electron beams carrying orbital angular momentum. *Nature* **464**, 737–739 (2010).

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Lost in a crowd

Introducing additional complexity to a simulation gives researchers better insight into how cellular signaling networks might operate

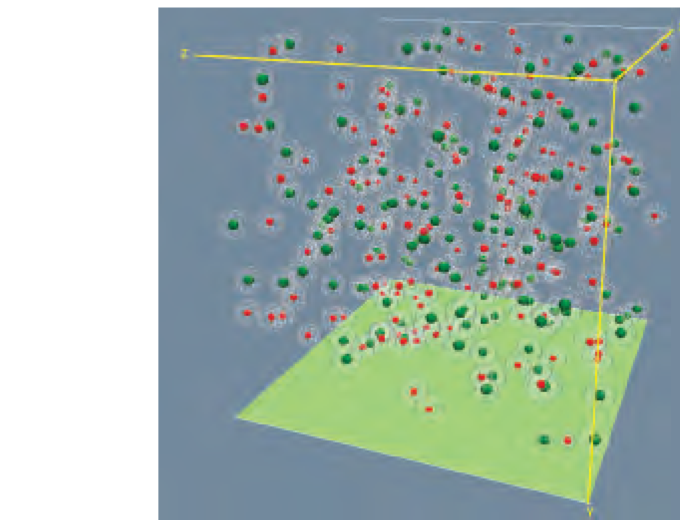

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Figure 1: A three-dimensional representation of simulated associations of different molecules of a cell, based on the enhanced GFRD algorithm.

One of the biggest challenges in simulating biological processes is developing mathematical models that accurately reflect the dynamics of real molecules. Koichi Takahashi, of the RIKEN Advanced Science Institute in Yokohama, is in a position to know—as a founding member of the E-Cell Project, he and his colleagues have spent the last 14 years working towards development of a comprehensive simulation of a whole functioning cell.

Takahashi's work has focused on dynamic behavior of populations of signaling factors. Such analyses are typically based on 'mean-field' descriptions, which assume an essentially averaged distribution of molecules throughout the cellular volume. This shortcut overlooks a lot of real-world complexity, but is made necessary by the excessive computational demands of more accurate models.

Things have now changed, thanks to a breakthrough from Pieter Rein ten Wolde at AMOLF in the Netherlands, whose team developed an algorithm called Green's Function Reaction Dynamics (GFRD) that enables sophisticated single-particle-level simulations with considerably reduced computing power[1]. Together, Takahashi and ten Wolde developed a faster and more accurate version of GFRD (Fig. 1), and applied it to mitogen-activated protein kinase (MAPK) cascades, a type of signaling pathway associated with diverse functions in the eukaryotic cell[2].

MAPK signaling is a multi-stage process; at each step, one kinase enzyme activates another, downstream kinase through two sequential chemical modifications. Predictions from mean-field-based simulations of MAPK dynamics have depended heavily on whether the enzyme is assumed to act 'processively', introducing both modifications without releasing the substrate, or 'distributively', releasing the substrate after each modification.

However, Takahashi and ten Wolde found strikingly different results with their model, which introduces the possibility of re-binding—a scenario in which substrates receive their second modification from the same enzyme that introduced the first. For example, slow diffusion of enzymes and substrate within the cytosol could lead to much more rapid overall activation kinetics by keeping substrates within easy reach of their upstream kinases. "'Walking slower' can speed up the response of the system," says Takahashi. This is the opposite of mean-field model predictions, and essentially erases the distinction between distributive and processive in this context.

These data demonstrate the importance of increasing complexity in models, a direction in which the authors are continuing to move. "We are extending our method so that we can also include structures such as membranes and organelles," says Takahashi. "We are also working to make the method even more high-performance, because that will enable us to represent intracellular molecular crowding." ■

1. van Zon, J.S. & ten Wolde, P.R. Simulating biochemical networks at the particle level and in time and space: Green's function reaction dynamics. *Physical Review Letters* **94**, 128103 (2005).
2. Takahashi, K., Tănase-Nicola, S. & ten Wolde, P.R. Spatio-temporal correlations can drastically change the response of a MAPK pathway. *Proceedings of the National Academy of Sciences USA* **107**, 2473–2478 (2010).

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Sweet surprises

By characterizing the sugar content of cells, researchers have begun to reconstruct important 'quality control' mechanisms for protein production

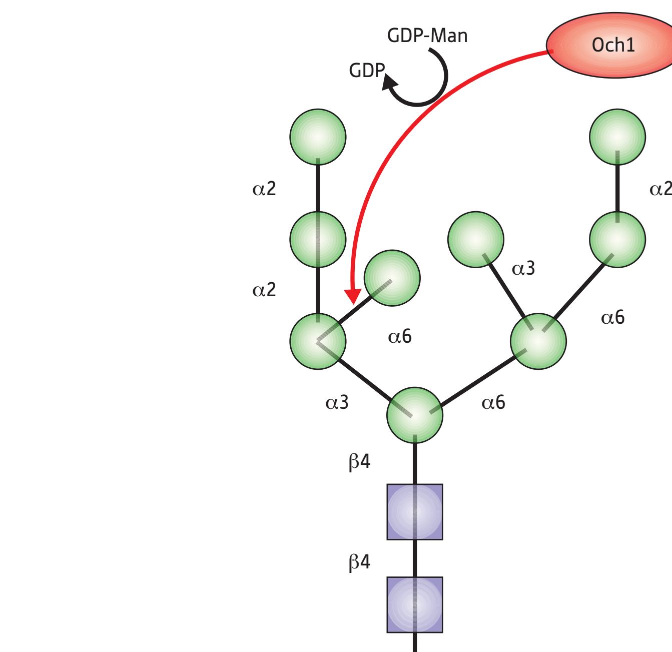

Figure 1: Schematic of a misfolded protein decorated with a diverse array of *N*-glycosylations prior to degradation in the ERAD pathway. Among these is a mannose carbohydrate introduced by the Golgi enzyme Och1, which might play a role in a novel late-stage protein 'quality control' system.

Many proteins undergo *N*-glycosylation, in which they are decorated with combinations of carbohydrates. These modifications not only contribute directly to normal protein function but also act as a flag for defective proteins, which get steered into a pathway known as endoplasmic reticulum-associated degradation (ERAD) with the assistance of enzymes that remove glycosylations to release free oligosaccharides (fOSs).

As an undergraduate, Tadashi Suzuki discovered the enzyme peptide:*N*-glycanase (PNGase) in the cytosol of mammalian cells; now, as a team leader at the RIKEN Advanced Science Institute, Wako, his group has uncovered valuable details about this enzyme's critical contribution to the early stages of ERAD.

Suzuki and postdoctoral researcher Hiroto Hirayama recently turned to brewer's yeast, a popular model organism, as a means to study an enigmatic PNGase-independent pathway for fOS production initially identified in mammalian cells. To achieve this, they developed an approach to selectively isolate these molecules, combining chemical labeling of oligosaccharides with a method for eliminating background contamination from β-1,6-glucans, a component of the yeast cell wall[1].

This strategy yielded a full library of yeast cytosolic fOSs—and some unexpected results. "To our surprise, we only detected PNGase-dependent fOSs under our experimental conditions," says Suzuki. "This clearly indicates that mechanisms for generation of fOSs are quite distinct between mammals and yeast."

To ensure that the full range of fOS diversity was represented, they performed their analysis in yeast lacking expression of the cytosol/vacuolar α-mannosidase (Ams1p), the only enzyme known to break down fOSs. "Very sophisticated and complicated glycan-recognition mechanisms for ERAD have been uncovered, but these conclusions have been drawn using a few model proteins," says Suzuki. "On the other hand, we analyzed the whole population of fOSs, which means we can get information about glycan structures for all ERAD substrates."

These data revealed that misfolded proteins can undergo diverse modifications prior to ERAD, including glycosylation by an enzyme within the Golgi apparatus (Fig. 1), a cellular structure in which proteins typically undergo their final modifications. This suggests the existence of a previously unidentified screening mechanism at this late stage in protein synthesis that selectively redirects misfolded molecules to the ERAD system.

These and other findings suggest a great deal of hidden complexity remaining to be uncovered, and Suzuki's team is now analyzing yeast strains with mutations in various proteins that help 'read' and interpret protein glycosylations. "Hopefully, through comparative fOS analysis for these strains, we can provide more precise mechanisms for the role of *N*-glycans in ERAD," he says. ■

1. Hirayama, H., Seino, J., Kitajima, T., Jigami, Y. & Suzuki, T. Free oligosaccharides to monitor glycoprotein endoplasmic reticulum-associated degradation in Saccharomyces cerevisiae. *The Journal of Biological Chemistry* **285**, 12390–12404 (2010).

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Eyes wide shut

Different cell types in the visual cortex respond differently to changes in visual experience

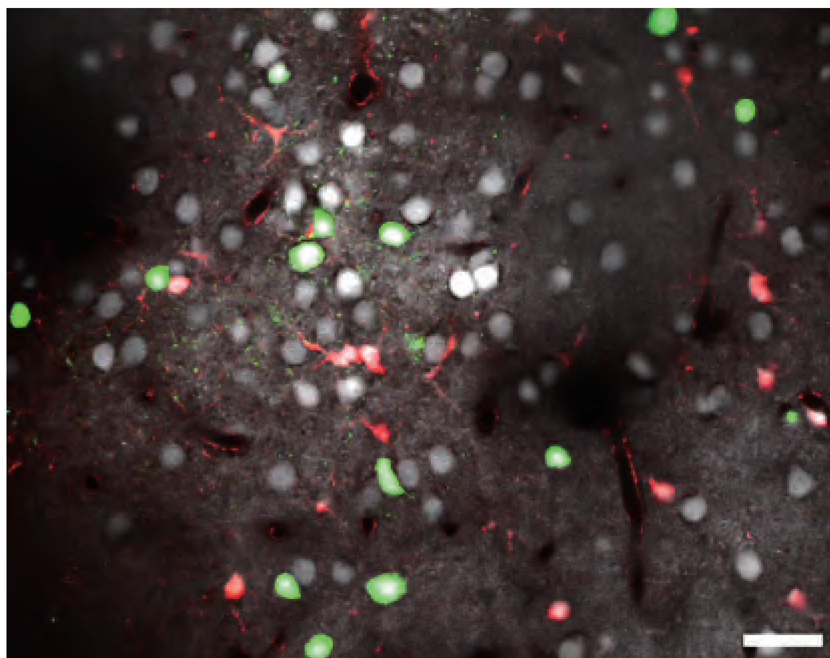

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Figure 1: A two-photon calcium image of the visual cortex of a mouse, at a depth of 120 μm from the cortical surface. Inhibitory interneurons are shown in green and excitatory neurons are shown in white; red cells are astrocytes (scale bar, 30 μm).

In the primary visual cortex of the brain, neurons are organized into alternating columns that receive inputs from either the left or right eye. This organization is strongly dependent on early visual experience. When one eye is deprived of visual inputs during a critical developmental period, the corresponding columns fail to develop properly, whereas those receiving inputs from the unaffected eye grow larger than normal.

The cortex consists primarily of two different types of neuron: excitatory neurons that synthesize and release the neurotransmitter glutamate, and inhibitory interneurons which use the transmitter γ-aminobutyric acid (GABA). How each of these cell types contributes to experience-dependent changes in the visual cortex is, however, unknown.

To investigate this, Tadaharu Tsumoto of the RIKEN Brain Science Institute, Wako, and his colleagues injected a calcium-sensitive dye, Fura-2, into the visual cortex of genetically engineered mice whose inhibitory interneurons express a fluorescent protein called Venus[1]. The intensity of Fura-2 fluorescence changes in response to the increase in calcium ion concentration that is characteristic of neuronal activity.

This approach enabled the researchers to both identify the interneurons in the visual cortex and monitor their activity (Fig. 1). In animals reared normally, they first identified the 'binocular' regions of the primary visual cortex by visually stimulating each eye in turn, and using two-photon laser-scanning microscopy to locate the cells that responded to both. This revealed that inhibitory interneurons are more responsive to inputs from both eyes than excitatory neurons.

The responses in mice deprived of visual inputs to one eye for two days during the critical period were then examined. The change in the responses of both cell types was found to be similar—both had become more responsive to inputs from the open eye.

When mice were deprived of visual inputs to one eye after the critical period, however, the effect observed was far stronger on the inhibitory interneurons. They tended to receive inputs from the open eye, and their responses to inputs from the closed eye were also depressed, whereas those of the excitatory neurons remained almost stable. The interneurons normally act to inhibit the excitatory neurons, so their depressed responses may contribute to the stability of excitatory neuron responses to the deprived eye.

"Inhibitory interneurons are divided into several subtypes," says Tsumoto. So, the next step is to determine which particular subtypes are involved in maintaining plasticity after the critical period. ■

1. Kameyama, K., Sohya, K., Ebina, T., Fukuda, A., Yanagawa, Y. & Tsumoto, T. Difference in binocularity and ocular dominance plasticity between GABAergic and excitatory cortical neurons. *Journal of Neuroscience* **30**, 1551–1559 (2010).

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Deactivating the alarm

Therapeutic strategies that block inflammatory response to islet cell transplantation may accelerate treatment of diabetic patients

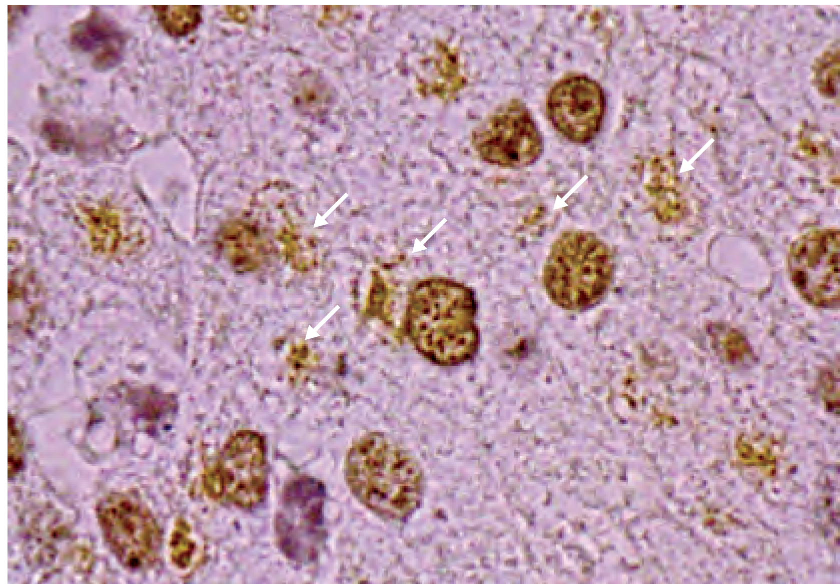

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Figure 1: Transplanted islet cells express HMGB1 in the nucleus (brown), as observed under normal physiological conditions, but also secrete it into the extracellular environment (white arrows).

For patients with insulin-dependent diabetes mellitus, the only route to full recovery without daily injections of insulin is by transplantation of pancreatic islet cells. This can be achieved non-surgically via injection of donor cells into the liver, but such treatment also elicits a vigorous negative response from the body.

"Transplantation tolerance can be controlled by immunosuppressive drugs such as FK506," says Masaru Taniguchi of the RIKEN Research Center for Allergy and Immunology in Yokohama. "However, transplanted islets are rejected soon after transplantation even with the use of FK506." The mechanism for this rejection is unknown, and patients must typically receive several injections from multiple donors for transplantation to succeed.

High-mobility group box 1 (HMGB1) was first identified as a DNA-binding factor in the cell nucleus, but it is also secreted by immune cells as an apparent trigger for inflammation in response to tissue damage. Taniguchi's team recently joined forces with Yohichi Yasunami at Fukuoka University to demonstrate the impact of this protein on transplanted islet survival[1].

They produced diabetic mice by treating the animals with an islet-killing drug, and then transplanted varying numbers of donor islet cells. Animals receiving 200 cells normally developed diabetic symptoms, but these could be averted by simultaneous treatment with HMGB1-blocking antibodies. This treatment also prevented accumulation of immune cells in the liver and countered the production of inflammatory cytokines—typical outcomes of islet transplantation.

The researchers were surprised to note that HMGB1 expression was highly specific to islet cells, with protein levels 20-fold higher than any other organ or tissue examined, further supporting its particular role in islet rejection. In fact, they noted two strong peaks in plasma levels of HMGB1 in transplant recipients: one 24 hours after chemical destruction of islets, and another 6 hours after islet injection. These results suggest that islet stress or damage directly triggers HMGB1 secretion (Fig. 1), which in turn activates the inflammatory response pathways that initiate destruction of the transplanted cells.

These findings provide strong hope for improving transplant efficiency. "We can use antibodies in humans without any side effects, because HMGB1 is not present in the serum under physiological conditions," says Taniguchi, who adds that Yasunami's team is now exploring clinical strategies based on this approach. However, Taniguchi also hopes to develop chemical inhibitors that preemptively block HMGB1 secretion by donor cells prior to transplantation. "This is ideal," he says, "because then we do not need to treat patients with any drugs or antibodies." ■

1. Matsuoka, N., Itoh, T., Watarai, H., Sekine-Kondo, E., Nagata, N., Okamoto, K., Mera, T., Yamamoto, H., Yamada, S., Maruyama, I. *et al.* High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice. *The Journal of Clinical Investigation* **120**, 735–743 (2010).

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Bringing leukemia out of hiding

Two new strategies target a deadly cancer that eludes conventional chemotherapy

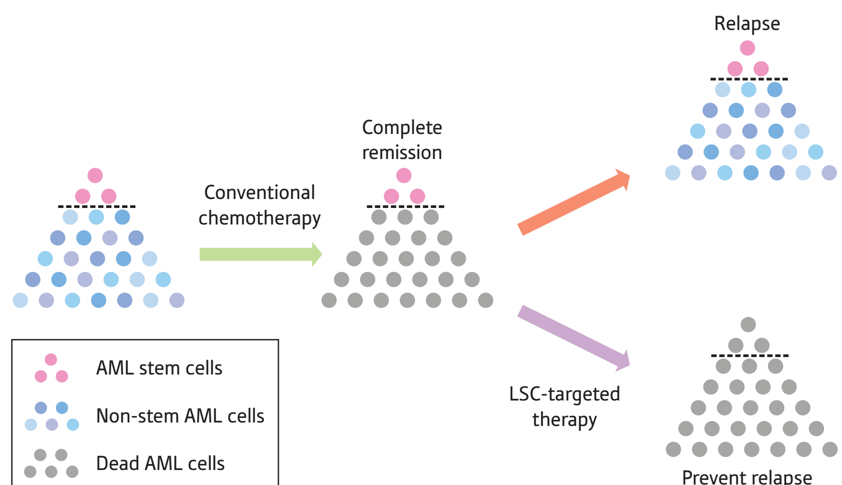

Figure 1: AML (left) can initially be driven into remission with chemotherapy, but this leaves behind a subpopulation of resistant LSCs (middle), with a high potential for initiating a fatal relapse (top right). Complete elimination of AML from patients may eventually be possible by directly targeting LSCs through more effective chemotherapeutic strategies or the identification of clinically useful LSC-specific markers (bottom right).

Acute myeloid leukemia (AML) is the most common form of adult leukemia, and with an estimated five-year survival rate of 20%, the long-term prognosis for many patients is relatively grim.

"Current treatments for AML can initially reduce the number of AML cells to undetectable levels, a state referred to as 'complete remission,'" says Fumihiko Ishikawa of the RIKEN Research Center for Allergy and Immunology in Yokohama. "Unfortunately, in a substantial proportion of these patients, AML eventually comes back—and many that relapse succumb to the disease." The need for an improved arsenal to fight AML has guided much of Ishikawa's work, and two recently published articles from his team present promising strategies for tackling this dreaded cancer.

AML originates in bone marrow, and relapse is initiated from small pockets of chemotherapy-resistant 'leukemia stem cells' (LSCs) within the marrow, which can be identified by their distinctive profile of cell-surface markers. In an effort to identify other features of LSCs that might offer useful therapeutic targets, Ishikawa, Yoriko Saito and team performed a thorough comparative analysis between LSCs and normal blood stem cells to identify genes with functional characteristics pertinent to cancerous growth whose expression is specifically elevated in LSCs[1].

Their analysis revealed two candidate cell-surface proteins, CD25 and CD32; both of these are commonly overexpressed in chemotherapy-resistant LSCs, but can also be therapeutically targeted without negatively affecting blood cell development from healthy hematopoietic stem cells, making them potentially promising targets for thwarting relapse.

In parallel, Ishikawa and colleagues have also explored methods for boosting the efficiency of chemotherapy. Standard AML drugs such as cytosine arabinoside (Ara-C) work by targeting actively dividing cells, and LSCs are believed to elude chemotherapy by entering a quiescent, non-dividing state. The researchers hypothesized that LSCs could be made more vulnerable to Ara-C via simultaneous treatment with cytokines—naturally-occurring cell signaling molecules—that stimulate them into active division[2]. In fact, this two-pronged treatment led to a ten-fold increase in survival rate relative to chemotherapy alone for mice that had been transplanted with human LSCs.

Previous studies have suggested that despite some risk of toxicity, cytokine treatment is relatively safe for patients, and Ishikawa's team is actively investigating the practicality and safety of interventions based on both of their recent discoveries (Fig. 1). "We have been putting our best effort into the translation of these findings into medicine," he says. "At the same time, we are continuing to try to identify unknown aspects of human AML and AML stem cells." ■

1. Saito, Y., Kitamura, H., Hijikata, A., Tomizawa-Murasawa, M., Tanaka, S., Takagi, S., Uchida, N., Suzuki, N., Sone, A., Najima, Y. *et al.* Identification of therapeutic targets for quiescent chemotherapy-resistant human leukemia stem cells. *Science Translational Medicine* **2**, 17ra9 (2010).
2. Saito, Y., Uchida, N., Tanaka, S., Suzuki, N., Tomizawa-Murasawa, M., Sone, A., Najima, Y., Takagi, S., Aoki, Y., Wake, A. *et al.* Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. *Nature Biotechnology* **28**, 275–280 (2010).

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'Cell surgery' using nano-beams

Using a simple glass capillary, atomic physicists at RIKEN are developing an ultra-narrow ion beam that pinpoints a part of organelles in a living cell, enabling biologists to visualize how the damage affects cell activities.

From a nucleus to mitochondria, lysosomes and the nuclear pore complex, every animal cell contains a range of organelles within just 1–100 micrometers of space. How might cell functions change if one of these organelles becomes damaged? Despite rapid progress in molecular biology research, such experiments have yet to be fully developed because organelles are too small and fragile to be manipulated individually.

Physicist Walter Meissl has been trying to solve this problem by developing an ultra-narrow ion beam that can pinpoint a single organelle while leaving the surrounding cellular functions intact. Since joining RIKEN in March 2009, the Austrian postdoctoral fellow has succeeded in hitting a nucleus with the 'nano-beam', and is now preparing for his ultimate target: a centrosome, one of the smallest organelles. "If a nucleus was like a soccer ball, a centrosome would be only one little point on that ball," Meissl says.

Creating nano-beams using glass capillaries

Meissl is a central figure of the nano-beam project, which was originally established with a grant from the President's Fund for fiscal 2007 and 2008. The project was initiated by Yasunori Yamazaki, chief scientist of the Atomic Physics Laboratory at the RIKEN Advanced Science Institute, in collaboration with Naoko Imamoto, chief scientist of the Cellular Dynamics Laboratory at the same institute (Fig. 1). Although the grant ended in March 2009, the researchers still continue to work together in an effort to implement what they call 'cell surgery'.

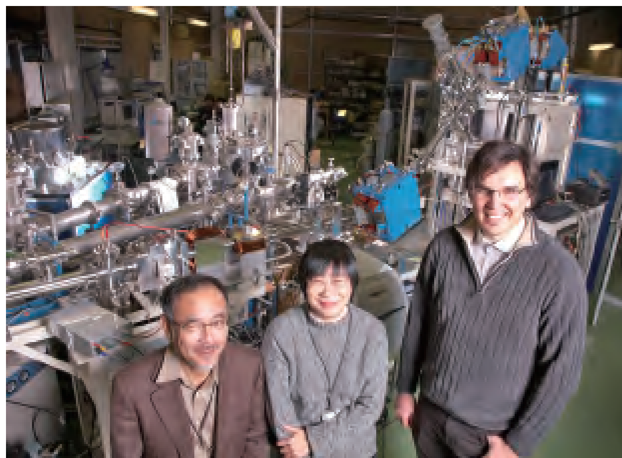

Figure 1: RIKEN's old accelerator, which had been barely holding on to life, was brought back to full operation by the nano-beam project (from right: Walter Meissl, Naoko Imamoto, Yasunori Yamazaki).

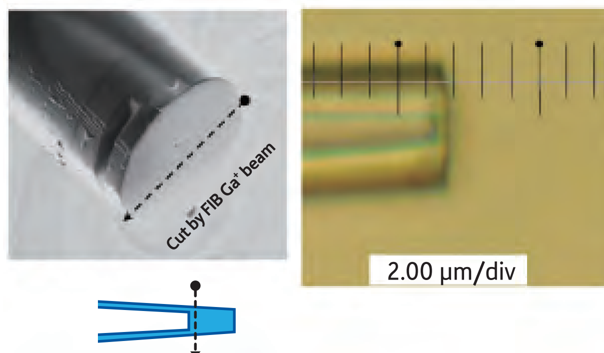

Figure 2: A thin glass cap at the tip of the glass capillary makes it possible to apply the nano-beam in biological experiments.

Previously from the Vienna University of Technology in Austria, Meissl first heard about the project when Yamazaki gave a talk at his institute in 2005. "I was intrigued with the idea of getting into the interdisciplinary work between physics and biology," says Meissl, who then decided to join Yamazaki's laboratory.

Meissl's work is a small but highly innovative product of Yamazaki's lab, the main focus of which is the investigation of exotic collision products such as antihydrogen atoms and the development of advanced cooling techniques to capture these particles. The lab has also been developing slow, highly charged ion beams (the aggregation of charged atoms and molecules) with nanometer-scale diameters using glass capillaries. Yamazaki says it is extremely difficult to focus highly charged ions into nano-sized spots, and other groups around the world have been attempting to do so using dedicated lenses that combine the effects of electric and magnetic fields. No-one had thought of using glass, because it does not conduct electricity and is susceptible to the build-up of static electricity, which deteriorates the quality of the ion beam, he says.

Instead, Yamazaki took advantage of glass's insulation properties. When ions are first injected into the inlet of a glass capillary, they accumulate on the capillary's inner wall; when the accumulation of ion charge on the inner walls becomes sufficient, subsequently injected ions are naturally guided all the way to the outlet. At a cost of 50 yen (US$0.40) per capillary, "it was so simple, like a joke, but we could confirm the beams were strong enough," says Yamazaki.

A tweak for biological applications

Nano-beams can be used to manipulate molecules and atoms on surfaces, so demand is growing for their use in

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Figure 3: Meissl manually prepares the cell and sets up the incident direction of the ion beam.

the fabrication of semiconductor materials. But Yamazaki wanted to use the technology for unconventional purposes, and sought ideas at one of RIKEN's informal chief scientist meetings, at which chief scientists with various backgrounds come together to learn about each other's activities. Yamazaki became intrigued with the potential biological applications of his nano-beams, and suggested a collaboration with Imamoto, whose primary area of study is in the regulation and maintenance of nuclear function. "But at first, my idea was rebuffed," Yamazaki says, "because the beams can only be produced in a vacuum chamber, and cells die without air."

Yet Yamazaki was undaunted and hit upon the idea of adding a thin glass cap at the capillary outlet so it can be immersed in a liquid while maintaining the capillary vacuum[1,2] (Fig. 2). The beam can be controlled so that it travels only 100 nanometers to several micrometers and the ions have enough energy to penetrate the window, allowing it to be used to irradiate a single organelle, or even a part of one, Yamazaki says. Another advantage of glass is that it is transparent and thus enables researchers to observe the irradiation point directly using an optical microscope. Compared to conventional ionizing radiation, which does not have the precision or selectivity of the nano-beam, "the new beam could lead us to observe more precisely how the damage to an organelle affects overall cell dynamics," Imamoto says.

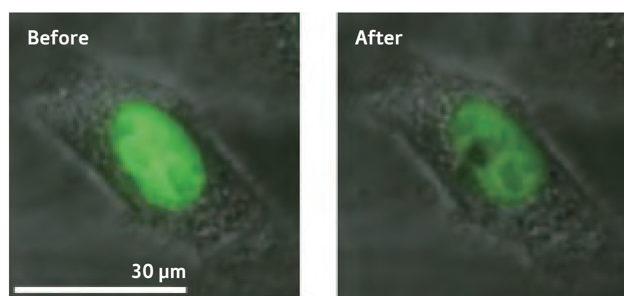

Figure 4: The nano-beam has been successfully used to selectively damage a nucleus (fluorescent green) in a cancer cell.

A physicist learns how to culture cells

Yoshio Iwai, a postdoctoral researcher who recently left the laboratory, spent the first 18 months of the project constructing a dedicated beam line for a small tandem accelerator at RIKEN. Now, much of the work has been handed over to Meissl. Unlikely for a physicist, he started with learning from Imamoto how to culture a cell, multiply it, and prepare solutions for experiments. "It is very exciting and totally different from my previous work because a living cell shows unpredictable results, a drastic change from surface physics," Meissl says.

Meissl also made some additional changes to the beamlines. "Usually in physics, you fight for more ions, more intensity. But for biological experiments, we need as little radiation as possible because even a single particle can harm a cell." He installed a fast beam switch that allows attenuation of the ion beam down to short packets containing as little as a single ion.

From nucleus to centrosome

The most difficult part of the experiments is manually setting up the cell in the best position for nano-beam irradiation, Meissl says (Fig. 3). An equally important step is to optimize the strength of the ion beam and calculate the right irradiation time. By switching the beam on and off in less than one microsecond, Meissl takes multiple shots at the cell surface, but each time he obtains different results. "I need much more target practice," he says. "It's not as easy an experiment as it looks. A lot of patience is required," Imamoto adds.

In the summer of 2009, Meissl succeeded in hitting a nucleus, and the cell died immediately. After a number of attempts, he has fine-tuned the strength of the beam and is now able to hit the nucleus while keeping the cell alive (Fig. 4). He is now preparing to target a centrosome. At less than one micrometer in diameter, the centrosome exists as a pair of organelles floating near the nucleus, and organizes microtubules to divide chromosomes into daughter cells during cell division. Yamazaki and Imamoto are curious to see what will happen if one of the pair is damaged, but Meissl says it is incomparably more difficult than targeting a nucleus.

"The project is just becoming science," Yamazaki says. "We have just begun to explore the potential of this new technique that can lead to unprecedented applications bridging biology and physics." ■

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Simulating the dynamics of proteins to understand protein functions

Theoretical simulations of protein structures using better computational methods provide important information on the biological functions that make life possible.

Yuji Sugita

Team Leader
Biomolecular Dynamics Simulation Research Team
Associate Chief Scientist
Theoretical Biochemistry Laboratory
RIKEN Advanced Science Institute

Proteins are found everywhere in living bodies, and are vital and essential for life. Scientists since the latter half of the twentieth century have been conducting research into structural biology to clarify the three-dimensional structure and to understand the functions of proteins based on their structures. Research by the many scientists involved in the field has brought about a significant advance in our understanding of life phenomena. "However, the knowledge obtained solely by observing the structures of proteins is insufficient for a full understanding of the mechanism of how proteins function in the body," says Yuji Sugita, associate chief scientist and leader of the Biomolecular Dynamics Simulation Research Team in the Theoretical Biochemistry Laboratory at the RIKEN Advanced Science Institute. With the aim of understanding protein functions at the atomic or molecular level, the laboratory has simulated the behavior of proteins using various theoretical calculation methods, specifically focusing on molecular dynamics. The laboratory is also working on the development of a new calculation method in close collaboration with structural biologists from within RIKEN and from other institutions.

Structural biology and simulation

Sugita is inundated with applications for research collaboration from many structural biologists within and outside RIKEN. "We use computers to simulate structural changes in biomacromolecules such as proteins and cellular membranes so as to clarify their functions. These people say, 'I have just solved the three-dimensional structure of a new protein, so why don't we conduct a joint simulation?'" he says. Proteins, responsible for many of life's mechanisms, consist of a number of amino acids and fold into the specific three dimensional structures. The three-dimensional structure of a protein is closely related to its function, and so protein functions can be investigated by clarifying the three-dimensional structure. This is the essence of structural biology.

It was in 1958 that the three-dimensional structure of a protein was first elucidated. John Kendrew, a British biochemist, and others created crystals of myoglobin, which stores and carries oxygen in the blood. They exposed the crystals to x-rays to clarify their three-dimensional structure at the atomic level, for which they were awarded the 1962 Nobel Prize in Chemistry. This technique, x-ray crystallographic analysis, is still a mainstream technique in three-dimensional structural analysis. Nuclear magnetic resonance (NMR) is also widely used for three-dimensional structural analyses of solutions.

As Sugita points out, "So far, more than 60,000 proteins have been clarified in terms of their three-dimensional structure, contributing significantly to our understanding of protein functions. However, the artificially arranged three-dimensional structure of protein crystals is not an exact match to the structure within the living organism. Proteins in a living organism either exist in solution in the cytoplasm or are embedded in biological membranes such as the cellular membrane and the endoplasmic reticulum membrane. Furthermore, a protein can change its structure dynamically by itself when it functions. However, when we want to observe these changes in greater detail, there is a limit to observation based only on x-ray analysis and NMR. Thus, much attention has been paid to computer-based molecular simulation techniques as a new approach that allows observation of the dynamics of proteins at the atomic level."

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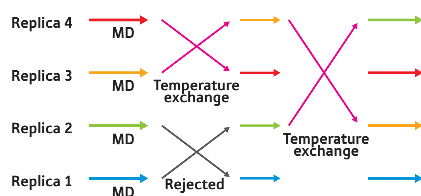

Figure 1: Replica-exchange molecular dynamics method.
First, multiple replicas of the target protein are prepared. Then, molecular dynamics (MD) calculations are performed in parallel for these individual replicas at different temperatures, which are exchanged during the calculations.

How the three-dimensional structure of proteins is computed

To simulate the structural changes in proteins, atomic-level information on the three-dimensional structure of the target protein is required. The resolution of the information should be equivalent to that obtained by x-ray analysis or NMR. As the information on the three-dimensional structure does not provide sufficient information with regard to hydrogen atoms, the positions of the hydrogen atoms around the target protein are first predicted theoretically before creating the full atomic model. When the target is a water-soluble protein, the water molecules are properly arranged around the protein in the model. When the target is a membrane protein, it is embedded in a lipid bilayer membrane, and water molecules are properly arranged around the target protein. Finally, the forces between the atoms are calculated by solving the classical Newton's equation to analyze and observe the dynamics of proteins, that is, how the positions of the atoms change with time. This procedure is known as molecular dynamics simulation.

The molecular dynamics simulation of proteins started in 1977. "The first report was of results calculated for a small protein called BPTI, a chain of 58 amino acids, in a vacuum for a period of one picosecond. Accurate representation of the motion of atoms, however, requires calculation at intervals of one femtosecond. The amount of calculation required increases from being proportional to the number of atoms or the square of the number of atoms. Computers at that time did not have sufficient computing power for such computations, and so the interaction between water molecules had to be neglected, and the calculation time was limited to very short time periods."

Current-day computing power makes it easy to simulate a target protein and surrounding water molecules for periods of one microsecond, and a BPTI protein in water can even be simulated for a period of up to one millisecond. Molecular dynamics simulations are now being applied to more complex, large-scale models such as membrane proteins embedded in biological membranes and DNA-protein complexes.

Sugita notes that there are three factors contributing to the rapid development in the molecular dynamics simulation of proteins. The first factor is the dramatic improvement in computing power, which at present is least a million times what it was when molecular dynamics simulation first began. The second factor is the amount of information accumulated on three-dimensional structures with the development of structural biology, and the third factor is the development of new calculation methods. "The molecular dynamics simulation of proteins has developed rapidly, supported by the multiplied computing power, three-dimensional structure information, and various calculation methods. We have also made a contribution through the development of calculation methods."

Development of an epoch-making replica-exchange molecular dynamics method

Sugita successfully developed a new calculation technique called the 'replica-exchange molecular dynamics method' when he worked for the Institute for Molecular Science. "The three-dimensional structure of a protein is most stable when it is at the lowest energy level. However, the energy level of a protein is constantly changing, and its energy distribution is like a rough landscape with many valleys and cliffs. Thus, it has many metastable states. We may be trapped in a valley during calculation and lost in the middle of this endless energy distribution. We could get out of the valley if we used an ultra-high performance computer for an indefinite period of time, but that is not realistic. Under these circumstances, the replica-exchange molecular dynamics method, which I and Prof. Yuko Okamoto at the Institute for Molecular Science (at present, Nagoya University) jointly developed, is one of the most effective calculation methods to solve this problem.

The replica-exchange molecular dynamics method is based on a technique developed in theoretical solid-state physics. The technique was then modified and applied to molecular dynamics calculations. In the replica-exchange molecular dynamics method, multiple replicas of a target protein are prepared. These individual replicas are then simulated simultaneously at different temperatures. These temperatures are exchanged during the calculations, and the operation is repeated (Fig. 1). "We can obtain energetically stable structures when calculations are performed at low temperatures, but it is difficult to get out of the valleys. In contract, we can easily get out of the valleys when calculations are performed at high temperatures, but we tend to obtain unstable structures. So we exchange the temperatures so as to take advantage of the two calculation methods and to drive various structures. We use these results to restructure the structural changes of the protein at a constant temperature and to achieve a long simulation period. A normal molecular dynamics calculation would require several hundred to several thousand times the computing time needed for the replica-exchange molecular dynamics method."

The replica-exchange molecular dynamics method is computationally very efficient because individual replicas are calculated in parallel. This method has been included in major molecular simulation software packages and is used widely around the world. The original article has been cited more than 600 times, and that number is

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still increasing. "Thanks to the replica-exchange molecular dynamics method, we are now able to simulate the folding of proteins in water, which had been considered impossible. The development of calculation methods for simulation can be compared to the development of equipment for experimental science. As advanced research results are created by a group with new equipment, so new simulations are created by a group that is actively working on the development of new calculation methods."

Moving proteins as if they were alive

"The advantage of research into simulation is high versatility. We can apply the calculation methods to various life phenomena after they have been examined in detail," says Sugita. In his laboratory, researchers are working on various simulations for phenomena such as structural changes in membrane proteins, structural prediction of amyloid proteins (considered to be a major cause of Alzheimer's disease), folding and degenerative processes of proteins in water, and the behavior of lipid molecules in biological membranes. "I have been amazed by the ingenious functions of many biomacromolecules. In particular, I was deeply impressed by the complex molecular mechanism by which the calcium ion pump changes its structure to enable active transport of Ca^{2+}. This is the research project I have been continuing in collaboration with Prof. Chikashi Toyoshima of the Institute of Molecular and Cellular Biosciences at The University of Tokyo since I once worked for the institute."

The Ca^{2+} pump (Fig. 2) is a membrane protein embedded in the endoplasmic reticulum membrane in muscle cells that transports Ca^{2+} from the cytoplasm into the endoplasmic reticulum. The application of x-ray crystallographic analysis to membrane proteins is very difficult because they do not crystallize easily. Toyoshima and others, however, successfully determined multiple three-dimensional structures of the Ca^{2+} pump in different states. Sugita is using computer simulation to connect and arrange all of these 'snapshots' in order. "I can really see the true meaning of 'understanding functions through three-dimensional structures' when I discuss the structures of the Ca^{2+} pump with Prof. Toyoshima."

However, they are only half way toward meeting the challenge of understanding the functions of the Ca^{2+} pump through its three-dimensional structure. The driving force that the Ca^{2+} pump requires for ion transport is the chemical energy produced when adenosine triphosphate (ATP) is hydrolyzed into adenosine diphosphate (ADP). However, the molecular dynamics simulation based on classical mechanics are unable to deal with chemical reactions. Thus, the effects of the chemical reactions cannot be taken into account. "We need to combine multiple theoretical calculation methods, including quantum-chemistry calculations." Setting this challenge as one of his goals, Sugita started the RIKEN Theoretical Biochemistry Laboratory in 2007.

One of the laboratory's recent research targets is related to the simulation of a membrane protein called translocon, which has the dual function of transporting proteins across a biological membrane and embedding other membrane proteins in biological membranes (Fig. 3). The three-dimensional structure of translocon has been clarified to have a closed form before a protein is transported, but Osamu Nureki of The University of Tokyo and others discovered another three-dimensional structure for translocon, one involving the binding of an antibody molecule. They wanted to prove that this three-dimensional structure is the stage following the closed form, and asked Sugita for his cooperation. "Dr Takaharu Mori, a contract researcher in our laboratory, and his team successfully conducted a simulation for a period of 100 nanoseconds. We found that the new structure changed into the closed form when the antibody molecule was removed. The article including pictures generated by our simulations was published in *Nature* in 2008." The team is now moving forward with joint research toward elucidating the molecular mechanism of how translocon transports proteins.

Sugita's laboratory is also making progress on the simulation of several membrane proteins with as yet undisclosed three-dimensional structures. These studies have been made possible by close collaboration between the theoretical biochemistry laboratory and structural biologists.

The field of protein simulation is very competitive, but Sugita has confidence in his laboratory. "In our laboratory, we work on both the development of calculation methods and computer simulation. We also have a close relationship with structural biologists. Furthermore,

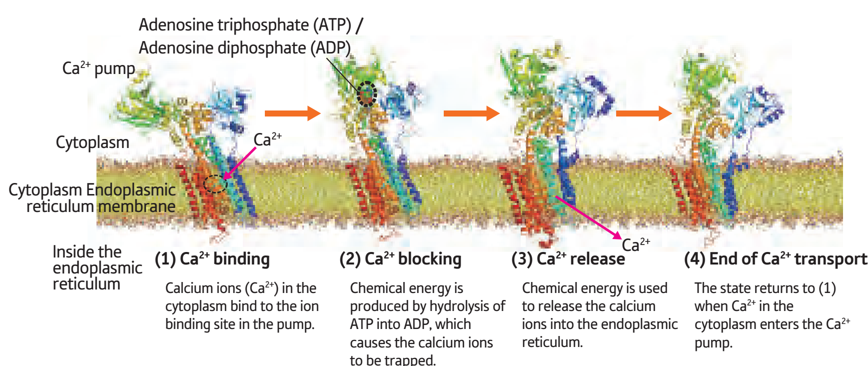

Figure 2: Structural change of the calcium ion pump.
Calcium ions (Ca^{2+}) in the muscle cells, related to the contraction and flaccidity of the muscles, are stored in the endoplasmic reticulum in high concentrations. The Ca^{2+} pump, which is embedded in the endoplasmic reticulum membrane, changes its structure dynamically to transport calcium ions in the cytoplasm into the endoplasmic reticulum against the concentration gradient of Ca^{2+}.

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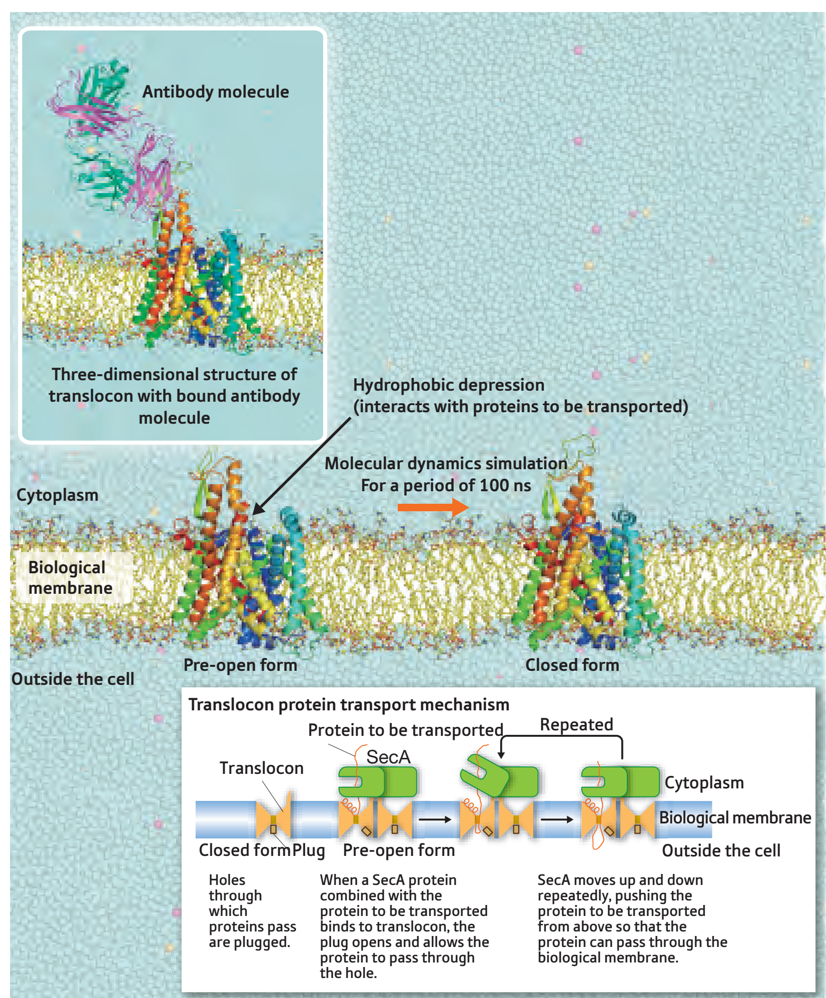

Figure 3: Molecular dynamics simulation of translocon.
Translocon is a membrane protein that transports proteins in the cytoplasm of a cell across its biological membrane. When a translocon is isolated, it is in a closed form in which the hole of the translocon is plugged. The plug opens when a partner protein, such as SecA, binds to translocon, allowing the transport of proteins (right). A recently found *Thermus thermophilus*-derived translocon, however, combines with antibody molecules (Fab) to assume a special form different to the closed form (upper left). Simulation of the molecular dynamics of translocon without antibody molecules for a period of 100 ns has revealed that translocon changes its structure and returns to the closed form when the antibody is removed. The simulation also showed that the structure with bound antibody molecule is in a 'pre-open' form at the early protein transport stage.

RIKEN's computer environment is wonderful. We have no parallel in the world in our ability to make progress with our research because of these conditions." In addition to their own computers, they can use MDGRAPE-3, a special-purpose computer system for molecular dynamics simulations incorporated into the RICC, the RIKEN Supercomputer System. The RICC supercomputer is very powerful, and MDGRAPE-3 is the largest system in Japan for molecular dynamics simulations of proteins.

Suyong Re, another contract researcher, and his team, who work on simulations based on quantum chemistry, are beginning to produce visible results. However, they need a faster computer because the amount of calculation required in quantum chemistry is proportional to the fourth power of the number of atoms. "We will get molecular dynamics and quantum chemistry simulations into full swing using the Next-Generation Supercomputer that RIKEN is now developing. We want to move large and complex proteins such as membrane proteins in the simulation as if they were alive. We have decided on a time length of one millisecond as a target because that time length allows us to observe the entire function cycle of the protein. Next, we would like to attempt the simulation of life phenomena involving multiple proteins," says Sugita.

Inspiration for theoretical biochemistry

In 1987, shortly before Sugita entered the Faculty of Science at Kyoto University, Susumu Tonegawa, director of the RIKEN Brain Science Institute, won the Nobel Prize for Physiology or Medicine. Sugita seemed to be interested in biology, but he could not give up his interest in physics. So he enrolled in Nobuhiro Go's laboratory, which was conducting research applying theory and calculation in the field of biology. "Go's laboratory belonged to the Department of Chemistry. At first, I was not interested in chemistry because I thought it consisted of purely memorization study. However, as I studied it further, I found it more and more interesting. In a broader sense, chemistry is the study of various phenomena driven by the interaction between atoms and molecules. We use the information obtained through theoretical calculations and experiments to understand the functions and structures of proteins at the atomic or molecular level. I once asked Dr Ryoji Noyori, president of RIKEN, whether our research activities were in the area of chemistry. I was very pleased when he told me that our research activities were exactly in the area of chemistry," says Sugita. The Theoretical Biochemistry Laboratory symbolizes Sugita's research interests. "I would like to focus on understanding living organisms from the perspective of chemistry, based on molecules and atoms." ■

About the researcher

Yuji Sugita was born in Niigata, Japan, in 1969. He graduated from the Faculty of Science, Kyoto University, in 1993, and obtained his PhD in 1998 from the same university. After half a year postdoctoral training at RIKEN, he worked as a research associate at the Institute for Molecular Science in Japan. In 2002, he moved to The University of Tokyo as a lecturer. From 2007, he has served as associate chief scientist at the RIKEN ASI, directing his own research group. His research focuses on computer simulation of biomolecules and the development of new simulation methodologies.

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Exploring new horizons of cerebellum research

The cerebellum is a region of the brain with a curious history. Most well known for its role in motor control, the cerebellum is today known to be involved in everything from cognition to emotion and language. Masao Ito, founder of the RIKEN Brain Science Institute (BSI), is famous as one of the pioneers in a research movement that has shed light on the deeper functions of the cerebellum.

It was to commemorate Ito's great contributions to brain science that the BSI hosted "The New Horizon of Cerebellar Research" symposium on March 29, 2010, featuring leading scientists discussing their latest findings on the cerebellum. Each from a different perspective, the presenters painted an intricate picture of cerebellar function and its complex relation to cognition.

Richard Ivry of the University of California, Berkley introduced this picture with a review of many decades of research, establishing the cerebellum's involvement in a range of cognitive functions. Peter L. Strick of the University of Pittsburgh supplied evidence for this involvement through his studies using virus-tracing technology, which confirm the conception that the cerebellum is made up of functional modules each communicating along parallel channels with areas of the cerebral cortex.

On more conceptual ground, Chris Miall of the University of Birmingham identified a strong cerebellar role in the forward modeling and prediction of outcomes. This predictive model was given a more tangible form by Mitsuo Kawato of the Advanced Telecommunications Research Institute, whose group explores internal models of the cerebellum through robot experiments.

In his own presentation, Ito recalled his nearly 50 years of experience researching the cerebellum, stressing the importance of evolution in understanding the brain's capacity to handle movement and knowledge through common mechanisms. For his many years of tireless work, Ito was rewarded with a standing ovation by the audience in attendance, many of whom have built careers on the basis of his pioneering research. Ito recounts the story of this research in his own words below.

Unlocking the secrets of the cerebellum: a message from Masao Ito

What is it about the cerebellum that so many researchers—including myself—find so fascinating? The answer is to be found in the many mysteries it promises to resolve. The brain is filled with intricate neuronal circuits, but the relatively simple, precise and geometrically exquisite circuits of the cerebellum are particularly intriguing. Those of us who study the brain believe that the cerebellum may provide the clues we need to attain our long-standing

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objective to decipher the meaning of the brain's neuronal circuits. To do so would utterly transform our understanding of brain function.

The era in which I began my career in neuroscience, in the 1960s and 1970s, was one that saw rapid advances in this area of research. Neuronal circuits of the cerebellum were dissected in great detail, and the revolutionary Marr–Albus network models, today's most widely accepted theories of cerebellar function, were first proposed. I made my debut in this field with the finding that Purkinje cells, a class of large neurons in the cerebellar cortex, are inhibitory in nature. The title of the monograph where this finding was reported, "The Cerebellum as a Neuronal Machine" (Eccles, Ito and Szentagothai, 1967), conveys a sense of the spirit of that era.

These discoveries were followed by many more. Our group obtained the first evidence that Purkinje cells are equipped with a type of synaptic plasticity called long-term depression, which depresses the action of synapses that cause erroneous movement. Complex molecular processes underlying synaptic plasticity were also uncovered, and models of the functional modules that make up the cerebellum advanced to the extent that they are today successfully employed to reproduce learning of motor skills in robots.

A number of unexpected findings were also uncovered along the way. Among these is the discovery that only 3% of the 175,000 excitatory synapses on a single Purkinje cell are actually functional. This suggests that while all synapses are initially functional, the majority become long-term depressed through learning, the functional remainder being sufficient to form a specific receptor field for each Purkinje cell.

Another great surprise was the discovery, spotlighted at the New Horizon of Cerebellar Research symposium, that the involvement of the cerebellum may go beyond motor control, to higher-level cognitive functions. Evidence for this involvement is found in a relatively recent evolutionary development, a region of the cerebellum that forms a loop connection with the headquarters of cognitive function—the cerebral prefrontal cortex. Many brain-imaging studies have since revealed that activation of the cerebellum is associated with non-motor cognitive activities such as language.

These discoveries demand that we reevaluate the long-standing dogma of the cerebellum to incorporate not only motor control, but also cognition. Furthermore, it is in learning that we find evidence for a connection between these two types of brain function: repetition of a particular pattern of thought enables us to learn the pattern and reproduce it intuitively, without conscious effort, just as we learn to execute patterns of movement without knowing in detail the motor control mechanisms involved. Many important cognitive functions, including language, intuition, inspiration and giftedness, can be attributed in this way to learning capabilities of the cerebellum.

The connection above suggests the possibility of a common mechanism underlying both motor control in the physical domain, and manipulation of knowledge in the mental domain. The implications of such a mechanism would be profound, touching on a fundamental proposition in science dating back to the time of Descartes that our physical brain embodies the conscious mind. The cerebellum thus holds the key to one of the innermost secrets in science, and one of the greatest mysteries of the brain. ■

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RIKEN holds Second Biosupercomputing Symposium

On March 18–19, RIKEN held its second symposium on biosupercomputing in downtown Tokyo, focusing on new directions in life science research made possible by high-performance supercomputing. Figuring centrally at the symposium was the RIKEN Next-Generation Supercomputer currently under construction in Kobe, which will elevate simulations to an entirely new level of depth and detail.

In a keynote lecture, Peter Coveney of the University College of London provided a glimpse of how supercomputing would impact the future of medicine. "By the end of this century, biomedicine won't look much like it does today," he predicted. Coveney described a future in which high-fidelity simulations would be used in clinical medicine for diagnosis and treatment, highlighting changes to supercomputer policy that such applications would require.

In a session on molecular scale simulations, Siewert Jan Marrink of the University of Groningen discussed simulations of lipid nano-containers using coarse-grained methods, and Helmut Grubmüller of the Max Planck Institute for Biophysical Chemistry presented more fine-grained atomic simulations of protein folding. At the cellular scale, Dirk Drasdo of the French National Institute for Research in Computer Science and Control discussed agent-based models for simulating tumor growth. Hideo Yokota of RIKEN followed with an outline of the cell-scale simulations to be performed using the new RIKEN Next-Generation Supercomputer.

Shifting focus to organ- and body-scale simulations, Grace Peng of the US National Institutes of Health presented an overview of high-performance computing applications in biomedical research. James Bower of the University of Texas and Shin Ishii of RIKEN followed with a discussion of applications of supercomputing technology to the study of neurons and neural network models.

While still in the relatively early stages of its evolution, supercomputer technology and the class of powerful simulations it enables promise to revolutionize the life sciences in years to come. Attendees to the Second Biosupercomputing Symposium witnessed a unique preview of this new era of high-performance simulations, one in which RIKEN is positioned to play a leading role. ■

Launch of RIKEN Channel on YouTube

On April 1, as part of efforts to reach a broader audience in Japan and across the world, RIKEN launched its first ever official channel on the video distribution website YouTube. The channel features new and previously unreleased videos covering a wide array of research by RIKEN centers and institutes.

A visit to the RIKEN Channel presents viewers with a diverse set of perspectives on RIKEN's past, present and future. One video presents an overview of RIKEN's history, its organization and its objectives, stretching from its foundation in 1917 to its modern-day form. Other videos introduce specific projects at RIKEN: one takes the viewer on a tour of the new X-ray Free Electron Laser (XFEL) facility at the SPring-8 Center in Harima, another introduces a robot (RIBA) developed to assist personnel and patients at care facilities, and still another introduces the fundamentals of biology through Japanese robot anime.

Through the sharing of this multimedia content, the new YouTube channel enables RIKEN and its researchers to interact with its Internet audiences in a way never before possible. It is hoped that this new form of interaction, by removing barriers to access, will contribute to further enhancing the dissemination of information and knowledge about RIKEN researchers and their important findings. ■

RIKEN Open Day: A day for discovery

As Japan's flagship research institute, one of RIKEN's missions is to share its research findings with the general public to boost awareness and curiosity about science. One way RIKEN does this is by opening its doors to the public in its annual Open Days held at each of its campuses. The Wako Institute, RIKEN's administrative headquarters and home to a number of its major research centers, greeted crowds of visitors to its Open Day on April 17 with an array of hands-on experiments and events, and more than a few surprises.

Each area of the campus covered different areas of science. Those who headed south were introduced to nanoscience, superconductivity, lasers and biotechnology. In the central area, visitors learned about brain science, supercomputing, molecular simulations and magnetism, while a trip eastwards brought encounters with heavy ion beams and nuclear physics. Venturing from lab to lab, visitors extracted DNA from broccoli, poked mysterious moving gels, estimated the age of their gut and played magic ball in a virtual baseball stadium.

The Open Day also featured two lectures by prominent researchers at RIKEN. Tohru Motobayashi of the Nishina Center for Accelerator-Based Science explored the mysteries of heavy ion physics through instruments at the RIKEN Radioactive Isotope Beam Facility. Naotaka Fujii, team leader of the Laboratory for Adaptive Intelligence at the Brain Science Institute, explored how brains communicate with each other and how this affects the design of technology for interfacing brains and machines.

Beyond the grounds of the Wako Institute, Open Day events also took place at the Tsukuba Institute on April 17–18, and later at the Harima Institute on April 29. The Yokohama Institute and Sendai Facility open their doors to the public later this summer, on July 3 and 31, so those who missed the events at Wako still have a chance to experience this unique event. ■

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Dr Kimitoshi Kono
Chief Scientist
Low Temperature Physics Laboratory
RIKEN Advanced Science Institute, Wako, Saitama, Japan

Dear Dr Kono,

I take great pleasure in writing to you after such a long time. I have recently been reminiscing about when we first met, in 1994: I was completing a seven-month sabbatical at The University of Tokyo with Professor Shun-ichi Kobayashi, at a time when very few Taiwanese scientists visited Japan for an extended period to perform research in the natural sciences. I became acquainted with a number of Japanese professors and graduate students — one of them being you.

After you moved your Low Temperature Physics Laboratory to RIKEN, my visits to and collaborations with RIKEN became routine. As you know, I have visited RIKEN on average one to two times every year since 2002, and yourself and Dr Keiji Ono have also visited the NCTU a couple of times in recent years.

Through the RIKEN–NCTU Joint Graduate School Program, two of my graduate students, Mr Shiu-Ming Huang and Ms Yu-Chen Sun, have carried out extensive parts of their doctoral experiments at RIKEN. Shiu-Ming finished his PhD in July 2008 and is currently a postdoctoral researcher at RIKEN. I find it very impressive that, through the close collaborations between RIKEN and the NCTU, Shiu-Ming has recently published two papers in the prestigious international journal *Physical Review Letters*. The first of these was highlighted in the 19 October 2007 issue of the *RIKEN RESEARCH* newsletter, with the title 'Against the Flow' — perhaps you read it?

Yu-Chen started her experiments at RIKEN in September 2008. I believe that sooner or later we will have joint publications based on her work on spin-dependent transport in semiconductor quantum dots at ultra-low temperatures. When I visited RIKEN last spring (during the cherry blossom season!), I could see that Yu-Chen treasures her stay at RIKEN, and it is clear that she has been doing her best in the name of good science. She has also learned to speak Japanese to quite a reasonable extent over this period.

Nowadays, the collaborations between RIKEN and the NCTU extend further than just these institutions alone. In fact, I think that the wider Japanese and Taiwanese scientific communities have significantly benefited from our efforts. For instance, together with Prof. Kobayashi and you we have so far organized three RIKEN–Taiwan Workshops on Nanoscience and Nanotechnology (2001 at NCTU, Hsinchu, Taiwan; 2002 at RIKEN; and 2005 at Academia Sinica, Taipei). In addition to the speakers from RIKEN and the NCTU, we have always taken care to invite scientists from various Japanese and Taiwanese institutions and universities to participate. This series of Workshops has definitely helped us foster more frequent and fruitful exchanges between Japanese and Taiwanese researchers in recent years. I am very grateful to RIKEN for its enthusiastic and kind support of these Workshops.

Personally, I always enjoy my stay at the RIKEN campus at Wako-shi. It is a very quiet place, especially during the evenings. The campus is a perfect place for concentrating on my research, collecting my thoughts, as well as focusing on writing the most demanding papers. On the occasions when there is moonlight, strolling around the guest house in the evening is a very spiritually soothing and refreshing experience. One of my favorite things to do is to spend the evening reading Chinese and Japanese classics in the quietness.

I wish you all the best, and hope to see you very soon.

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P.S. I just learned that RIKEN recently issued a Press Release (29 March 2010) on Shiu-Ming's second paper. Congratulations.

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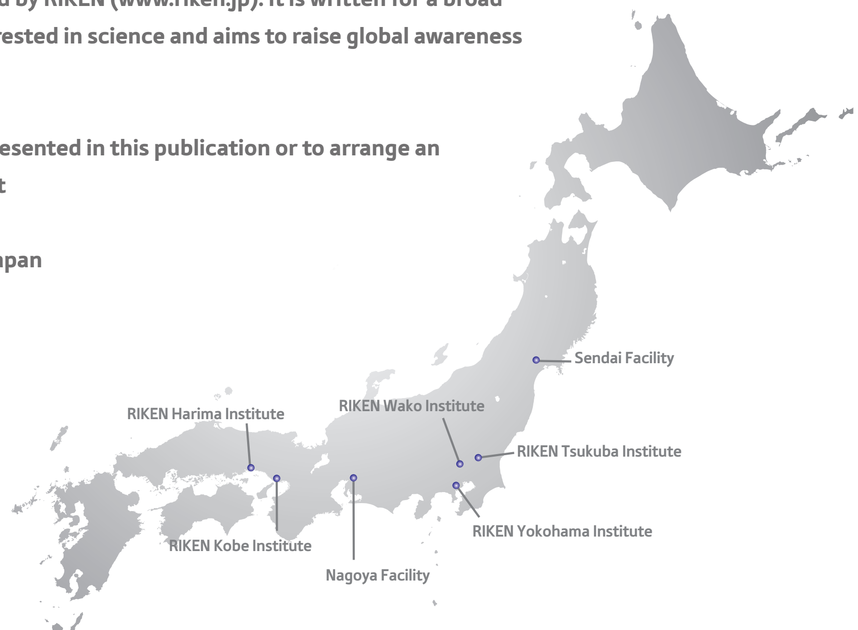

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