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Filling out the map

Recent findings from the FANTOM consortium spotlight new mysteries and challenge old assumptions about the mammalian genome

Even with a complete sequence at their disposal, scientists are still laboring to unlock the secrets of the human genome—but data from a landmark international research effort headed by the RIKEN Omics Science Center (OSC) in Yokohama offer surprising new insights and promising foundations for future work.

A primary mission of the Functional Annotation of the Mammalian Genome (FANTOM) Consortium (Fig. 1) is to exhaustively catalogue human and mouse genes and their activity. In previous projects, FANTOM has obtained full-length sequence data for over 100,000 mouse gene transcripts, but their latest iteration, FANTOM4, is even more ambitious. "FANTOM4's main targets have been to demonstrate that it is possible to use sequencing to detect not only DNA or RNA sequences, but also to detect expression and—much more importantly—the networks that control transcription," explains OSC researcher Harukazu Suzuki.

A key weapon in FANTOM's arsenal is their 'deepCAGE' strategy, combining a method called 5' cap analysis of gene expression (CAGE), which allows scientists to accurately identify and quantify activity of transcriptional start sites (TSSs), with next-generation sequencing technology. Now, in three new articles from *Nature Genetics*, FANTOM describes striking findings achieved through their pairing of sophisticated experimental and analytical techniques.

Network news

Every gene is regulated by a stretch of DNA called the promoter, containing binding sites for various transcription factor proteins that contribute to gene activation or repression, and whose combined activity ensures that transcription occurs at the right time and place.

The ability to accurately map which factors control which genes and how these regulatory networks interact would be immensely useful in helping scientists to understand the mechanisms underlying virtually any cellular process, and FANTOM achieved major progress on this front with a pilot study investigating chemically induced differentiation of human leukemia cells into mature immune cells[1].

The team located promoters throughout the genome based on the clustering of TSSs identified via deepCAGE, and then identified known transcription factor binding sites within those promoters. They collected data from numerous time-points to chart changes in activity during differentiation, and correlated those changes with involvement of specific transcription factors. The result was a detailed, experimentally testable network of regulatory pathways involved in the differentiation process (Fig. 2). "We showed that a network inferred using only experimental data but no previous knowledge can identify all known key transcription factors for THP-1 differentiation and many known—as well as previously unknown—regulatory processes," says OSC researcher Carsten Daub. "This method can now be applied to biological systems that are poorly understood."

Double agents

As much as half of the genome is composed of repetitive sequences, derived largely from retrotransposons—DNA elements that can self-replicate and insert themselves into other chromosomal sites with potentially damaging consequences. Scientists have generally looked on these disruptive 'jumping genes' uncharitably. "Retrotransposons have been thought mainly to have a parasitic role in the genome," explains Piero Carninci.

Figure 1: The FANTOM4 team—represented in part here—is comprised of scientists at more than 50 research institutions in 15 nations.

As such, Carninci and his FANTOM4 colleagues were surprised to uncover compelling evidence that the influence of retrotransposons may be far more pervasive—and beneficial—than was previously understood[2]. Although retrotransposons contain promoters, these were generally assumed to be non-functional and to have little direct effect on gene regulation. However, deepCAGE data revealed that up to 18.1 and 31.4% of TSSs in mice and humans respectively lie within these repetitive elements, and in many cases the positioning of these TSSs suggests that retrotransposon promoters directly contribute to regulation of protein-coding genes.

In addition, more than a quarter of the protein-coding mRNAs examined contained retrotransposon-derived repetitive elements; the presence of these sequences is strongly associated with downregulation of expression, suggesting that retrotransposons may also help 'silence' specific genes. Carninci describes these findings as a paradigm shift: "Although the general idea is that retrotransposons are passive—or even harmful— elements of the genome, cells have learned how to use them in symbiotic mechanisms."

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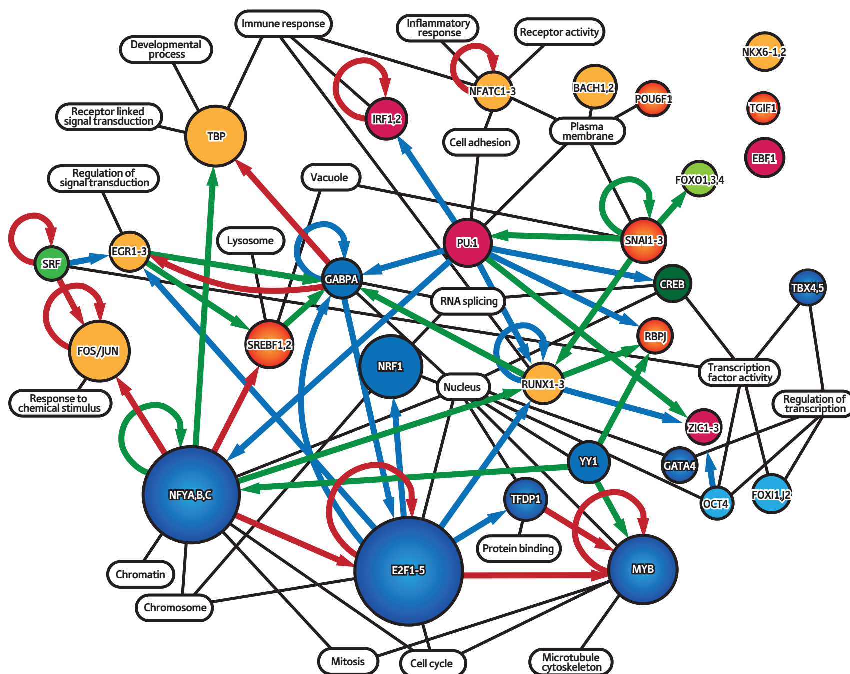

Figure 2: The complicated transcription network uncovered by the latest FANTOM study.

Teeming with tiny transcripts

Little RNAs are big news these days, as scientists continue to uncover an increasingly large array of species of small, non-protein-coding RNA molecules that execute some of the cell's most important regulatory functions—and FANTOM has now added one more to the list: the tiny transcription initiation RNA (tiRNA)[3].

Working in collaboration with University of Queensland investigator John Mattick, a member of the FANTOM consortium, the researchers discovered numerous tiRNAs, averaging 18 nucleotides in length, apparently produced from sites directly adjacent to TSSs in humans, mice, chickens, and even fruit flies. In this last species, up to half of the genes analyzed had tiRNAs associated with them, indicating the ubiquity of these molecules. Subsequent analysis indicated that tiRNAs appear to be deliberately processed by the cell, but not by the same mechanisms used by other known small RNAs classes.

At present it remains unclear exactly what role these molecules fulfill, and further study will clearly be needed. "We do not really know the mechanisms by which they are produced," says Carninci. "They could be produced by RNA polymerase stalling over promoters, and may be there to influence transcription at this level."

Outside the CAGE

These findings offer numerous starting points for further studies, and the FANTOM team is following up on the investigation of these phenomena both internally and in collaboration with unaffiliated institutions and researchers. According to Suzuki, RIKEN's Life Science Accelerator initiative for high-throughput biological analysis will be a direct beneficiary of this work. "We have established a pipeline to analyze transcriptional regulation networks solely by using experimental data without advance knowledge," he says, "and this pipeline is applicable to any biological event." ■

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2. Faulkner, G.J., Kimura, Y., Daub, C.O., Wani, S., Plessy, C., Irvine, K.M., Schroder, K., Cloonan, N., Steptoe, A.L., Lassmann, T. *et al.* The regulated retrotransposon transcriptome of mammalian cells. *Nature Genetics* **41**, 563–571 (2009).
3. Taft, R.J., Glazov, E.A., Cloonan, N., Simons, C., Stephen, S., Faulkner, G.J., Lassmann, T., Forrest, A.R.R., Grimmond, S.M., Schroder, K. *et al.* Tiny RNAs associated with transcription start sites in animals. *Nature Genetics* **41**, 572–578 (2009).

About the researchers

Carsten Oliver Daub is the facility director of the Life Science Accelerator (LSA) Core Bioinformatics Facility in Omics Science Center. He was born in Berlin, Germany in 1972 and obtained his chemistry diploma at the Technical University of Berlin and his PhD in Bioinformatics in 2004 at the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany. He has been with RIKEN since April 2006, when he arrived following post-doctoral work in genomics and bioinformatics at the Karolinska Institutet in Stockholm, Sweden. He has led efforts at recruiting bioinformaticians to OSC from across the globe. This strong bioinformatics team has continued to apply distinct and targeted strategies to analyze the transcriptome as they develop new tools to reveal its unknown regulatory functions and readily make these tools available to the scientific community.

Harukazu Suzuki was born in Ono, Fukui prefecture in 1960. His studies were in pharmaceutical sciences at Kyoto University where he received his PhD in 1988. He spent a number of years at a major Japanese pharmaceutical company before joining RIKEN's Genomic Sciences Center in 1998 as a team leader. He became a deputy project director in 2005 and project director in 2009. He has been a key researcher in the FANTOM consortium since its inception. As scientific coordinator, he guided the FANTOM4 project and his focus is now the life sciences analysis pipeline.

Piero Carninci is the deputy project director of the LSA Technology Development Group and team leader of the Functional Genomics Group in Omics Science Center. He was born in Trieste, Italy in 1965 and received his PhD from the University of Trieste in biological sciences in 1989. Upon graduation he worked in the National Laboratory of Italian Consortium for Biotechnology and for a private biotechnology venture. He joined the Genome Science Laboratory at RIKEN in April 1995 and since has published prodigiously and received numerous accolades. In addition to his development of Cap Analysis of Gene Expression (CAGE), which has been integral to the success of the FANTOM projects, he is highly sought after as a world authority on non-coding RNA (see *Nature*, 19 February 2009) and most recently on repetitive-element associated transcription start sites.

Pictured: Carsten Daub (left), Harukazu Suzuki (center) and Piero Carninci (right).

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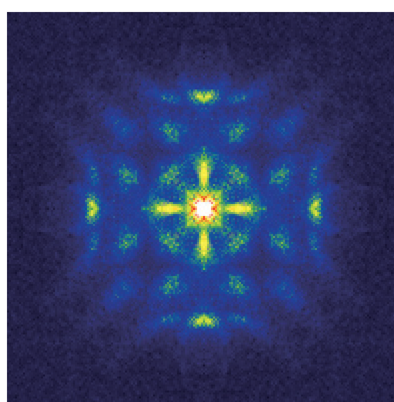
The pairing habits of superconductors

A microscopy technique unveils previously hidden information on the nature of superconductivity

Superconductivity is caused by electrons forming pairs in particular types of materials. This pairing makes the electrons insensitive to the perturbations in their pathway and leads to the disappearance of any electrical resistance. Although electron pairing is relatively well understood for conventional superconductors, many questions remain for the more unconventional class of high-temperature superconductors. Researchers from RIKEN's Advanced Science Institute in Wako, in collaboration with researchers from Rutgers University, US, and Kyoto University, have now developed an experimental method that enables the investigation of the electron pairing mechanism even for these more complex superconductors.

The experimentally measurable quantity that reflects the electron pairing in superconductors is governed by the so-called coherence factor—a measure of coordinated movement of the paired electrons. However, in the high-temperature superconductors the coherence factor is obscured by other effects and therefore impossible to measure with conventional techniques. "Our study establishes a new method to investigate the coherence factor in these complicated superconductors," says Tetsuo Hanaguri from the research team.

The technique Hanaguri and colleagues developed, which is published in the journal *Science*[1], is a scanning microscopy-based method that derives the coherence factor from the unique reaction of electron pairs to magnetic fields. The magnetic field enters the superconductor in the form of tiny bundles called vortices. In the center of a vortex, the superconducting state ceases to exist so that if the superconducting electron pairs encounter a vortex, the pairs get scattered. This type of scattering is different to that caused by other sources such as impurities in the sample.



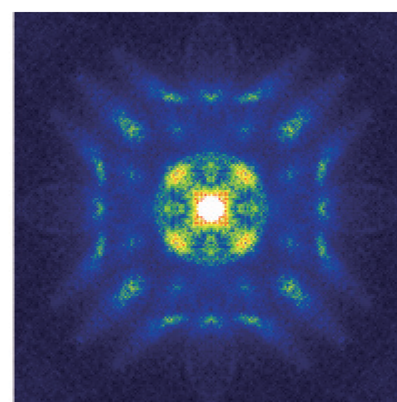


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Figure 1: Scattering patterns of superconducting electron pairs revealed. The patterns depicting scattering mechanisms without vortices (left) appear markedly different to those in the presence of vortices (right), providing direct clues to the nature of the electron pairs.

To distinguish between these different scattering properties, the researches mapped the surface of a high-temperature superconductor. The comparison between scattering patterns with and without magnetic fields shows remarkable differences (Fig. 1). These differences are unique signatures of the coherence factor and provide important clues on the symmetry properties of the electron pairs.

However, the impact of this technique extends beyond these results: it also offers an insight into the nature of electron pairs in other classes of unconventional superconductors. An example is the recently discovered class of iron-based superconductors, whose precise properties are under intense debate.

Furthermore, these experiments may enable the observation of other electronic phenomena that may exist in superconductors, says Hanaguri. "Different coherence factors may appear for other phenomena. By examining their influence on the coherence-factor we will be able to infer the presence of states which may compete or coexist with superconductivity." ■

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Mild-mannered reagents

Comparing aluminate and zincate compounds has revealed their versatility, which provides new tools for chemists

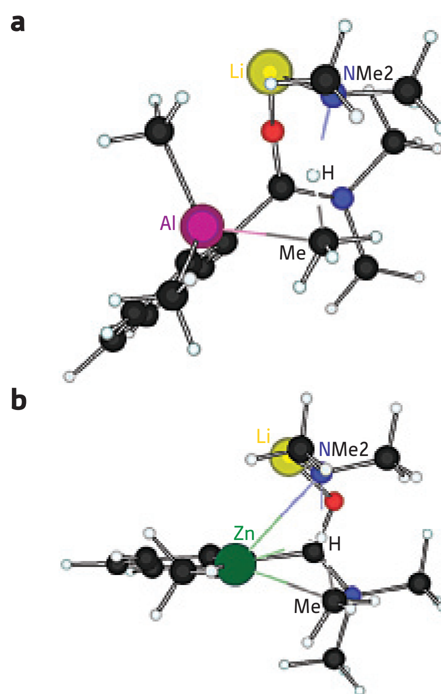

Figure 1: Schematic of the structures of the aluminate (a) and zincate (b) reagents. The aluminum atom (purple) is less able to attract electrons from the nitrogen atom (blue) in this intermediate molecule. This leads to a different end product than the zinc-based reagent.

An aluminum-based chemical reagent designed by a RIKEN scientist could prove to be a useful way of building complex carbon compounds, such as novel pharmaceuticals.

The aluminate reagent (i-Bu$_3$Al(TMP)Li) is able to pluck a hydrogen atom away from a carbon atom to create a new carbon–aluminum bond. The aluminum can then be replaced by a wide variety of other chemical groups, allowing new compounds to be constructed.

Masanobu Uchiyama of RIKEN's Advanced Science Institute in Wako, and colleagues at Tohuku University, Japan, and the University of Cambridge, UK, have now uncovered exactly how the aluminate reagent works, and for which reactions it is most suitable[1].

Uchiyama and colleagues used density functional theory to calculate how chemical reactions involving the aluminum reagent were likely to proceed. This technique relies on quantum theory to determine how electrons are spread around the molecules involved in a reaction.

This revealed that it is specifically the ring-shaped TMP portion of the aluminate reagent that is responsible for removing a hydrogen atom at the beginning of the reaction; a conclusion confirmed by subsequent experiments.

The aluminate also requires only a single chemical step to remove the hydrogen atom from its target. But this process is markedly different when using an analogous zinc-based reagent investigated by the team.

By creating detailed computer models of both reagents caught in mid-reaction (Fig. 1), the scientists found that their reaction pathways diverge because the aluminum atom is less able to attract electrons located on a nitrogen atom in a different part of the intermediate molecule.

The upshot is that while the zincate reagent tends to create the most energetically stable product molecule, the aluminate reagent simply replaces the most easily removed hydrogen atom, leading to a different end product.

Strong bases incorporating lithium or magnesium have been used traditionally for these reactions. But these reagents can inadvertently scramble part of the molecules involved in the reaction, and work only at very low temperatures.

Aluminates and zincates use much milder reaction conditions and are less likely to interfere with other parts of the reactant molecules, says Uchiyama. Choosing the appropriate reagent will give chemists the ability to control the course of chemical reactions that may have more than one possible product.

Knowing the precise path of the aluminate's reaction should allow the scientists to improve the yields of compounds it generates, he adds. The team is now testing both reagents to assess how widely they can be used by chemists. ■

1. Naka, H., Morey, J.V., Haywood, J., Eisler, D.J., McPartlin, M., Garcia, F., Kudo, H., Kondo, Y., Uchiyama, M. & Wheatley, A.E.H. Mixed alkylamido aluminate as a kinetically controlled base. *Journal of the American Chemical Society* **130**, 16193–16200 (2008).

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No pores in the war on epilepsy

A significant form of epilepsy is genetically linked to a non-membrane channel protein

A RIKEN-led research team has gathered strong biological evidence that mutations in the gene *EFHC1* trigger the onset of a common form of adolescent epilepsy for which there is currently no explanation. The mutations also increase susceptibility to epileptic seizures. The gene encodes myoclonin1, a protein found in adults in cilia—the hair-like projections that line the windpipe and the ventricles or cavities in the brain. During development, myoclonin1 is also found in the cells that produce the cerebrospinal fluid in the ventricles. *EFHC1* is one of the few genes known to be directly involved in the onset of epilepsy that does not code for a protein associated with the ion channels or pores in the plasma membrane. The researchers hope their findings can one day be translated into better treatment of juvenile myoclonic epilepsy.

The research group, led by Kazuhiro Yamakawa of RIKEN's Brain Science Institute in Wako, had previously found an association between *EFHC1* mutants and epilepsy. It had also determined the tissues in which myoclonin1 was produced. While work by other groups supported these findings, there was no direct biological or physiological evidence that *EFHC1* deficiency caused epilepsy. Details of how the researchers gathered that evidence were recently published in *Human Molecular Genetics*[1].

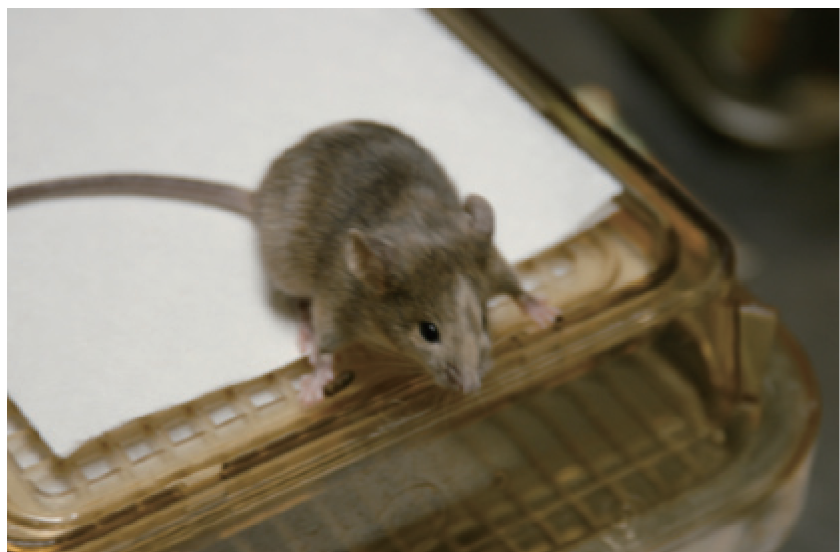

Figure 1: An *Efhc1* knockout mouse that appears to be normal but suffers from an abnormal number of involuntary muscle twitches.

Initially, the researchers generated *Efhc1*-deficient mice (Fig. 1). These mutant mice appeared normal and were fertile both in the null form where two copies of the *Efhc1* gene were defective and in the heterozygous form that carried only one defective copy. As they grew, however, mice of both forms began to display increased levels of the involuntary muscle twitches known as myoclonus, and both began to show increased susceptibility to a chemical known to trigger epileptic seizures.

When the researchers investigated the impact of *Efhc1* deficiency on the null form they found enlarged ventricles in the brain and a reduced beating frequency of the cilia—both of which suggested that the onset of epilepsy may have something to do with the circulation of cerebrospinal fluid. But in the heterozygous form, neither of these two abnormalities was apparent, yet the mice showed the same susceptibility to development of epilepsy.

"So we don't yet have a clear picture of the pathological cascade or mechanism," says Yamakawa. "But in the knockout mouse we have provided a very important tool to investigate this further. Our next step is to clarify the pathological cascade. That would make a huge contribution to our understanding." ■

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The color and the shape

A new twist on a fluorescence-based method for monitoring cell division provides scientists with information about accompanying changes in cell morphology

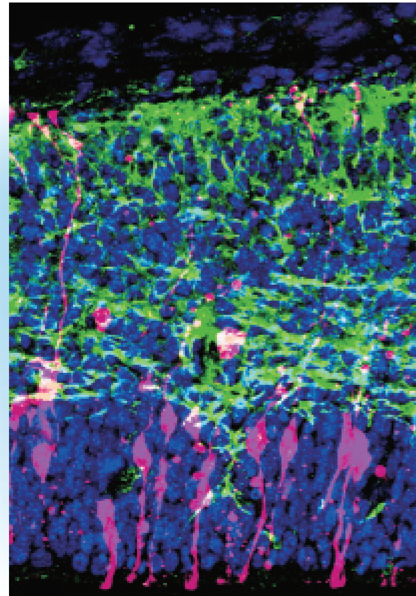

Figure 1: Demonstration of the modified Fucci method in neural progenitor cells within the developing cerebral cortex of a mouse. This image contains superimposed fluorescent signals from a modified Geminin derivative that localizes to both nucleus and cytoplasm (pink), the neuronal cell marker Tuj1 (green), and a generic stain for cell nuclei (blue).

As with higher-level organisms, reproduction is among the primary biological imperatives of the cell. This process is regulated through a multi-stage cell cycle; the more rapidly this cycle proceeds, the more quickly division takes place. A stalled cycle may be indicative of terminal cell differentiation, while rapid cycling might denote either healthy tissue growth or tumor formation, depending on the context.

The 'Fucci' method developed by Atsushi Miyawaki and colleagues at the RIKEN Brain Science Institute in Wako offers a powerful tool for monitoring cell cycle procession[1]. Fucci uses two fluorescently tagged proteins, Cdt1 and Geminin, that are differentially expressed throughout the cell cycle as a visual indicator of cell cycle phase. Cdt1 is predominantly expressed in the G_1 phase, in which cells idle prior to S-phase DNA replication; as replication begins, Cdt1 levels drop and Geminin levels rise, indicating onset of active cell division.

Fucci has proven effective in numerous applications, but Miyawaki's team ran into complications while studying the differentiation of neuronal progenitors. "The nuclear targeting of the fluorescence signal did not permit us to identify cell types and differentiation states by their characteristic morphologies," explains Miyawaki. To address this, his team developed a Fucci variant that illuminates the cytoplasm, enabling simultaneous monitoring of transitions in cell shape and cell cycle state[2].

Normally, Geminin is strictly localized to the nucleus; however, by trimming a segment from the end of the protein, Miyawaki's team was able to engineer Fucci-ready variants that linger in the cytoplasm. Upon testing their new Fucci markers in neural progenitors in the developing mouse brain, they found that they could now classify different types of neuroepithelial progenitors based on changes in growth and morphology as well as their cycling behavior (Fig. 1).

For Fucci to maintain an accurate readout, the cell must degrade labeled Geminin at the end of each cycle, and Miyawaki's team found that this requires the protein to be in the nucleus. Fortunately, the modified Geminin is only transiently located in the cytoplasm before being shuttled back to the nucleus, minimizing the overall impact on Fucci's effectiveness.

Miyawaki's future goals include expansion of the Fucci palette to incorporate additional color tags and spotlight different, specific segments of the cell cycle, but he sees immediate value in this latest variant. "This type of indicator, which traces the silhouette of individual cells with fluorescence in a cell cycle-dependent manner, will be useful for imaging mitotic cells with interesting shapes, including neural progenitor cells," he says. ■

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2. Sakaue-Sawano, A., Ohtawa, K., Hama, H., Kawano, M., Ogawa, M. & Miyawaki, A. Tracing the silhouette of individual cells in S/G_2/M phases with fluorescence. *Chemistry & Biology* **15,** 1243–1248 (2008).

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Homing in on heart attacks

Single-nucleotide changes in the genome can greatly increase an individual's susceptibility to heart attacks

Kouichi Ozaki at RIKEN's Center for Genomic Medicine in Yokohama and co-workers have identified small changes in a gene called *BRAP* that can greatly increase the risk of heart attacks in Asian people[1].

A heart attack, technically known as myocardial infarction (MI), is a sudden interruption of the blood supply to the heart. Scientists have been searching for genetic markers of the condition, which may be as small as single nucleotide polymorphisms (SNPs)—genetic variations that are commonly present in our genome.

"There are several reports associating MI with many SNPs, however most of these reports were conducted with small samples," says Ozaki. "In 2001, we started genome-wide association studies using nearly 90,000 gene-based SNPs, and identified several genes … that confer risk of MI."

One of these genes, called *LGALS2*, codes for a protein called galectin-2. Ozaki and co-workers searched for proteins that interact with galectin-2, and identified BRAP protein as a possible binding partner.

They found 26 previously unknown SNPs in the BRAP gene, and counted how often these SNPs occurred in the genomes of 2,475 Japanese MI sufferers and 2,778 control subjects. This process revealed two particular SNPs that were very strongly linked to MI.

The link was confirmed in another Japanese dataset and a dataset from Taiwan. However, the two critical SNPs were not found in datasets from North America or Africa, implying that they may be unique to Asian populations (Fig. 1).

Figure 1: Tiny changes in a gene called *BRAP* can increase an individual's risk to heart attack.

"The results indicate that these SNPs are likely to be present only in Asian populations," says Ozaki. "However, the possibility cannot be excluded that different variations in this gene confer risk of MI in other populations."

The BRAP protein was found alongside galectin-2 in both the cytoplasm and nucleus of human coronary artery muscle cells. It was also expressed in myocardial lesions—abnormal tissue growth in the artery caused by a massive inflammatory response.

"The function of galectin-2 is largely unknown," says Ozaki. "However, we found that galectin-2 binds to and regulates secretion of lymphotoxin-alpha, a proinflammatory cytokine produced in an early stage of vascular inflammation. Considering that it also interacts with BRAP protein, galectin-2 may be a key player in the vascular inflammatory system."

The study opens the question of whether scientists could eventually alter individual SNPs in a genome in order to prevent conditions like myocardial infarction.

"It is impossible with present technologies and ethics," says Ozaki. "However, innovative technologies might be developed in the future." ■

1. Ozaki, K., Sato, H., Inoue, K., Tsunoda, T., Sakata, Y., Mizuno, H., Lin, T.-H., Miyamoto, Y., Aoki, A., Onouchi, Y. et al. SNPs in *BRAP* associated with risk of myocardial infarction in Asian populations. *Nature Genetics* **41**, 329–333 (2009).

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Marking metabolism

A unique metabolic fingerprint of an individual can be built up by using a common spectroscopy technique to identify the molecules involved

Recent advances in DNA sequencing have made it relatively easy to acquire the full genotype of an individual, but it is equally important to match those genes to their functions. One useful step is to build up a 'metabolic phenotype' outlining all the processes operating to sustain the individual's life.

Jun Kikuchi and co-workers at the RIKEN Plant Science Center in Yokohama, Yokohama City University and Nagoya University have developed a systematic method to characterize metabolic pathways in plants and animals. Their method involves measuring nuclear magnetic resonance (NMR) of samples and comparing them against an extensive database of molecules associated with metabolism, known as metabolites.

NMR works by detecting the response of atoms or molecules to a magnetic field. Normal carbon atoms show no response, so cells must be labeled with the stable isotope carbon-13.

Kikuchi and co-workers fed *Arabidopsis* plants and silkworm larvae (Fig. 1) with glucose and amino acids that had carbon-13 atoms in place of the normal carbon. After this incubation process, almost all the metabolites produced by the cells contained carbon-13. Importantly, carbon-13 displays a slightly different magnetic response depending on the structure of the molecule it is in, so each metabolite provided a unique NMR spectrum.

The researchers compared the spectra of their samples against a database of spectra for known metabolites. They identified 57 unique metabolites in the silkworm larvae, and 61 in *Arabidopsis*.

The team then used a technique called Principal Component Analysis to identify correlations between metabolites in the silkworm. These correlations represent metabolic pathways related to key stages in the larval development.

Figure 1: An image of silkworm larva. A technique using NMR to reveal the metabolic pathways that act to keep silkworm larvae alive could soon be applied to humans.

In particular, the results showed a random pattern of metabolic pathways over the first six days of the study, giving way to some correlations later. This suggests that better metabolic organization emerged as the larvae grew.

The study represents the first 'top-down' method of analyzing whole metabolic pathways. It provides a macroscopic phenotype describing cells, fluids and tissues, rather than looking at specific reactions from the atomic level upwards. What's more, the technique is relatively quick.

"After an NMR measurement, typically taking about 1 hour, computation of the metabolic pathways finishes within half a day," explains Eisuke Chikayama, who wrote the team's recent paper in *PLoS ONE*[1].

Chikayama is also hopeful that the technique could be extended to other plants and animals, including humans.

"Our method is not restricted to any particular organism, if adequate NMR samples are ready." ■

1. Chikayama, E., Suto, M., Nishihara, T., Shinozaki, K., Hirayama, T. & Kikuchi J. Systematic NMR analysis of stable isotope labeled metabolite mixtures in plant and animal systems: Coarse grained views of metabolic pathways. *PLoS ONE* **3(11)**, e3805 (2008).

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Networking for survival

A metabolic study reveals that plant-based compounds cooperate to overcome dehydration

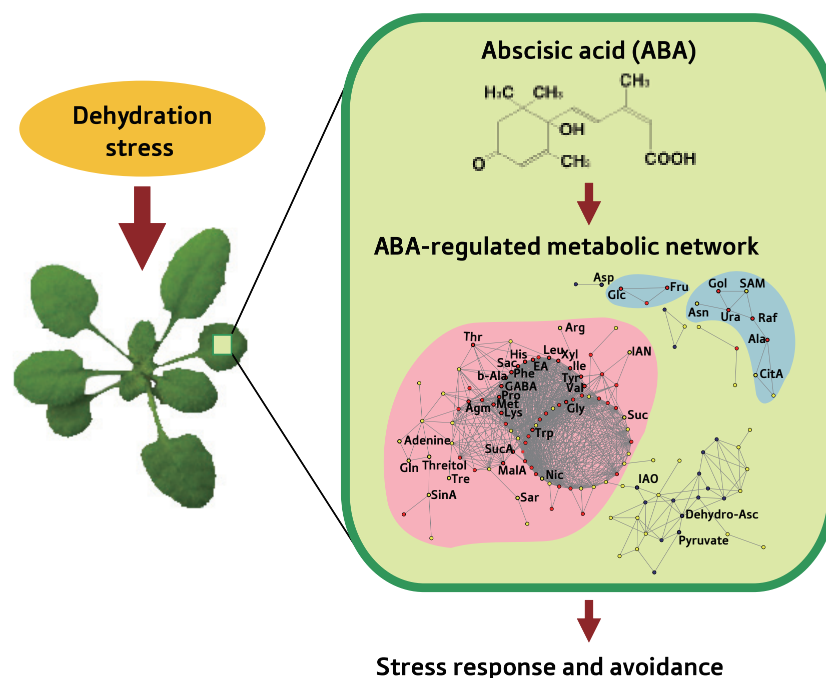

Figure 1: Metabolic networking in response to dehydration stress in *Arabidopsis*. The metabolic network analysis revealed that dehydration-increased amino acids (red dots) contribute more significantly to the dehydration-stress response when they have a global correlation with each other (pink area), whereas sugars show a modest network that is independent of amino acids (blue area). (blue dots, dehydration-decreased metabolites; yellow dots, metabolites with no change, pairs show significantly correlated metabolites)

Japanese plant biologists have exposed dynamic networks of small molecules that respond to dehydration stress in plants. Worldwide, drought is a major limitation to crop productivity, which results in economic loss and food shortages.

The researchers, led by Kazuo Shinozaki of the RIKEN Plant Science Center in Tsukuba, analyzed the so-called dehydration metabolome, which includes the complete set of small molecules, or metabolites, in drought-stressed examples of the model plant *Arabidopsis thaliana*. Metabolomics is a powerful tool for understanding highly complex cellular processes. Using this approach, the researchers found that networks of metabolites interact in adaptation to dry conditions. Their results are published in *The Plant Journal*[1].

Abscisic acid (ABA) is a phytohormone that plays a prominent role in regulating the dehydration response. Shinozaki and colleagues investigated its effect on metabolic changes in response to drought by working with a genetic mutant of *Arabidopsis*, *nc3-2* in which ABA accumulation is significantly reduced. They found that an increased rate of accumulation of metabolites such as amino acids in response to a lack of water is dependent on ABA, and depletion of this hormone alters metabolite concentrations.

The team found that dehydration-increased amino acids in wild-type plants correlated with each other, and that this interaction contributed significantly to the stress response. This suggests that metabolic engineering of amino acid biosynthesis is a promising approach for improving drought tolerance. Sugars showed no correlations with amino acid groups but did interact with each other, demonstrating for the first time that these two types of metabolite respond to dehydration stress through different metabolic networks (Fig. 1).

Shinozaki and colleagues showed that nc3-2 metabolites had to interact more widely than those in wild-type plants. Despite having lower dehydration-induced metabolite concentrations than wild-type plants, ABA-deficient mutants adapted somewhat to dry conditions through extensive correlation between certain sugars and other small molecules.

The study has revealed many more ABA-dependent metabolites and metabolic pathways than previously reported. "Soon, combining mathematics, computational biology and molecular biology should provide more insight into the complex metabolic networks that constitute the response to dehydration stress," says team-member Kaoru Urano. Understanding the sophisticated interactions between metabolites in dehydrated plants will ultimately enable better management of crops in harsh environments. ■

1. Urano, K., Maruyama, K., Ogata, Y., Morishita, Y., Takeda, M., Sakurai, N., Suzuki, H. Saito, K., Shibata, D., Kobayashi, M., *et al.* Characterization of the ABA-regulated global responses to dehydration in *Arabidopsis* by metabolomics. *The Plant Journal* **57,** 1065–1078 (2009).

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Building a soy gene catalogue

A RIKEN-led consortium of scientists has compiled a massive collection of complete gene sequences for the invaluable soybean plant

Figure 1: The life and times of the soybean plant—a soybean crop, flowers, maturing pods and roots and nodules (from top left to bottom right).

In the thousands of years since the soybean was first cultivated, it has only become more useful and important, providing nutrition for billions of humans and animals as well as raw material for a numerous industrial applications, including lubricants, inks and plastics. As valuable as this crop already is, however, a better understanding of its genomic content could enable scientists to cultivate still more useful strains that are hardier or better suited for specific applications (Fig. 1).

A first draft of the soybean genome was recently made publicly available, but an even more useful resource would be a complete database of full-length, gene sequences—containing not only protein-coding regions, but also the regulatory sequences that govern when and where a protein is produced. A consortium of scientists from across Japan, led by Kazuo Shinozaki and colleagues at the RIKEN Plant Science Center in Yokohama, has pooled their resources to tackle this task, and recently announced a major step forward: the successful sequencing of more than 6,500 complete gene transcripts[1].

They began by pooling RNA isolated from plants cultivated under a wide variety of conditions, such as low temperature or high salt, to ensure expression of as many different genes as possible. They subsequently converted these RNAs into complementary DNA (cDNA), which makes them suitable for cloning and sequencing. They obtained sequence data from nearly 40,000 clones, which were subsequently computationally assembled into overlapping 'sequence scaffolds'. From these, they identified a total of 6,570 full-length cDNAs.

The resulting dataset is important not only in terms of magnitude, but novelty as well. "Our collection is the first full-length cDNA resource of soybean in the world," explains Taishi Umezawa, co-lead author on this work, along with Tetsuya Sakurai. Importantly, many of these sequences represent previously uncharacterized transcripts, as well as quite a few expressed sequences that appear to be soybean-specific—from the raw sequence data, Shinozaki's team identified more than 500 sequences with no apparent equivalent in other plant species.

The team has deposited their data with Japan's National Bioresource Project (NBRP), making them publicly available for broader analysis, and is also collaborating with American researchers towards the annotation of their genomic data. Their findings have also borne commercial fruit, however, in the form of soybean-specific 'DNA chips', now available to the scientific community from Agilent Technologies. "These will be useful for studying gene expression profiles in soybean," says Umezawa, "and we are using them to investigate environmental stress-responsive gene expression." ■

1. Umezawa, T., Sakurai, T., Totoki, Y., Toyoda, A., Seki, M., Ishiwata, A., Akiyama, K., Kurotani, A., Yoshida, T., Mochida, K. *et al.* Sequencing and analysis of approximately 40 000 soybean cDNA clones from a full-length-enriched cDNA library. *DNA Research* **15,** 333–346 (2008).

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Building new connections

Two newly discovered proteins may offer a breakthrough in understanding the function of an enigmatic network of protein fibers

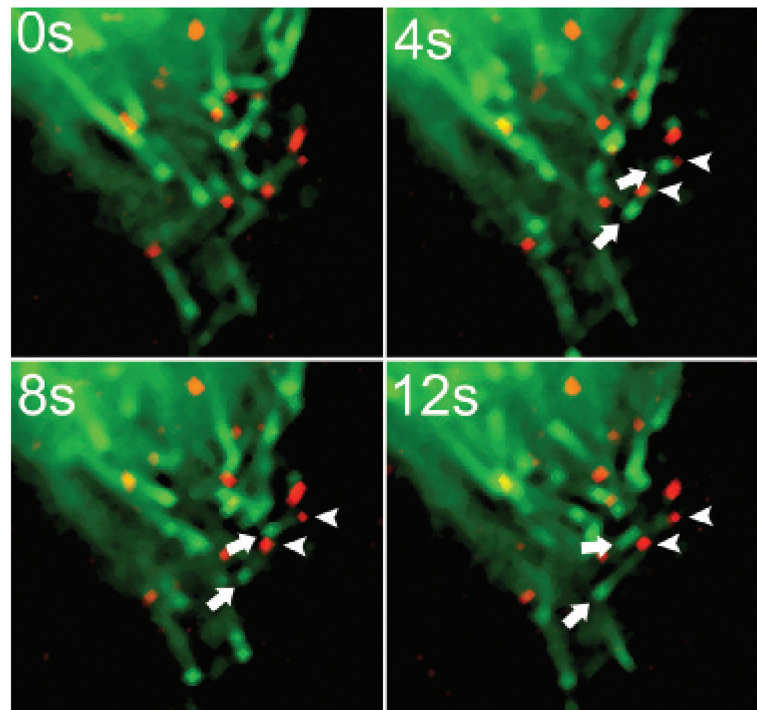

Figure 1: Time-lapse image series of live cells expressing fluorescently labeled Nezha (red, arrowheads) and EB1 (green, arrows). EB1 marks the plus-ends of microtubules, illustrating the growth of microtubules away from minus-end-associated Nezha.

Cells are crisscrossed by microtubules, protein cables that provide infrastructure, which facilitate cellular migration and assist in transport of molecular cargo, among other functions. Most microtubules radiate out from structures known as centrosomes, but many cells also contain non-centrosomal microtubules of ambiguous function that are anchored to yet-unknown cellular targets.

For example, in epithelia—cell sheets that compose tissues including the skin and digestive tract—evidence has suggested that microtubules may interact with adherens junctions (AJs), protein complexes that connect epithelial cells together. "However, it was not clearly understood whether and how microtubules were involved in AJ formation," says Masatoshi Takeichi, of the RIKEN Center for Developmental Biology in Kobe.

Fortunately, a new study by Takeichi's team, including lead author Wenxiang Meng, offers some illumination. The researchers were looking for interacting partners for p120-catenin, a protein that participates in formation of the zonula adherens (ZA)—bands of AJs that encircle epithelial cells, reinforcing their shape and linking them tightly into two-dimensional sheets.

Their search led to the identification of PLEKHA7 and Nezha, two novel proteins that appear to provide the 'missing link' between the ZA and the microtubule network[1]. Nezha binds to PLEKHA7, which interacts directly with p120, and both Nezha and PLEKHA7 localize to the ZA, where they appear to play an important role in maintaining its integrity.

Meng and Takeichi subsequently found that Nezha interacts directly with non-centrosomal microtubules. Every microtubule has a defined 'minus' and 'plus' end, with fiber growth occurring exclusively taking place at the latter. Nezha binds specifically to microtubule minus ends, enabling further extension at the plus end (Fig. 1), and this association seems to play an essential part in enabling PLEKHA7-Nezha stabilization of the ZA.

Although the details of microtubule involvement in the ZA are still unclear, the researchers uncovered a promising lead when they identified a motor protein, KIFC3, which travels along microtubules towards PLEKHA7-Nezha-associated junctions. "Minus-end directed motors like KIFC3 may utilize these microtubules as a 'rail' to transport cargo necessary to maintain the ZA," says Takeichi.

These findings raise many new questions, but also represent major progress in cell biology, confirming the involvement of microtubules in maintenance of cell-cell junctions and revealing factors that help mediate this function. "To my knowledge, Nezha is the first non-centrosomal protein shown to tether the microtubule minus-ends," says Takeichi. "These findings are thus a breakthrough for our deeper understanding of the dynamics and biological roles of non-centrosomal microtubules." ■

1. Meng, W., Mushika, Y., Ichii, T. & Takeichi, M. Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. *Cell* **135**, 948–959 (2008).

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Unraveling fibers

High-resolution structural data about an essential protein reveal new insights into how some cells transform fiber into force

The actin protein exists in two major forms in the cell: as individual molecules of globular (G)-actin, or linked together as long filaments of fibrous (F)-actin. Actin microfilaments provide the primary scaffolding for contractile muscle fibers, and act as a key component of cellular infrastructure in general. However, many cell types also derive their mobility from directional microfilament growth and disassembly, a process powered in part by the hydrolysis of energy-providing adenosine triphosphate (ATP) molecules.

Although crude structural data on actin have been available for well over a decade, these have proven insufficient to provide a detailed understanding of the mechanism of the G-to-F-actin transition and ATP binding and hydrolysis. As such, a high-resolution structure of F-actin published recently in *Nature* by RIKEN SPring-8 Center researcher Toshiro Oda and his colleagues represents an important leap forward in understanding the function of this essential protein[1].

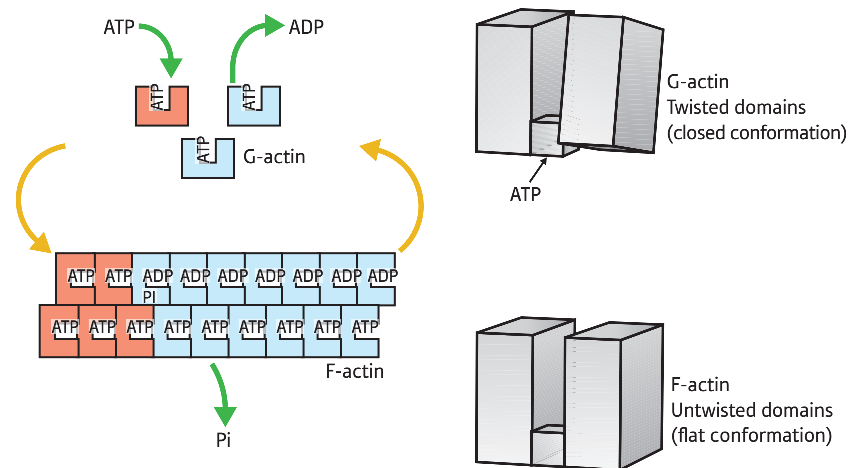

Figure 1: Overview of the actin assembly/disassembly process. G-actin (top right) is bound by ATP (top right) and undergoes a transition into the F-state, enabling it to assemble into fibers (bottom left). This transition involves rotation of the two major domains into a 'flattened' state (bottom right). This shift also enables ATP hydrolysis (top left), subsequently driving subunit dissociation and a return to G-actin conformation.

Their findings revealed a major structural difference between the two states, an overall 'flattening' of the actin molecule as it enters the F state, brought about by a large shift in the relative positioning of the protein's two segments. "A simple rotation of the two major domains of the actin molecule is the essence of the G- to F-actin transition," explains Oda. "This simple rotation produces the flat conformation."

In the current model for microfilament assembly, the association of ATP with F-actin has a stabilizing effect, while the hydrolysis of ATP by actin's enzymatic subdomain is believed to destabilize the F-actin and induce localized disruption of the microfilament. The data from Oda's team indicate that the flattening of F-actin not only drives fiber polymerization, but also leads to other internal rearrangements favorable to subsequent ATP hydrolysis (Fig. 1), although additional analysis will be required to confirm this.

"Our structure in this paper gives us the basis for understanding the biological processes in which actin participates," says Oda, who adds that their data should prove a major asset for muscle researchers as a complement to the abundant high-resolution structural data on myosin—the other major protein component of muscle fibers.

Oda's group, on the other hand, is following up with investigations into the ramifications of their structure for clarifying the process of actin-driven cell-motility. "We're checking our ideas about ATP cleavage and energy usage," says Oda. "Understanding the ATP usage is important for understanding the driving force of the 'actin motor.'" ■

1. Oda, T., Iwasa, M., Aihara, T., Maeda, Y. & Narita, A. The nature of the globular- to fibrous-actin transition. *Nature* **457**, 441–445 (2009).

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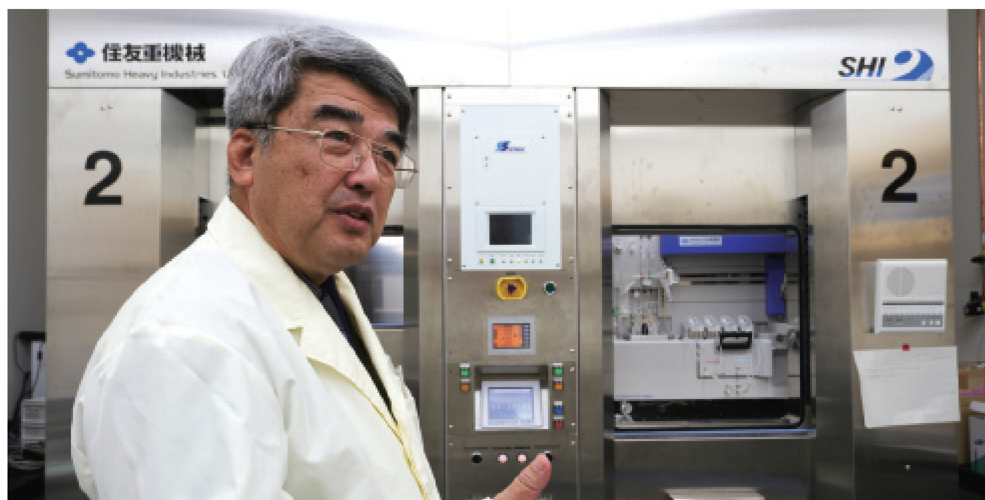
Molecular imaging leads a revolution in diagnosis and drug discovery

Masaaki Suzuki

Team Leader, Molecular Imaging Medicinal Chemistry Laboratory
RIKEN Center for Molecular Imaging Science (CMIS)



Molecular imaging is a technology that helps us probe the location of target molecules in living organisms, including human beings. As the various phenomena in living beings result from interactions among molecules, molecular imaging is expected to become essential for developing a better understanding of life and its processes. Masaaki Suzuki, of the Molecular Imaging Medicinal Chemistry Laboratory, says, "Molecular imaging is the ultimate goal of life science." Molecular imaging is expected to assist in the early detection of lifestyle-related diseases, such as cancer, dementia, and diabetes, as well as in the development of improved drugs with the fewest side-effects much more quickly. This article reports on the forefront of molecular imaging research supported by Japan's "world's best capabilities in chemistry."

Observing molecules in the human body

"To begin with, please take a look at our laboratory, and you will easily understand what we are doing. No other research institute in the world has molecular imaging facilities better than ours," says Suzuki with confidence and a smile.

The RIKEN Center for Molecular Imaging Science (CMIS), formerly the Molecular Imaging Research Program (MIRP), was established in August 2008. The research base of CMIS was moved to the Kobe Molecular Imaging Research and Development Center on Port Island in Kobe City, close to the RIKEN Center for Developmental Biology. Research and development at CMIS is actively promoted by the collaboration of the following five teams and two units:

- Molecular Imaging Medicinal Chemistry Laboratory (Team Leader: Masaaki Suzuki)
- Molecular Imaging Labeling Chemistry Laboratory (Team Leader: Hisashi Doi)
- Functional Probe Research Laboratory (Team Leader: Hirotaka Onoe)
- Molecular Probe Dynamics Laboratory (Team Leader: Yasuyoshi Watanabe)
- Cellular Function Imaging Laboratory: Team Leader: Yosky Kataoka)
- Molecular Imaging Integration Unit (Unit Leader: Kazuhiro Takahashi)
- Metallomics Imaging Research Unit (Unit Leader: Shuichi Enomoto)

"When you look up at the night sky, you can see the moon and stars, and distant galaxies radiating out. Scientists in the twentieth century had a dream of finding new celestial bodies. In the twenty-first century, we are trying to observe the molecules that control life functions in the living human body. This is what we call 'molecular imaging.'"

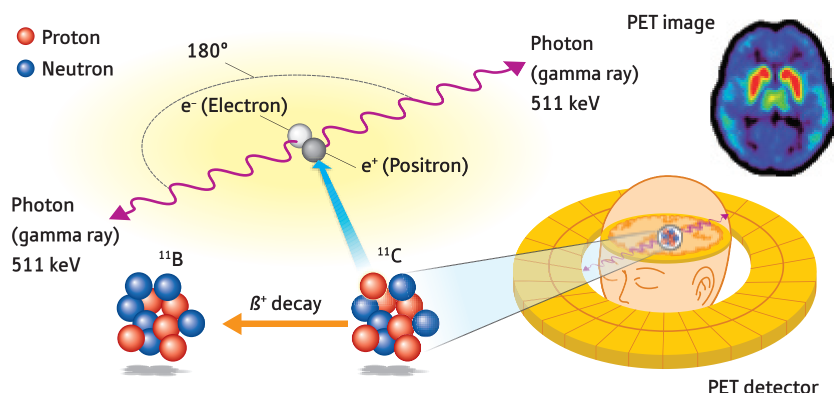


Figure 1: How positron emission tomography (PET) works.
A tiny amount of a positron-emitting radionuclide is combined with a target molecule, and the radiolabeled chemical is then administered by intravenous injection. Carbon-11 (^{11}C) emits a single positron upon decaying to boron-11 (^{11}B). The positron collides with nearby electrons, emitting gamma rays in opposing directions, and the gamma rays are detected by the PET instrument. Tomographic images show the distribution and quantity of target molecules in the organism.

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How, then, can we observe molecules in the human body? A powerful technique is positron emission tomography (PET). PET images are constructed based on the distribution of gamma rays produced by the collision between positrons and electrons (Fig. 1). In the PET process, a tiny amount of a positron-emitting radionuclide is combined with the molecule under investigation, and the radiolabeled product is administered to an organism. The combination of radionuclide and target molecule is called a 'molecular probe'. Once administered, the radionuclide gradually decays to a different nuclide, emitting positrons that collide with nearby electrons. These positron-electron collisions in turn cause the emission of gamma rays, which can be readily detected. This technique therefore makes it possible to determine where, and in what quantities, the target molecules are located in the organism.

PET is frequently used in the diagnosis of cancer. The molecular probe used for cancer diagnosis is fluorodeoxyglucose (FDG), which is a combination of deoxyglucose and fluorine-18 (^{18}F). As proliferating cancer cells take in much glucose as a source of energy, PET can be used to detect the intense gamma emissions generated by the accumulation of FDG in cancer cells. PET has thus improved the accuracy of cancer detection compared to conventional methods of cancer diagnosis based on X-ray and computed tomography scanning techniques.

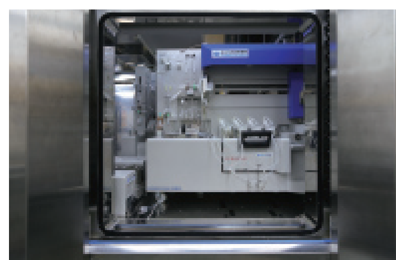
However, as Suzuki points out, "The latent utility of PET is not limited to diagnosis. We have not yet exploited PET to its fullest. We may make a mistake in diagnosis because FDG accumulates not only in cancer cells but also in larger internal organs or normal actively working cells. If there were a molecular probe that interacts with molecules that are expressed only in cancer cells, a more accurate diagnosis could be obtained. Our target is therefore to develop new molecular probes that help PET work more effectively, thus contributing to the imaging of various molecules in the human body."

Rapid C-methylation reaction that achieved the seemingly impossible

What is the process used to construct molecular probes? CMIS has two compact cyclotrons that are used for this purpose (Fig. 2-1). The radionuclide, in this case carbon-11 (^{11}C), is formed by bombarding nitrogen gas with high-speed protons accelerated in the cyclotron. Various nuclides can be produced by changing the target being bombarded according to the specific nuclear reactions. The radionuclides thus produced are fed rapidly to a 'hot cell' for the synthesis of radiolabeled chemicals. The hot cell, referring to a cell used to handle radioactive materials, is completely shielded to prevent radiation leakage into the environment. An automated synthesizer installed in the hot cell (Fig. 2-2) then combines the radionuclides from the cyclotron with target molecules to afford radiolabeled chemicals. This synthesis, however, is a very difficult process.

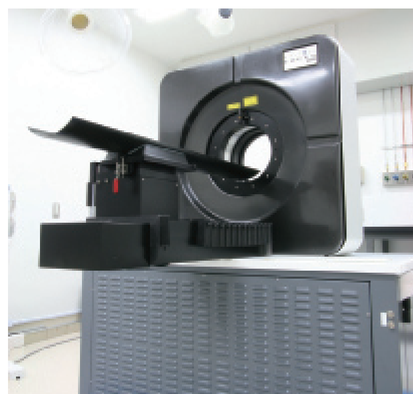
One of the problems encountered in the development of this procedure is the

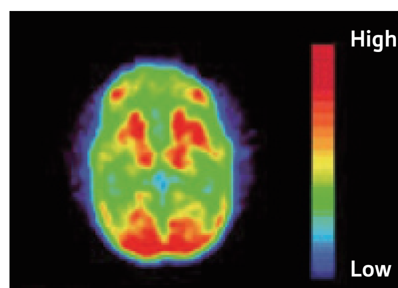
2-1 Self-shielded compact cyclotron
Radionuclides such as ^{11}C or ^{18}F are created in a self-shielded cyclotron with no radiation leakage into the environment.



2-2 Hot cell and automated synthesizer
The hot cell at CMIS has 5 cm-thick lead walls to prevent radiation exposure. The automated synthesizer installed in the hot cell combines the radionuclide and target molecule to produce a molecular probe. All operations are controlled remotely.

2-3 Linear-motor-driven transfer system
The molecular probe produced in the automated synthesizer is stored in a shielded container and transferred to the PET area by a linear-motor-driven cartridge conveyance system.





2-4 Micro-PET system for animals and example tomographic image
The molecular probe is administered to a living animal to monitor the movement of the radiolabeled chemical within the animal's body. The PET image shows the distribution of FDG in the brain of a marmoset monkey.

Figure 2: From constructing molecular probes to taking PET images.

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length of time needed for synthesis. The ^{11}C radionuclide has a radioactive half-life (the period over which the number of radionuclides decreases by one-half due to radioactive decay to another nuclide) of only 20 minutes. Taking account of the time required to purify the molecular probes and transfer the radiolabeled chemical to another area for diagnostic use, a time window of only five minutes remains for combining the ^{11}C and the target molecule. You might think that radionuclides with a longer half-life could be used. However, Suzuki asserts, "We use only radionuclides with a short half-life. If we dealt with radionuclides with a longer half-life, there would be a risk of radiation exposure. Long-half-life radionuclides cannot be used in humans. We should instead choose radionuclides that are already present in our bodies and which have as short a half-life as possible. Therefore, ^{11}C is the best choice."

However, there were major difficulties in applying ^{11}C in this procedure because no method was previously known for the efficient combination of ^{11}C atoms with carbon-containing target molecules at time scales of a few minutes. Furthermore, none of the labelling sites in the molecules were expected to be metabolized immediately. "Someone even told me that my idea was out of the question. However, the more difficult a problem seems, the more likely you are to take up the challenge." Eventually, Suzuki succeeded in developing a 'rapid C-methylation reaction' that combines ^{11}C-methyl groups ($^{11}CH_3$–) with carbon atoms in organic molecules in just five minutes (Fig. 3). By conventional chemical reactions, this combination reaction would take from a few hours to several dozen hours to complete. Furthermore, this highly efficient reaction can be used to combine ^{11}C-methyl groups with almost all organic molecules. "It took five years to successfully develop a chemical reaction that takes just five minutes." What was the key to this success? "Inspiration and perseverance," answers Suzuki.

Purification and enrichment of the radiolabeled chemicals are conducted automatically in an automated synthesizer, and the molecular probe bearing the radionuclide at the desired atomic sites is isolated in a glass vessel housed within a shielded lead container. The molecular probe is then transferred by the most recent linear-motor-driven conveyance system to a PET system for animals within the same laboratory (Fig. 2-3). The transfer of the molecular probe takes about one minute, and once loaded into the PET system, the molecular probe is promptly administered (Fig. 2-4). Mice are used as test subjects to monitor whether the probes move as expected, and the results are fed back to the team at the Molecular Imaging Medicinal Chemistry Laboratory for design changes as required. This process is repeated several times during the development of a new molecular probe (Fig. 4).

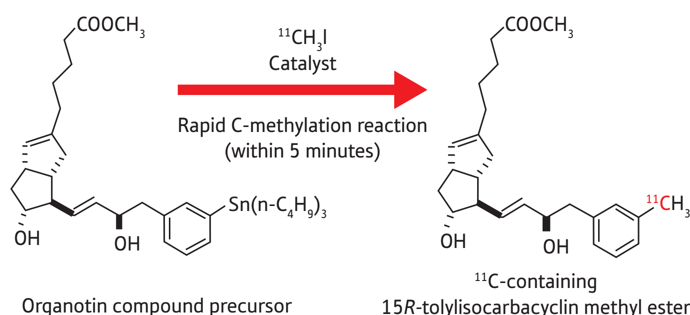

Figure 3: Rapid C-methylation reactions.
Carbon-11 (^{11}C) produced in the cyclotron reacts with a tiny amount of oxygen to afford carbon dioxide ($^{11}CO_2$) bearing the ^{11}C radionuclide. In the automated synthesizer, $^{11}CO_2$ is converted to methanol ($^{11}CH_3OH$) in the presence of a reducing agent. The methanol is then reacted with iodic acid (HI) to afford methyl iodide ($^{11}CH_3I$). Methyl groups (CH_3–) in the target molecule are then substituted with radiolabeled methyl groups by reaction with the ^{11}C-containing methyl iodide.

Straight through from basic study to human beings

At the next stage of development, molecular probes that have proved useful in animal experiments are administered to humans. CMIS has installed areas that conform to Good Maintenance Practice (GMP) standards, which are a set of manufacturing and quality control regulations established for the provision of safe and high-quality drugs and medicines, and these areas will become operational in the near future. Molecular probes created with the utmost care in the GMP synthesis area will be transferred to the Institute of Biomedical Research and Innovation, which is linked to CMIS via a connecting bridge. There, the molecular probes will be administered to humans.

Port Island in Kobe City hosts the Kobe Medical Industry Development Project. The RIKEN Center for Developmental Biology, the Institute of Biomedical Research and Innovation, and civilian hospitals are also located close by in the same cluster. "That is the reason why RIKEN chose this site. This location provides us with opportunities in the medical field to immediately test the most advanced results obtained through fundamental studies. We conduct basic studies at RIKEN from start to finish, but we can only contribute to society if the results can be successfully applied to humans." The Next Generation Supercomputer being developed by RIKEN, due for completion in 2012, is also to be located on Port Island, and collaboration with this new facility is anticipated.

How will molecular imaging change the medical field? "In 10 years, we expect PET to be used regularly for diagnosis and treatment. It will allow the early detection of various diseases, including lifestyle-related diseases such as dementia, cancer, and diabetes," says Suzuki.

To that end, researchers need to develop a library of disease-specific molecular probes. "If candidate molecules for diagnosis can be found, it remains only for chemists to process these into molecular probes. This is not a problem

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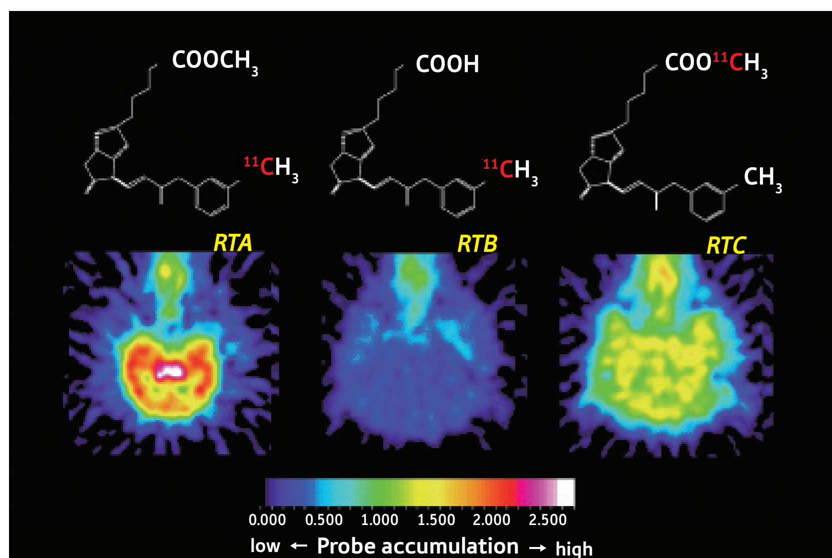

Figure 4: Examples of molecular probes.
Examples of PET brain images of monkeys after administering ^{11}C-substituted 15*R*-tolylisocarbacyclin methyl ester as a molecular probe. Depending on the site of ^{11}C substitution in the target molecule, the molecular probe may fail to reach the brain (center) or fail to accumulate in a specific area (right). The molecular design is indispensable to ensuring that the molecular probe accumulates in the desired manner.

for us," says Suzuki confidently. Recent advances in genome analysis also back up his confidence because it has become easier to find candidate molecules. "Japan is aging at a rapid pace," says Suzuki. "We will surely face health problems with the elderly, such as dementia. We want to expand the molecular probe library so that many more patients can be diagnosed."

Another item that is high on the list of aims is the contribution to drug discovery. "A drug discovery revolution will occur," asserts Suzuki. At present, candidate drugs are administered to mice, and only when proved to be very efficient are the drugs finally administered to humans in clinical trials. Many candidate drugs are eliminated at this stage because of side effects or ineffectiveness in the human body, even if they have proved effective in mice. The development of even a single new drug is thus very expensive and laborious, and is a task that takes many years. "Molecular imaging will be the solution to this problem," says Suzuki.

By preparing molecular probes from candidate drugs, researchers can confirm the effects of the drug on the human body before starting clinical trials. Such a molecular probe will interact with target receptor molecules, but also possibly with unexpected molecules. If the unexpected interactions prove to induce side effects, the developer has an opportunity to change the structure of the candidate drug such that it interacts more specifically with the target receptor molecules. In this manner it is possible to considerably reduce the time and cost incurred in the development of new drugs.

On 28 December 2007, the Japanese Ministry of Health, Labour and Welfare released a draft version of Guidelines for the Conduct of Microdose Clinical Trials, and also a revised draft version of the Building and Facility Standards for Manufacturing Facilities for Investigational Medical Products (Investigational Medical Product GMP). A microdose clinical trial is a clinical study conducted in the early stages of drug development, and involves the administering of a single 'microdose' of one or more test substances to humans in order to examine its intended and side effects. The conventional Investigational Medical Product GMP targets only the bulk production of drugs, whereas the revised version permits more flexible operations, such as microdose clinical trials. The formal version of the guidelines was released on 3 June 2008. With these developments, molecular imaging is becoming a reality.

Japan has the world's best capabilities in chemistry

Many international scientists visit CMIS. "They are all astounded at the high level of our advanced studies," says Suzuki. Why is CMIS taking the lead in molecular imaging research? "The reason is that Japan has the world's best capabilities in chemistry. Successful research on molecular imaging requires the fusion of all fields of sciences, including chemistry, medicine, pharmacy, biology and engineering. Among these, chemistry is the most important," says Suzuki. The combination of radionuclides with molecules and the alteration of molecular structures are both based on chemical reactions. "Japan has the world's best capabilities in chemistry; Japanese scientists have won the Nobel Prize in Chemistry for three consecutive years. In fact, the president of RIKEN, Ryoji Noyori, is himself a Nobel Laureate in Chemistry," says Suzuki.

Noyori presented PET images of human brains during his Nobel lecture in 2001. For these images, Suzuki himself served as the human subject. "Dr Noyori listed molecular imaging as one of the most important technologies that should be promoted in chemistry. Dr Noyori was my teacher. I want to use the advantages of chemistry to promote molecular imaging so that it can yield significant results." ■

About the researcher

Masaaki Suzuki was born in Gifu, Japan in 1947. He graduated from Nagoya University in 1970 and obtained his PhD in 1975 (Prof. Y. Hirata) from the same university. After postdoctoral training at Harvard University (Prof. R.B. Woodward) until 1977, he returned to Nagoya University and served as assistant professor with Prof. R. Noyori until 1983. He then worked as associate professor at the same university before attaining full professorship at Gifu University, Japan, in 1993. He served as a professor there until 2007, when he was appointed vice program director of the RIKEN Molecular Imaging Research Program. He is now vice director of RIKEN CMIS in Kobe, Japan.

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RIKEN adopts Semantic Web as data-release standard for database-construction platform

RIKEN has developed a common infrastructure called RIKEN SciNeS (Science Networking System) to execute large-scale releases of data in a format conforming to the international Semantic Web standard. This infrastructure unifies the various platforms used within RIKEN for the construction of databases in the life sciences. Development of this infrastructure was carried out by a research team led by Tetsuro Toyoda of RIKEN Bioinformatics and Systems Engineering (BASE) division.

In recent years the amount of data handled in life science research has grown considerably. This in turn has led to increased opportunities for individuals to present their findings not only in the form of research papers, but also as databases accessible via the Web. However, while sharing of data through research papers is facilitated by specialized publications such as scientific journals, an individual researcher wishing to publish and release a database must launch and manage their own website. The operational costs involved in maintaining a database after it has been made available online have proved to be particularly burdensome for researchers. Moreover, as the number of individual researchers independently launching websites increases, so too does the number of websites that fail to comply with international standards. Such non-standard sites have caused confusion for users, and steps toward the integrated application of services have likewise been hampered.

In response to this situation, RIKEN BASE has developed RIKEN SciNeS as a common infrastructure that makes it possible for researchers to publish databases containing their entire set of research results without requiring them to maintain web servers themselves. Envisioned to ultimately accommodate tens of thousands of research groups or more, RIKEN SciNeS is designed to assist researchers in independently organizing virtual research projects in cyberspace. The newly developed database-construction platform isolates each research project, maintaining confidentiality of data while granting each project flexible setup functionality for management of unpublished information. The system also provides control over the operational flow of research mediated by large-scale data sets. RIKEN SciNeS is expected to improve data management within research projects, and to serve as a new medium for academic work. Clusters of databases constructed with this platform, moreover, follow international standards, thus facilitating the public release of data. RIKEN will proceed with intensive operation and maintenance of these databases via the RIKEN Hub Database, enabling researchers at RIKEN from various academic fields to play a central role in international collaborative research.

The present research was conducted as an internal collaborative promotional project using RIKEN funds for strategic discretionary research. The RIKEN Hub Database and a trial version of RIKEN SciNeS compatible with the Firefox Web browser were released on 31 March 2009 (http://database.riken.jp/). ■

RIKEN–University of Edinburgh Joint Workshop for Computational and Systems Biology

A two-day joint workshop organized by RIKEN and the University of Edinburgh was held at the British Embassy in Tokyo on 14–15 May, featuring presentations by leading researchers in the fields of Systems Biology, Computational Biology and Metagenomics.

In an opening speech, Igor Goryanin, Director of the Edinburgh Centre for Bioinformatics, described collaboration between Japan and the UK in the area of systems biology as among the strongest in the world. "Systems biology is recognized as a science of the future, and in the UK and Europe there is huge investment," he said. "Nowadays high-speed, high-performance computing is essential for simulations, and RIKEN is a leader in this area."

In the area of metagenomics, Masahira Hattori of the University of Tokyo's Department of Computational Biology outlined findings on gut microbiota. "The challenge is to analyze genetic and biological features of gut microbiology," Hattori explained, "and identify molecules and genes involved in important interactions."

Todd Taylor, leader of the MetaSystems Research Team at RIKEN Advanced Science Institute, highlighted the difficulty of "trying to catch up with the deluge of metagenomic data" being produced by metagenomic analyses. One solution is the comprehensive Metagenomic BioMining Engine (MetaBioME), a search engine for mining metagenomic data under development at RIKEN Advanced Science Institute. MetaBioME will allow users to explore categorized data sets and potentially discover useful enzymes.

David Harrison, head of the Division of Pathology at University of Edinburgh, stressed in a presentation on the "moving target" of pathology that current "protocol-driven, static treatments" to cancer must be replaced by a focus on temporal and spatial change. "The major problem," he said, "is how to predict what will happen after we have taken the static step of removing the tumor."

In a presentation on the systems biology approach to breast and ovarian cancer prognosis, Simon Langdon of the Edinburgh Cancer Research Centre's Cell Biology Group provided one potential solution to this problem in the form of cancer xenograft models, which are used to study cell signaling and response prediction.

The two-day workshop ended with closing remarks by Taiji Makoto of RIKEN Advanced Science Institute, who spoke about the application of molecular dynamics simulations in molecular biology. ■

RIKEN and the Indian Institute of Technology Bombay launch International Joint Graduate School Program

RIKEN has signed a renewable five-year agreement with the Indian Institute of Technology Bombay (IITB) to launch an International Joint Graduate School Program for PhD students in science and technology. The program aims to strengthen collaborative ties between RIKEN and IITB by offering a top-class research environment for talented young IITB graduate students.

PhD research by the IITB students will be supervised by a RIKEN scientist appointed as visiting professor at IITB during the term of the agreement. One or more academic staff or professors will be designated to act as co-supervisors, and a second RIKEN scientist may also be appointed to the position of Associate Visiting Professor at IITB.

PhD students or suitable candidates interested in the Joint Graduate School Program must submit a written research proposal to the visiting professor and to the designated IITB academic staff. Successful applicants will be offered the opportunity to visit RIKEN for up to three years and make use of RIKEN state-of-the-art facilities and equipment under the supervision of the visiting professor and designated IITB academic staff.

An IITB doctoral degree will be awarded to PhD students who successfully complete their research to the standards of IITB, and an additional joint certificate will also be awarded to the student by RIKEN and IITB. ■

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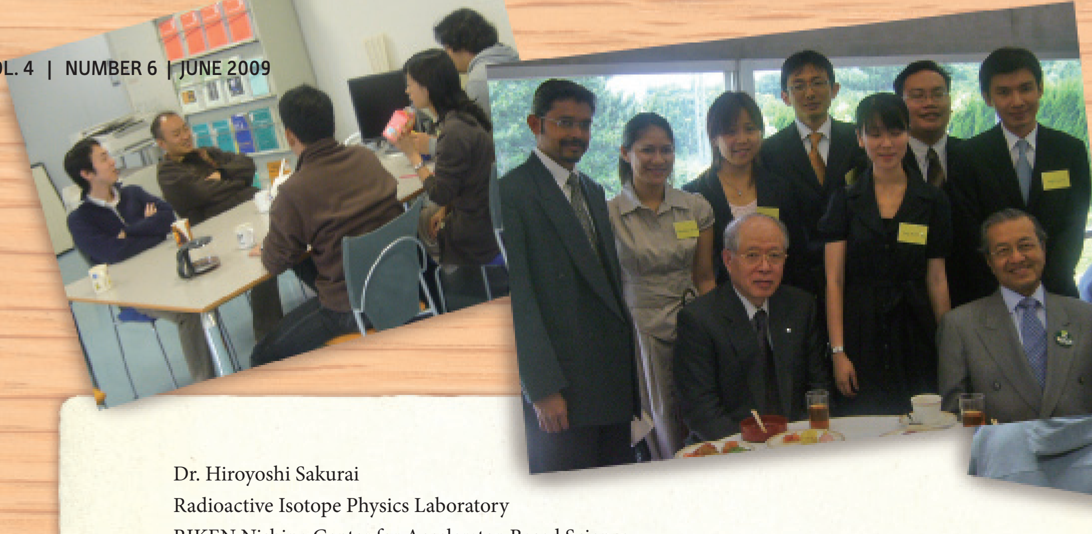

Dr. Hiroyoshi Sakurai
Radioactive Isotope Physics Laboratory
RIKEN Nishina Center for Accelerator-Based Science
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351-0198 JAPAN

Dear Dr. Sakurai,
How are you? Some time ago, I was asked about my research life at RIKEN. Sitting at home watching the Beijing Olympics then, I thought to myself, "If having the opportunity to come and study at a Japanese university is like qualifying for an Olympic games, then having the opportunity to do research at the world-class RIKEN would be like winning a gold medal." Yes, that's how I would recall my unexpected arrival at RIKEN. I have become more motivated, enthusiastic and confident thanks to my six years doing research at RIKEN, first as a graduate student and later as a postdoc. In fact, it was at RIKEN that I learned how to carry out nuclear physics experiments using radioactive nuclear beams, as well as how to become a good researcher.

I can still vividly recall my first day at RIKEN when I was given a guided tour to the renowned projectile fragment separator (RIPS) by my senior. There, I witnessed researchers and students working hand-in-hand in preparation for an experiment. You were my supervisor then, and always told us that we would be treated as equals at RIKEN, although you always reminded us to be professional.

At RIKEN, we were trained to do almost everything by ourselves: from writing an experiment proposal, and setting up the detectors and the data acquisition systems before the experiment, to writing the resultant paper for publication. Although still far from being perfect, I am proud that I have managed to achieve each step. For this, I am grateful to you and my former colleagues at the RIKEN Nishina Center. Without your dedication during the experiments and pertinent advice in times of need, I would not have achieved much.

My life at RIKEN was always full of joy and laughter. I miss, among others, the inexpensive lunch at the cafeteria, and the daily chat over a cup of coffee after lunch. Of the places I have been to, RIKEN is one of the best as far as opportunities for meeting and making new friends are concerned. There, I have befriended not only physicists but also many biologists, chemists and administrative staff. I enjoyed the RIKEN annual open house held every April. It was always refreshing talking and explaining our work to the visiting school children and students. I also enjoyed the many parties held throughout the year by the Radioactive Isotope Physics group and the Heavy Ion Nuclear Physics groups—as well as the cooking parties on the fourth floor of the RIBF building. All these activities and friendships helped brighten up my life and endure the otherwise challenging and stressful life of a researcher.

I have to admit that even after almost a year, I still reminisce about the days I spent at RIKEN. I have, on several occasions, returned to RIKEN on business trips. During each trip, I met friends and brewed coffee after lunch with the same coffee maker that I used to use, which unfortunately has been left unattended of late. Old habits die hard, and I think I will continue to brew coffee during my next trip(s).

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

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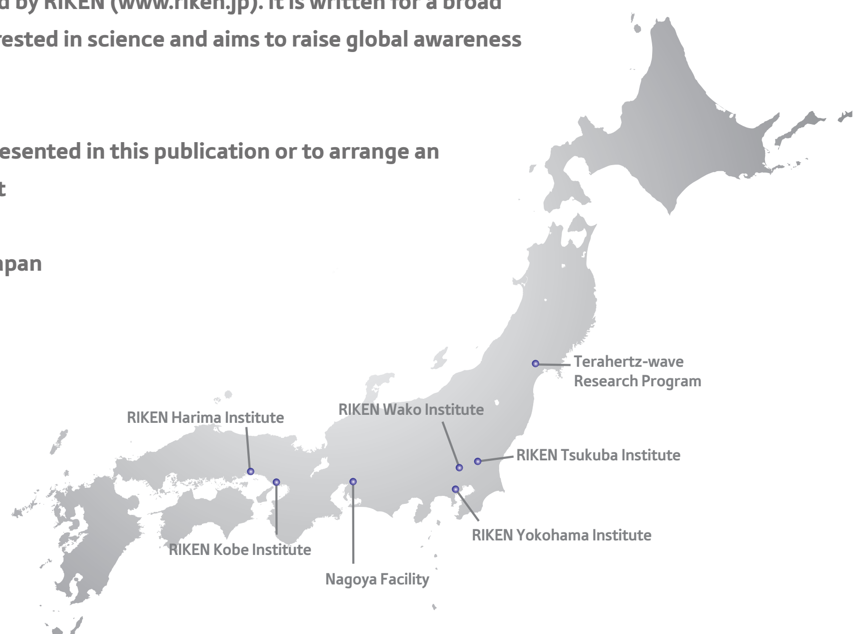

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