RIKEN RESEARCH

JULY
2007 Volume 2 Number 7

A taste of success in enzyme hunt

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Hunting for the genetic basis of arthritis

New work identifies a defective gene associated with osteoarthritis

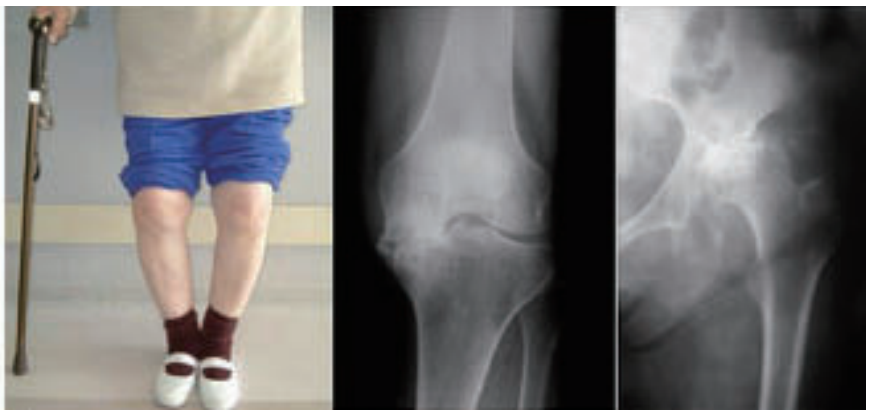

Figure 1: Deformed lower extremities of a patient with osteoarthritis (left) and radiographs of his knee (middle) and hip (right) joints. The cartilage is lost and aberrant bone is formed.

A recent study links osteoarthritis, the most prevalent form of arthritis in humans, with a defect in a gene encoding a protein found in human joints. Osteoarthritis patients exhibit mild but nevertheless damaging joint inflammation. This inflammation, which can occur in any joint in the body, destroys cartilage, a type of connective tissue that lines and maintains joints. Cartilage acts as a 'buffer', effectively preventing bones from grinding against one another. Bone-on-bone friction ultimately causes the chronic pain, as well as the frequent subsequent muscle and ligament weakening, that characterizes this debilitating disease.

A team led by Shiro Ikegawa, a scientist at RIKEN's SNP Research Center in Tokyo, linked a variation in the sequence of the gene *GDF5*, which encodes growth and differentiation factor 5 (also known as cartilage-derived morphogenetic protein 1), with the occurrence of osteoarthritis. Their work was recently published in *Nature Genetics*[1].

Preparing for the hunt

Cartilage consists primarily of collagen, a strong structural protein, and proteoglycans, which are proteins decorated with carbohydrates. The chondrocyte, the only type of cell found within cartilage, produces and sustains this collagen and proteoglycan 'matrix'. The researchers focused on GDF5 because previous work indicates that this protein, which is released from chondrocytes in an active dimeric, or paired form, promotes the growth and maturation of chrondrocytes, as well as chondrocyte production of proteoglycans that contribute to the collagen matrix.

Moreover, prior studies demonstrated that GDF5 mutations or *GDF5* deficiency result in impaired joint development in humans and mice. In contrast, mice expressing excessive amounts of GDF5 exhibit abnormally thick and expanded cartilage. In addition, some other human diseases characterized by joint degeneration, such as type C brachydactyly and angel-shaped phalangoepiphyseal dysplasia, are associated with mutations in *GDF5*.

The team analyzed the sequence of the gene encoding GDF5 in 24 Japanese patients suffering from hip osteoarthritis (Fig. 1). This analysis identified 52 variations or 'polymorphisms'. To determine if any of these variations hold potential to be disease-causing mutations, the researchers used statistical methods to calculate whether any one mutation occurred more frequently than the expected random frequency in osteoarthritis patients (Fig. 2).

One mutation appeared at significantly greater than random frequency in the genome of patients with hip osteoarthritis. Notably, the finding was replicated using independent strategies for detecting mutations, and was found in the genome of two much larger independent groups of Japanese and Chinese patients with knee osteoarthritis. Additional studies certified that the statistical link between the mutation and osteoarthritis remained strong regardless of age, sex or body mass index of patients and healthy controls.

Establishing the link

Next the researchers investigated how this mutation might influence osteoarthritis

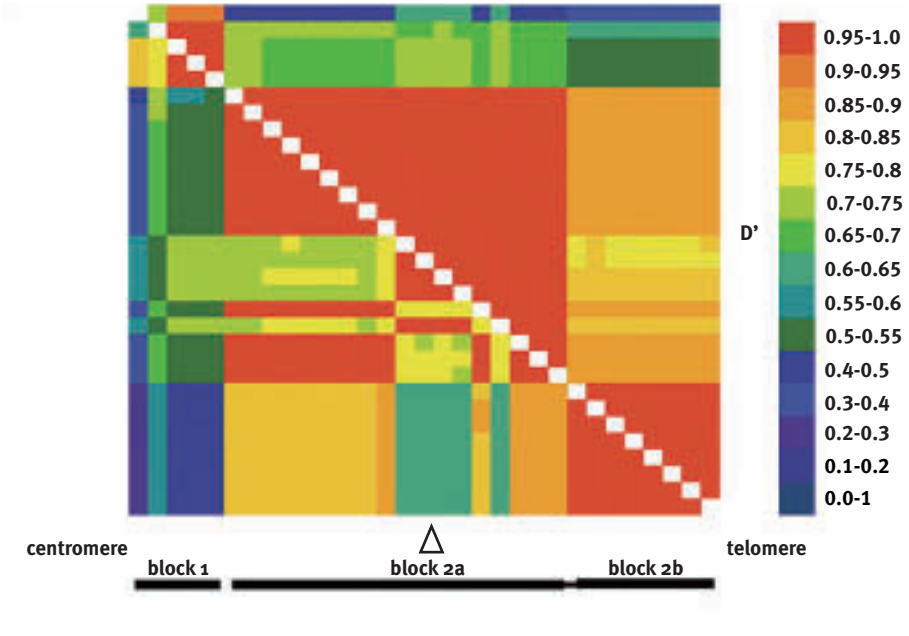

Figure 2: Statistical analysis of the occurrence of sequence variations in and around *GDF5* in samples from osteoarthritis patients. Higher numbers indicate more frequent occurrence.

development. They mapped the mutation to the *GDF5* promoter region, which is a stretch of DNA that controls the timing and level of gene expression. To identify the exact promoter sequences responsible for driving *GDF5* expression, the team tested the ability of small promoter fragments to trigger expression of a fluorescent beacon 'reporter' within chondrocyte-like cells.

Encouragingly, the promoter mutation linked with osteoarthritis susceptibility lies precisely within the sequence essential for *GDF5* promoter activity. Cartilage cells containing the mutant *GDF5* promoter, or promoter fragments lacking the region in which the mutation is found, expressed lower amounts of *GDF5* than cells containing an unaltered *GDF5* promoter.

Why this particular promoter mutation blunts *GDF5* expression is not known. This alteration might disrupt a conserved sequence recognized and bound by gene activation machinery. Alternatively, this mutation might unexpectedly create a sequence able to recruit gene suppression machinery. Regardless, these findings indicate that sufficient *GDF5* expression is required to maintain collagen production and joint health.

Advancing efforts for new treatments

The team's data also highlight *GDF5* as a potential focus of future studies to develop preventative, as well as ameliorative, osteoarthritis treatments. The disease is currently most commonly treated with anti-inflammatory medication, which can sometimes stave off additional cartilage destruction. However, a bona-fide cure for osteoarthritis awaits innovative technologies capable of regenerating lost cartilage.

Early detection of *GDF5* mutations and measurement of GDF5 production could help identify patients potentially at increased risk of developing osteoarthritis. Whether artificially replacing or 'tuning' *GDF5* expression to levels approximating those in healthy mice and humans might help postpone the onset of osteoarthritis, or reduce the severity of existing osteoarthritis, remains to be seen.

"Osteoarthritis is a serious world health problem, with its significance highlighted by the program called *Bone and Joint Decade*, which was launched in 2000 at the World Health Organization," explains Ikegawa. "The present discovery will open a new window for the study of etiology and pathogenesis of osteoarthritis, a major health problem in developed countries. We want to continue this work to further advance efforts to develop a cure for osteoarthritis patients, who are estimated to be more than 200 billion around the world." ■

1. Miyamoto, Y., Mabuchi, A., Shi, D., Kubo, T., Takatori, Y., Saito, S., Fujioka, M., Sudo, A., Uchida, A., Yamamoto, S., Ozaki, K., Takigawa, M., Tanaka, T., Nakamura, Y., Jiang, Q. & Ikegawa, S. A functional polymorphism in the 5' UTR of *GDF5* is associated with susceptibility to osteoarthritis. *Nature Genetics* **39**, 529–533 (2007).

About the researchers

Shiro Ikegawa was born in Osaka, Japan, in 1957, and graduated from the Faculty of Medicine, the University of Tokyo, in 1983. After spending 12 years as an orthopedic surgeon, he became a student in the Department of Biochemistry at the Japanese Foundation for Cancer Research in 1995. He then returned to clinical practice as the chief orthopedic surgeon at the National Rehabilitation Center for Disabled Children, and later became a research associate at the Institute of Medical Science, the University of Tokyo (Yusuke Nakamura's lab), in 1997. Since 2001, he has been the head of the Lab of Bone and Joint Diseases, SNP Research Center (SRC), RIKEN. His research interest is a genetic study of bone and joint diseases (monogenic and polygenic disease).

A new tool for tracking the tiniest changes

A robust new method for genomic analysis could extend the reach of personalized medicine within the developing world

Even the smallest genetic variations, known as single nucleotide polymorphisms (SNPs), can have important implications for an individual's predisposition to certain diseases or response to specific medicines. As such, analyzing the SNPs of a given patient can reveal important health information and make it possible to develop personalized therapeutic strategies.

Most techniques for SNP screening rely on a method called the polymerase chain reaction (PCR), which exploits a DNA-replicating enzyme to rapidly amplify genomic sequences for analysis. The amplified region is determined by a pair of short, single-stranded DNA molecules called 'primers', which are designed to match target sequences in the genome. In principle, an identical primer-target match is required for sequences to be amplified, enabling the use of specific primers to identify particular SNP variants.

PCR is a powerful tool for DNA amplification, but also suffers from a number of liabilities that can limit its clinical power. Assays may generate false-positive results due to contamination or the amplification of inappropriate targets, and therefore careful sample preparation is essential—especially for clinical applications. Each PCR reaction is a multi-step process, where samples need to be 'cycled' through a series of incubations at different temperatures, and identifying the proper reaction conditions for amplifying a given target sequence can be challenging. Because of these requirements, PCR requires the use of specialized and fairly expensive equipment, which makes it a less practical solution for clinical facilities with limited resources.

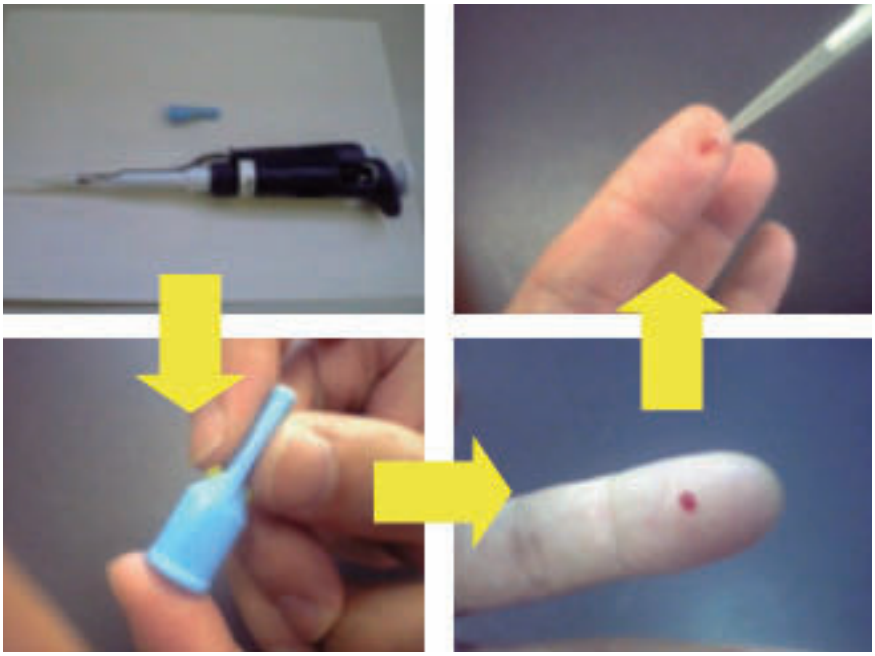

Figure 1: SMAP 2 diagnostic assays can be performed directly on blood samples, simplifying sample collection and preparation.

Enter SMAP 2—a new tool for point-of-care diagnostics

"Existing technologies are generally complex in design and consequently more expensive," explains Yoshihide Hayashizaki of the RIKEN Genomic Sciences Center in Yokohama. "They take longer, and require more preparation." In a recent article from *Nature Methods*, Hayashizaki and his colleagues describe an alternative DNA amplification method, smart amplification process version 2 (SMAP 2), which they suggest could provide a strong alternative to PCR for point-of-care diagnostics[1].

SMAP 2 uses five primers instead of two; one of these is selected to serve as the 'discrimination primer', which is designed to reveal the presence or absence of a target mutation. For each SMAP 2 assay, different versions of the discrimination primer are created, where each version can only bind to a specific SNP variant. By analyzing the amplification that takes place with different discrimination primers, it becomes possible to characterize a patient's genomic variations.

Advantages of SMAP 2

SMAP 2 is extremely precise, and much of this precision is gained from a unique feature of the assay—the addition of MutS, a purified bacterial protein that eliminates 'background signal' resulting from the amplification of inappropriate sequences. As a test of the discriminatory power of SMAP 2, Hayashizaki's group demonstrated the assay's ability to accurately detect minor variations in one member of the cytochrome P450 gene family, a group of closely related genes with very similar sequences.

SMAP 2 offers a number of other advantages over PCR as a clinical tool.

Figure 2: Hayashizaki's group and collaborators have developed a SMAP 2-based kit for screening EGFR gene mutations.

Unlike the multi-step process of PCR-based detection, SMAP 2 samples need only be incubated at a single temperature for 15 to 30 minutes. Additionally, no DNA purification is required to prepare samples for SMAP 2—assays can be performed directly on raw blood or tissue samples (Fig. 1), making this an ideal tool for rapid patient screening. Most importantly, SMAP 2 integrates the amplification and detection process, so that no signal is generated if amplification has not taken place, and sequence analysis therefore becomes simple and accurate.

The rules governing effective primer design are not entirely clear, and the initial development of a SMAP 2 assay can be somewhat complicated compared to PCR, but Hayashizaki and colleagues have developed a computer program that considerably simplifies this process. "It may take a week to a few months to create an optimized assay for routine diagnostic use," he says. "But with more experience and the creation of intelligent software algorithms, we expect to simplify this process enormously in the very near future."

Adapting SMAP 2 for clinical use

Since the initial publication of this work, the development and commercialization of effective diagnostic tools has become a top priority. Hayashizaki's team has already begun adapting SMAP 2 for clinical use, and recently applied the technology toward the detection and analysis of mutations in the epidermal growth factor receptor (EGFR) gene, a useful diagnostic indicator for lung cancer.

These results will be published later in the year, and Hayashizaki's group recently announced the launch of a collaboration with Singaporean researchers and doctors at the National University of Singapore and the National University Hospital, with the aim of developing a simple and rapid EGFR screen (Fig. 2). Accurately identifying such mutations can help doctors to apply an appropriate therapeutic strategy, but current diagnostic procedures require about three weeks—and surgery. SMAP 2-based diagnosis could reduce this time to as little as five hours, and would require only a tiny biopsy sample, making screening faster and more comfortable for patients.

Hayashizaki makes it clear that SMAP 2 is not intended as a replacement for PCR as a research tool, but initial findings clearly suggest that this technique could be a powerful method for extending the reach of genetic analysis as a clinical tool. "One of the greatest potentials for using knowledge of genetic information is in the area of personalized medicine," he says, "and SMAP has the added potential to be employed at point-of-care since the technology is very simple and very robust."

1. Mitani, Y., Lezhava, A., Kawai, Y., Kikuchi, T., Oguchi-Katayama, A., Kogo, Y., Itoh, M., Miyagi, T., Takakura, H., Hoshi, K. *et al.* Rapid SNP diagnostics using asymmetric isothermal amplification and a new mismatch suppression technology. *Nature Methods* **4**, 257–262 (2007).

About the researchers

Yoshihide Hayashizaki was born in Osaka, Japan, 1957. He received his MD and PhD from Osaka University Medical School in 1982 and 1986, respectively. From 1988 to 1992, he worked as a research scientist at the National Cardiovascular Center Research Institute (Department of Bioscience), in Osaka, and developed a new technology known as the Restriction Landmark Genome Scanning (RLGS) System. In 1992, he joined RIKEN, and was appointed Project Director for the RIKEN Genome Project in 1995. Since then he has been taking part in, and aiming for, the establishment of a Mouse Genome Encyclopedia. His present position is project director of the Genome Exploration Research Group, Genomic Sciences Center, RIKEN. He organized the FANTOM (Functional Annotation of Mouse cDNA) consortium to annotate all of the RIKEN mouse clones. During this work, he has been able to discover a large amount of non-protein coding RNAs, so-called RNA continent. He also developed the DNABookTM, which enables the efficient distribution of a large amount of clones. Currently he works toward an illumination of the gene transcriptional network.
In 2001 he was assigned as a foreign adjunctive professor of the Karolinska Institute (Sweden) and an honorary professor of the University of Queensland (Australia).

Tailoring for gold

Structurally specific monolayers with molecular conductivity are the first steps towards biosensors

A new, self-assembled monolayer (SAM) of cage-like compounds could provide a route to new molecular electronic devices. The SAM, formed from a layer of functionalized adamantane—a chemical compound known as a cycloalkane with three linked rings, was prepared on a flat gold surface by a team of Japanese researchers. Studies of the SAM show that there is a high level of organization of the adamantanes which means this SAM could be a starting point for devices to measure molecular conductivity.

As devices to measure molecular conductivity could be incorporated into new technologies in the future they attract much attention by scientists. Now, Yousoo Kim from the RIKEN Discovery Research Institute in Wako, and colleagues from several other Japanese research organizations, have developed a method that creates a regular yet complex pattern across a surface capable of this behavior.

The team used molecules of bromo adamantane trithiol on gold. These adamantane derivatives have a cage structure with a tripod base allowing it to stand proud of the surface. The tripod is formed from three thiol feet with the chemical formula CH_2SH that bond to the metal surface, by replacing the S–H bonds with S-metal bonds; a delicate procedure that requires careful handling to avoid destroying the molecules.

Using scanning tunneling microscopy (STM) the SAMs were found to be organized into a two-tiered hierarchy. The cages arrange into trimers that are chiral—structurally specific mirror images—and each trimer then forms hexagons with the chirality passed on

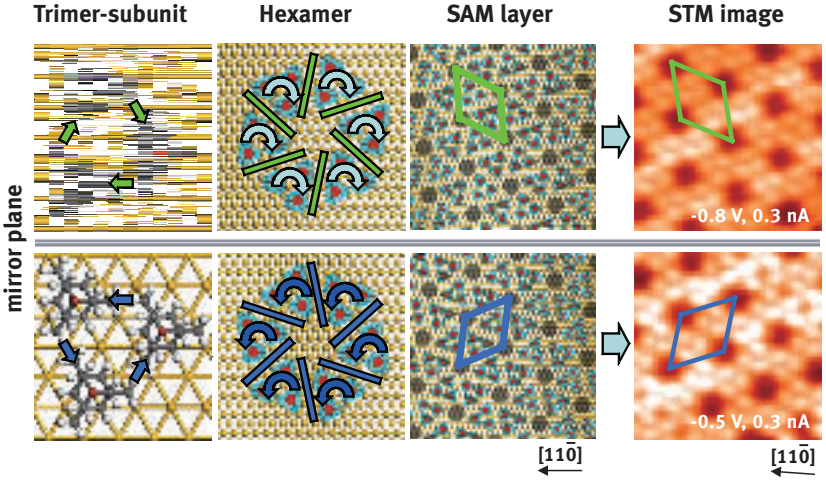

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Figure 1: Views of the hierarchical chiral assembly of the adamantane derivative on a gold surface—from molecule to microscopy.

(Fig. 1). Two possible chiral forms can be formed and STM measurements show that both exist.

This study is the first to demonstrate that a molecule with no chirality can make chiral SAMs in a hierarchical manner[1]. The chirality is believed to result from the CH_2 fragments of the tripod feet owing to a slight mismatch between the surface bonding points and the arrangement of the groups on the cage molecules.

Modification of surfaces by chiral molecules opens a promising path to develop biosensors at the single molecule level. "We can design a variety of functional groups on the adamantane cage, and this provides us with a good chance to measure electric conductivity of individual molecules," says Kim. The team is now investigating the effect on molecular conductance by altering the functional groups and looking at fixing bigger molecules on the gold surface. ■

1. Katano S., Kim Y., Matsubara H., Kitagawa T. & Kawai M. Hierarchical chiral framework based on a rigid adamantane tripod on Au(111). *Journal of the American Chemical Society* **129**, 2511–2515 (2007).

Left-handed waves for perfect two-dimensional imaging

Researchers predict that a unique behavior of electromagnetic waves at the surface of a material could enable novel applications

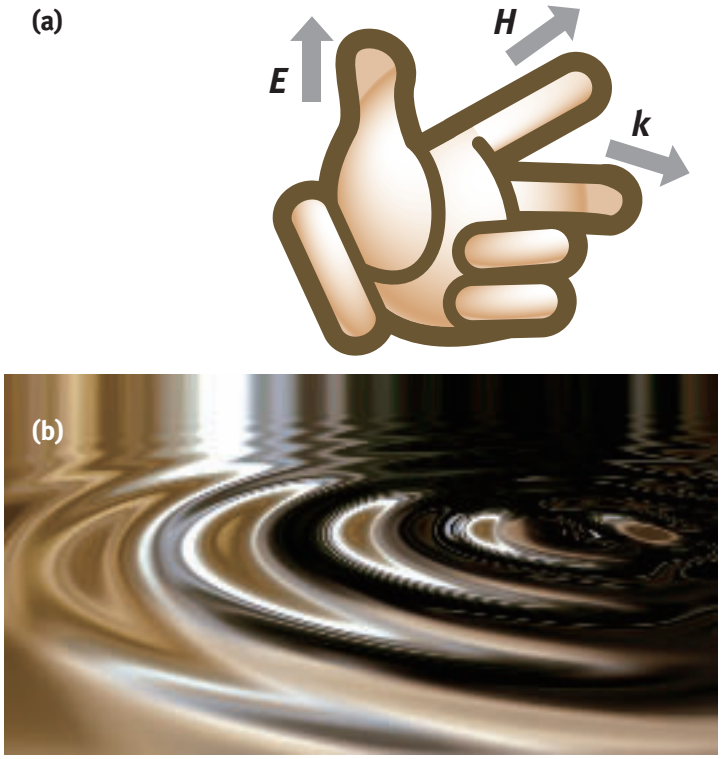


Figure 1: Left-handed surface waves. (a) Contrary to the usual right-handed media, the electric (*E*) and magnetic (*H*) components of a surface wave propagating along *k* can be constructed with the fingers of the left hand. (b) Two-dimensional electromagnetic surface waves can be seen as waves on the surface of water.

Civilization is based on our ability to shape and functionalize natural materials. Yet the laws of physics allow for much broader possibilities. Lenses, for example, are typically made of glass, but there are limits to the size of the objects they can resolve. Only recent progress in the design of artificial, so-called 'left-handed', materials has enabled the fabrication of 'superlenses' with no limit on the achievable resolution.

Superlensing is enabled by the way light, an electromagnetic wave, travels through a left-handed medium. The fixed relation between the direction that light is propagating and the electric and magnetic fields that comprise this light wave can be represented by the fingers of the left hand (Fig. 1a). This is contrary to natural materials, where their relative orientation can be represented by the fingers of the right hand. This seemingly minor difference leads to the unique properties of left-handed materials.

Now, a team of researchers from the RIKEN Frontier Research System in Wako and the University of Michigan has shown theoretically that both three-dimensional and two-dimensional electromagnetic waves traveling on the surface of a material (Fig. 1b) can be left-handed. Their work is reported in *Physical Review Letters*[1]. "Even though surface electromagnetic waves were studied intensively, as far as we know nobody has paid attention to some of the interesting analogues to three-dimensional waves," explains Franco Nori, a member of the research team.

Using the fundamental set of equations that describes the behavior of electromagnetic waves, Maxwell's equations, the team studied the properties of two-dimensional waves at the interface of two natural materials. This could be a metal in air, or the boundary between a metal and an insulating or left-handed material. The researchers show that when natural materials of suitable electric and magnetic properties are combined, electromagnetic waves at the interface between the two materials possess left-handed properties.

While there are a number of practical issues that need to be overcome, such as energy losses causing the dampening of the waves, Sergey Savel'ev from the team is convinced that these results "open new perspectives for optical devices with unprecedented properties". Superlensing, for example, could enable the miniaturization of optical lenses, with important implications for telecommunications applications. On a more general level, these theoretical findings demonstrate that some of the exciting three-dimensional features of left-handed materials can be preserved in a two-dimensional environment. ■

1. Kats, A. V., Savel'ev, S., Yampol'skii, V. A. & Nori, F. Left-handed interfaces for electromagnetic surface waves. *Physical Review Letters* **98**, 073901 (2007).

Translating DNA into protein

Researchers reveal an expanded role for RNA

RIKEN researchers and Canadian colleagues have unraveled details of a mechanism for ensuring accurate translation of the genetic code into proteins. The mechanism guarantees that the molecule which transports the amino acid glutamate to a site of protein manufacture carries it and no other amino acid.

Proteins are chains constructed from 20 different kinds of amino acids. The sequence of the chain is rigidly specified by the genetic code. Each different kind of amino acid is carried by a matching transfer RNA (tRNA) molecule which fits it onto the chain. But each type of tRNA must be specific for one amino acid only. This accuracy is fundamental to protein structure and function, and hence to all cellular and bodily function.

What the research team has shown is that glutamyl-tRNA synthetase—the enzyme which controls this reaction linking glutamate to the glutamate-tRNA—first binds glutamate-tRNA. This action stimulates a change in the synthetase structure which has two consequences. It creates a pocket or binding site specific to glutamate. And it ensures the glutamate is correctly positioned to be activated by linkage with the energy compound adenosine triphosphate (ATP).

In a paper published recently in *Structure*[1], researchers from RIKEN's SPring-8 Center in Harima and Genomic Sciences Center in Yokohama, and the University of Tokyo, together with colleagues from Laval University in Quebec, provided details of how they synthesized and analyzed the crystal structures of four complexes representing different combinations of the synthetase, the tRNA, glutamate and ATP. They also compared these structures with several previously described structures.

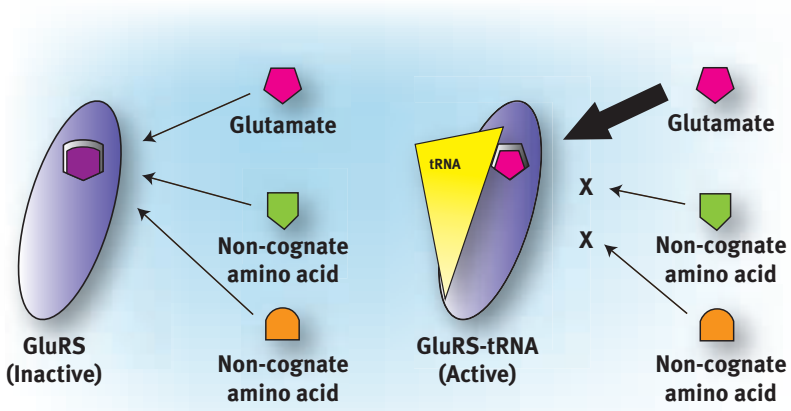


Figure 1: Specific amino acid recognition by glutamyl-tRNA synthetase depends upon it binding glutamate-tRNA.

The researchers found that the complex of the synthetase and the tRNA forms a binding site which accommodates only the right form of glutamate in terms of shape, size and distribution of electrostatic charge. When not linked to the tRNA, the site can bind other incorrect amino acids (Fig. 1). So the change in structure of the synthetase upon binding the tRNA selects the right amino acid.

Once bound to the synthetase-tRNA complex, the glutamate is positioned so that it can react with ATP. In the absence of the tRNA, the ATP is bound in such a way that it can react neither with glutamate, nor any other amino acid.

A similar mechanism also seems to be in operation for the closely related amino acid glutamine and perhaps also for argenine. "It may be a basic, general mechanism," says lead author, Shun-ichi Sekine. "I would like study the arginine complexes as a first step in checking this hypothesis." ■

1. Sekine, S., Shichiri, M., Bernier, S., Chênevert, R., Lapointe, J. & Yokoyama, S. Structural bases of transfer RNA-dependent amino acid recognition and activation by glutamyl-tRNA synthetase. *Structure* **14**, 1791–1799 (2006).

Insights into regulation of tumor suppression

Recent understanding of a protein structure could lead to new cancer drugs

Researchers from RIKEN have unraveled details of the interaction between an oncoprotein, involved in regulating cancer, and the 26S proteasome, which breaks down proteins. The complex they form is part of the degradation pathway of at least two important tumor suppressing proteins, p53 and retinoblastoma protein (pRb). The work has suggested a mechanism for the breakdown of the suppressors, and could lead to the design and development of new drugs to treat cancer.

The oncoprotein, gankyrin, is commonly associated with liver cancer. Earlier research has shown that it interacts directly with pRb and with components responsible for pRb's activation and degradation. Gankyrin is also involved in tagging the ubiquitous tumor suppressor p53 for degradation. It was first identified, however, as binding with a regulatory particle which unfolds and feeds specially tagged proteins into the barrel-shaped 26S proteasome where they are chopped up and destroyed.

Although gankyrin is known to associate with S6 ATPase, one of six different ATPase enzymes that are part of the regulatory particle, the details of this interaction have never been clear. In a recent paper in *Structure*[1], researchers from the RIKEN Genomic Sciences Center in Yokohama provide the first details of the gankyrin–S6 ATPase complex. They were obtained by means of x-ray crystallography using synchrotron radiation.

The structure shows how a concave region of gankyrin binds strongly to complementary electrostatic charges in a region close to one end of S6 ATPase. Mutations which change the charge distribution in this region of S6 ATPase result in a dramatic loss of binding affinity for gankyrin. And gankyrin does not bind to any of the other ATPases in the regulatory particle, presumably because they do not have the correct charge distribution.

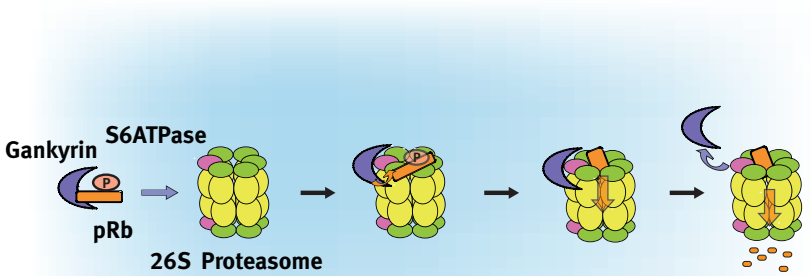


Figure 1: The proposed model of gankyrin's role in the degradation of the tumor suppressing protein pRb.

The researchers also found that gankyrin could interact with S6 ATPase whether it was attached to the proteasome or not. Gankyrin also interacts with pRb, whether associated with S6 ATPase or not. It does not bind as strongly to pRb, however, as to the ATPase. The suggestion from all this evidence is that gankyrin can function to carry phosphorylated pRb to the 26S proteasome where it binds strongly to S6 ATPase. Subsequently, gankyrin releases pRb for destruction in the proteasome (Fig. 1).

"We want to understand how this protein regulates cancer through interaction with other proteins," say research team members, Balasundaram Padmanabhan and Shigeyuki Yokoyama. "This is an expanding project. So far, only this one complex structure is known." ■

1. Nakamura, Y., Nakano, K., Umehara, T., Kimura, M., Hayashizaki, Y., Tanaka, A., Horikoshi, M., Padmanabhan, B. & Yokoyama, S. Structure of the oncoprotein gankyrin in complex with S6 ATPase of the 26S proteasome. *Structure* **15**, 179–189 (2007).

Narrowing bottleneck theories

Commonly accepted theory on mitochondria transmission ruled out

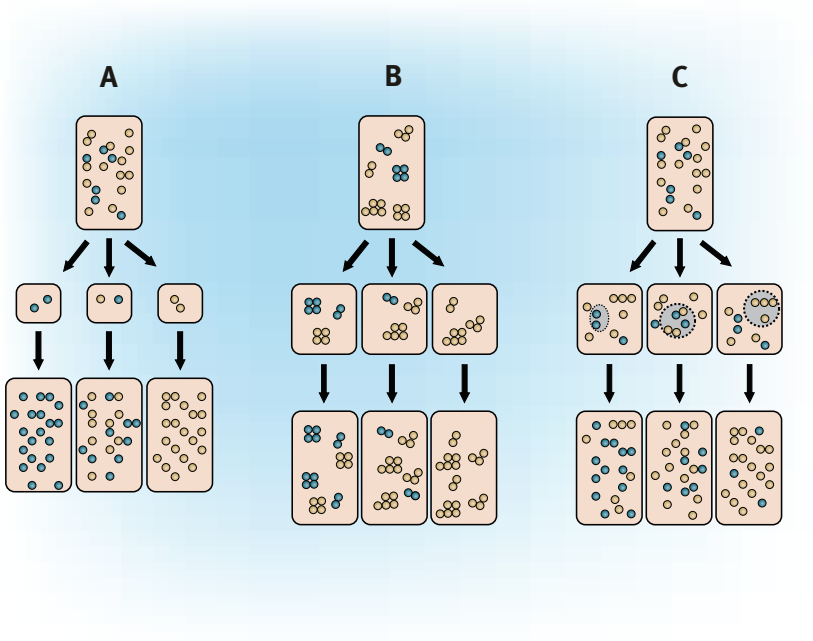


Figure 1: Three models for rapid mtDNA segregation during cell division in female mouse reproductive (germ) cells: (a) model A—reduction in the number of mtDNA copies; (b) model B—clumping together of like variants; and (c) model C—replication of sub-groups.

Researchers from the RIKEN BioResource Center in Tsukuba, the Tokyo Metropolitan Institute of Medical Science and two Japanese universities have ruled out one of the leading hypotheses as to how mutations in mitochondrial DNA (mtDNA) can become incorporated into the genome in just a few generations. The finding is an important step towards understanding the dynamics of mitochondrial genetic disease and predicting the risk of its occurrence in children.

Mitochondria are membrane complexes which serve as the energy production centers of cells. Any disruption to their function can cause serious illness. In mammals, mitochondria are inherited only from the mother. They carry their own DNA which has a higher mutation rate than the DNA in the nucleus.

Because of the high mutation rate, there are a large number of variants of mtDNA in any population. Most individuals, however, tend only to have one variant, which implies that variants are either accepted or rejected within a few generations. This observation has led to the suggestion that during mammalian development the mitochondria pass through a 'bottleneck' stage whereby the statistical chances of one competing variant becoming dominant are increased.

There are three different models as to how this could happen (Fig. 1). Two of them propose that at some point in development only a small number of mtDNA segregation units are involved in transmission, increasing the chances that different variants will be sorted unequally into daughter cells during cell division. This could occur if there were a large reduction in the total number of copies of mtDNA in cells (model A, Fig. 1a) or if like variants tended to clump together and were sorted as fewer units (model B, Fig. 1b). The third model proposes that copies of mtDNA in a small region of the cell replicate massively after cell division (model C, Fig. 1c).

In a recent paper in *Nature Genetics*[1], the researchers describe how they traced the mtDNA content of the cells which become germ cells in female mice during the first 13.5 days of development. They determined that the number of copies of mtDNA per cell never fell to a level that would make model A possible. In comparison, the mtDNA content of non-germ cells of the same age varied much more widely, and at times fell to much lower levels. This leaves only models B and C as possible explanations.

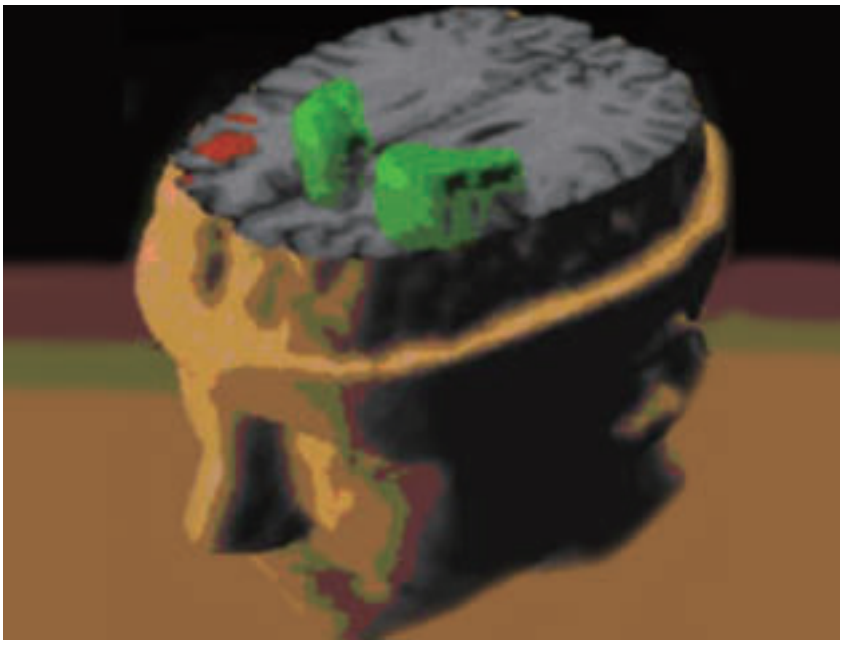
"Having ruled out the most commonly accepted theory of transmission," says lead author, Liqin Cao, "we now want to nail down exactly what is happening using *in vivo* imaging." ■

1. Cao, L., Shitara, H., Horii, T., Nagao, Y., Imai, H., Abe, K., Hara, T., Hayashi, J-I. & Yonekawa, H. The mitochondrial bottleneck occurs without reduction of mtDNA content in female mouse germ cells. *Nature Genetics* **39**, 386–390 (2007).

Nature Genetics

New suspected causes of schizophrenia

Genetic research uncovers more candidate schizophrenia genes



National Institute of Mental Health

Figure 1: PET scan of a schizophrenic brain, showing activated frontal lobes (red) during a working memory task; the striatum (green), a site of dopamine activity, is thought to be related to the neurocognitive deficits in schizophrenia.

Researchers have long known that close relatives of schizophrenia patients are also at risk of developing the disease. Now a collaboration of scientists studying 374 Japanese schizophrenics from 124 families has linked the disease to defects in a set of genes not previously associated with the disease.

The collaboration was led by Kazuo Yamada and Takeo Yoshikawa from the RIKEN Brain Science Institute, Wako, and Susumu Tonegawa from the RIKEN–MIT Neuroscience Research Center in Cambridge, US. "In 2003, Professor Tonegawa and collaborators in the US reported that a defect in one of the calcineurin genes, *PPP3CC*, is associated with schizophrenia," says Yoshikawa. "We wanted to test other components of the calcineurin pathway as possible additional susceptibility factors for schizophrenia."

Normally, calcineurin helps to regulate transmission of signals from outside to inside cells. Memory formation and other brain functions that require calcineurin activity are linked to two neuronal pathways that use the neurotransmitters dopamine and glutamate to propagate brain signals from neuron to neuron (Fig. 1). Defects in these two pathways have been previously associated with schizophrenia.

Yamada and colleagues focused their attention on a region of chromosome 8 near the calcineurin gene. They applied statistical methods to experimental data obtained from evaluating schizophrenic and normal individuals, and found correlations between subtle changes in three 'early growth response' (*EGR*) genes and the likelihood of having schizophrenia.

According to Yoshikawa, the schizophrenic families fell mainly into two groups: one with altered *EGR* genes and one with the previously identified altered calcineurin gene. "Only a few families carried alterations in both genes," he notes. Intriguingly, regulation of EGR proteins by calcineurin was known, leading Yoshikawa to speculate that, "a signaling pathway involving calcineurin and EGR proteins could underlie schizophrenia."

Further experiments by the RIKEN team found reduced EGR protein in the brains of deceased schizophrenics. This was significant because characteristics associated with schizophrenia, including impairments in attention, memory and language, are caused by altered brain activity that is regulated by EGR protein. Yoshikawa adds that a complementary recent report found that adult male mice that lack EGR3 protein display emotional behavior abnormalities and schizophrenic-like activity similar to those seen in mice that lack calcineurin.

At present, how altered EGR activity could lead to schizophrenia is not clear. Yet the work by Yoshikawa and colleagues raises the possibility that understanding more about the targets of EGR activity in the brain may provide insight into the cause and/or etiology of schizophrenia. ■

1. Yamada, K., Gerber, D.J., Iwayama, Y., Ohnishi, T., Ohba, H., Toyota, T., Aruga, J., Minabe, Y., Tonegawa, S. & Yoshikawa, T. Genetic analysis of the calcineurin pathway identifies members of the *EGR* gene family, specifically *EGR3*, as potential susceptibility candidates in schizophrenia. *Proceedings of the National Academy of Sciences USA* **104**, 2815–2820 (2007).

Exploring new pathways leading to gene expression in the brain

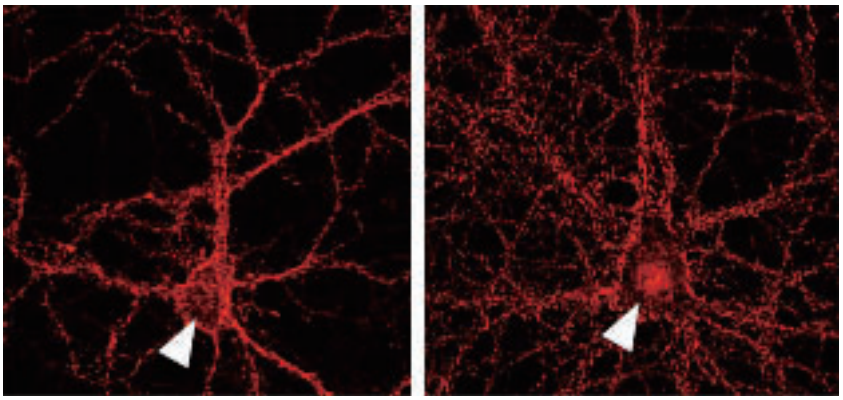
Recent work may help explain how the brain reacts to exposure to unfamiliar environments

Researchers have identified a signal transmitted within the brains of mice placed within novel surroundings. Neuronal excitements triggered by stimuli such as encounters with unfamiliar settings activate gene expression in nuclei, the cellular compartments where genes are located. Gene expression is suggested to play an important role in acquiring new memories of environments.

Kentaro Abe and Masatoshi Takeichi from the RIKEN Center for Developmental Biology in Kobe set out to trace the steps linking neuronal stimulation with gene expression. Their work appears in a recent issue of *Neuron*[1].

To mimic brain stimulation, the researchers used glutamate, an amino acid that excites neurons by binding to receptors displayed on the neuronal surface. Unexpectedly, glutamate stimulation resulted in truncation of β-catenin, a component of cadherin–catenin adhesion complex that physically sticks two neurons together at synapses, or gaps between neurons. Calpain, a protease enzyme controlled by glutamate, was responsible for cleaving β-catenin.

β-catenin plays essential roles in two distinct cell functions, specifically cell-cell adhesion as a component of the cadherin–catenin adhesion complex, and regulation of gene expression as a component of the Wnt signaling system. Wnt proteins send signals through receptors expressed on the surface of multiple cell types in diverse organisms. These signals culminate to stabilize β-catenin, by halting its truncation, and influence myriad biological processes including the immune cell development and cellular transformation. However, in mature neurons, although β-catenin acting in the cadherin–catenin complex was known to regulate the formation or the function of synapses, whether β-catenin functions as a regulator of gene expression was not fully known.



Neuron

Figure 1: In cultured hippocampal neurons, β-catenin (red) can be detected only at synapses (left). After stimulation with glutamate (right), β-catenin is also observed in the nucleus (indicated by arrowhead).

The pair next sought to determine if, like the intact β-catenin in other cell types, these β-catenin fragments were competent to activate gene expression in neurons. After glutamate stimulation, the truncated β-catenin proteins migrated into the nuclei (Fig. 1). Within the nucleus, the fragments worked with established β-catenin partner proteins to drive production of known β-catenin–responsive genes.

Lastly, the researchers investigated whether calpain-mediated cleavage of β-catenin occurs within the brains of live mice. Like glutamate, exposure to an unfamiliar environment stimulated calpain-mediated production of β-catenin fragments, and activated the gene expression in neurons located in the brains of live mice.

"Identification of the full spectrum of genes regulated by calpain-mediated β-catenin cleavage may be the subject of later investigation," says Abe. "As some mood stabilizing drugs can alter the cellular amounts of β-catenin, and some neurological disorders are associated with defective calpain activity, these findings may hold therapeutic relevance." ■

1. Abe, K. & Takeichi, M. NDMA-receptor activation induces calpain-mediated β-catenin cleavages for triggering gene expression. *Neuron* **53**, 387–397 (2007).

Honing in on plant 'flavor'

Researchers combine techniques to pinpoint a metabolic pathway in plants

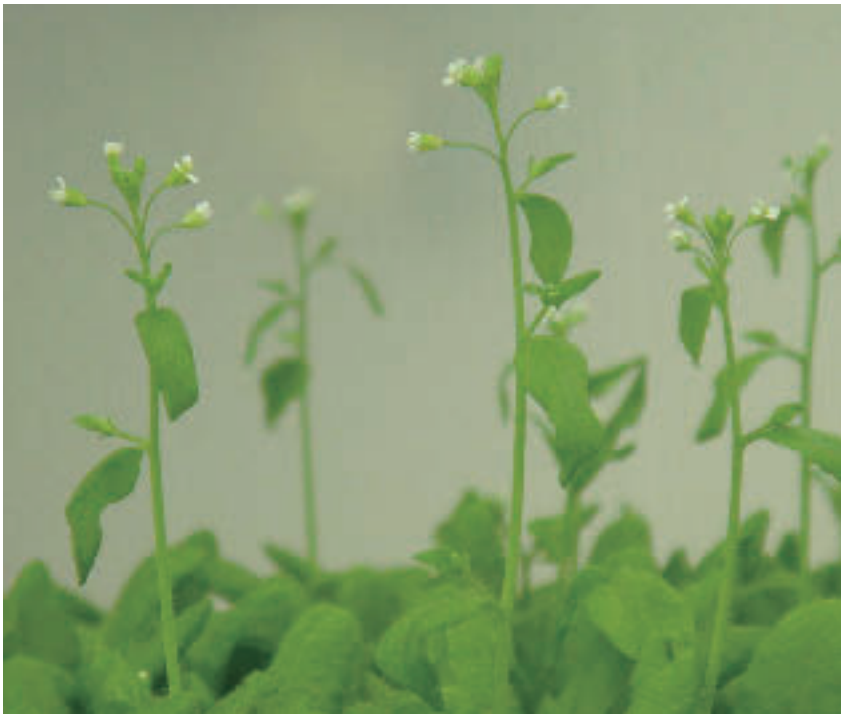


Figure 1: Seedlings of *Arabidopsis thaliana*.

Researchers at the RIKEN Plant Science Center in Yokohama have identified the enzyme responsible for a key step in the synthesis of secondary metabolites in the mustard plant, *Arabidopsis thaliana* (Fig. 1). They used a combination of techniques including transcriptomics, metabolomics, reverse genetics and biochemical analyses.

Secondary metabolites are small organic compounds that are not directly necessary for the normal health of an organism, but are typically derived enzymatically from primary metabolites. They can help the organism defend itself from predators or, in the case of plants, add color to its blooms and thus aid in pollination. Several of the plant-derived secondary metabolites, including flavonoids, also have potential medicinal effects.

Based on published information about the flavonoid biosynthetic pathway in *Arabidopsis*, the research team, led by Kazuki Saito, suspected the existence of a certain type of enzyme in the process. To confirm their theory, the researchers needed to identify the gene encoding this enzyme—a challenge considering that there were 107 candidates, as judged by analysis of the complete sequence of the *Arabidopsis* genome.

Initially, the team used publicly available databases that describe the expression patterns of the *Arabidopsis* genome to look for appropriate regulatory and enzymatic genes that are co-expressed in the flavonoid biosynthetic pathway across a wide-range of tissues and growth conditions. This transcriptomic analysis narrowed the candidate pool to four genes. One of these genes, *UGT89C1*, encodes a protein that also has strong amino acid sequence homology to similar enzymes involved in different biological processes, so the researchers used metabolomics to analyze the flavonoid profiles of mutant *Arabidopsis* strains lacking the expression of *UGT89C1*. These mutants were deficient in flavonoids generated by the enzyme that the team hypothesized to exist. This combination of metabolomics and reverse genetics indicated that *UGT89C1* encoded for the suspected enzyme[1].

The team then validated the function of this enzyme by showing that it behaves as expected when purified and tested in a test tube, that re-expression of the normal gene in the *UGT89C1* mutant strains restored normal levels of the missing flavonoids, and that the enzyme was expressed in the parts of the plant where the particular flavonoids occurred.

Saito believes "these results point to a proof-of-principle that combining several techniques will allow researchers to find a needle in a biological haystack", especially given the improvement in analytical techniques and public databases. He is particularly excited by the combination of transcriptomics with metabolomics, which may further reveal complete metabolic pathways and their regulators. ■

1. Yonekura-Sakakibara, K., Tohge, T., Niida, R. & Saito, K. Identification of a flavonol 7-*O*-rhamnosyltransferase gene determining flavonoid pattern in *Arabidopsis* by transcriptome coexpression analysis and reverse genetics. *Journal of Biological Chemistry* **282**, 14932–14941 (2007).

Creating Larger Plants

Minami Matsui

Team Leader and Group Director, Plant Functional Genomics Research Team
Plant Functional Genomics Research Group
Plant Science Center
RIKEN Yokohama Institute

The size of organisms depends on the size and number of their body cells. It is also known that a cell becomes larger as the amount of DNA in the nucleus of the cell increases. If we can control the amount of DNA in the nucleus, we will be able to make cells larger, and create crop plants with larger leaves that produce larger fruit. This issue introduces readers to the recent research achievements of the Plant Functional Genomics Research Team led by Minami Matsui.

How plants grow

"A plant seed changes into a long-stemmed seedling, or what is called a bean sprout, when grown in a dark place (Fig. 1), whereas it changes into a short-stemmed seedling when grown in bright light. What do you think lies behind the difference?" begins Matsui. Most people would assume that the long-stemmed seedling has more cells. However, Matsui explains that the number of cells is the same. "Plants grow by mechanisms that are different to those of mammals."

As one growth mechanism, Matsui draws attention to endoreduplication. This word stands for repetitive DNA duplication in a single cell without associated cell division.

A cell normally starts duplicating chromosomes following a preparation phase. Similarly, it will complete another preparation phase before cell division. This series of events is referred to as the 'cell cycle' (Fig. 2, left). "We, humans, have two sets of chromosomes in the nucleus of our body cells: a set of paternal chromosomes and a set of maternal chromosomes. These chromosome sets are doubled into four sets of chromosomes when they are duplicated, but as the cell is divided into two, each of the equivalent daughter cells still has only two sets. Most human body cells are diploid cells consisting of two sets of chromosomes. Plant cells, however, have a mixture of multiploid cells including diploids, 4-ploids, 8-ploids, 16-ploids, and so on, owing to endoreduplication. The amount of plant chromosome doubles because a plant cell does not break up even after the chromosome has duplicated (Fig. 2, right).

Figure 1 shows the comparison between two sample seedlings in terms of the amount of DNA in the hypocotyls (embryonic stems of seedlings without roots and cotyledons). One sample was grown in bright light, and the other was grown in the dark. The graph shows that the hypocotyls of the sample grown in the dark have more 16-ploid cells than the sample grown in bright light; even 32-ploid cells are found. "Endoreduplication is enhanced in the dark, which is the cause of the increase in the amount of DNA in this sample," Explains Matsui.

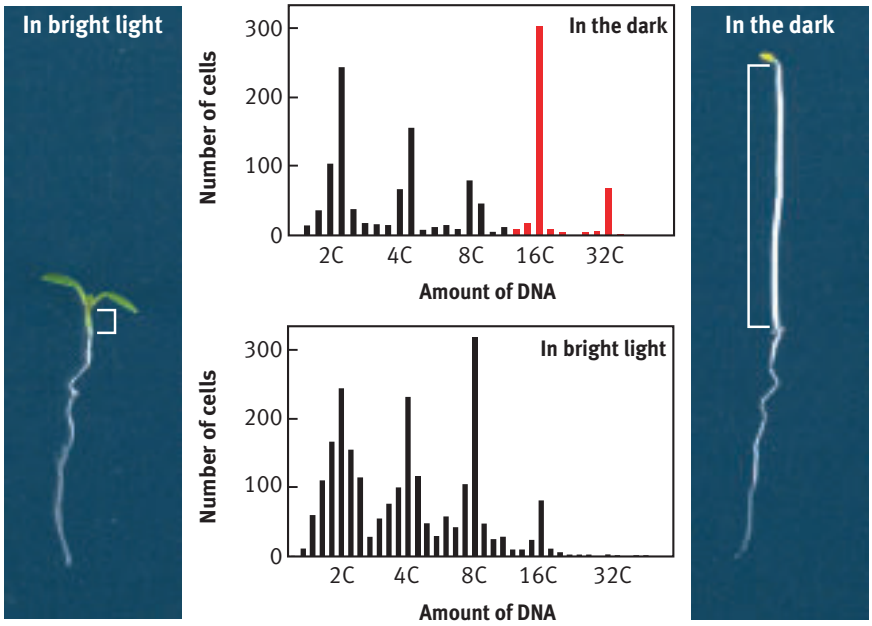


Figure 1 : Comparison of the length of the hypocotyls (embryonic stems) and the amount of DNA for a sample seedling grown in bright light and one grown in the dark.
The symbol C is the basic unit for the amount of DNA; 2C represents the amount for a diploid. The hypocotyls grown in bright light are shorter than those grown in the dark, but the number of cells remains the same. The sample grown in the dark contains more 16C cells and also contains 32C cells that do not appear in the sample grown in bright light.

Most cells become larger in size as the amount of DNA increases. "The hypocotyl of the sample seedling grown in the dark is longer because each cell is larger in size, although the total number of cells remains the same."

Matsui explains the reason why endoreduplication occurs in the hypocotyls grown in the dark as follows: "[In the dark] plants may want to grow as fast as possible, so that they can reach a place where they can get light." He explains that growth based on cell division requires a certain amount of time, and that cell growth based on doubling the amount of DNA is more efficient. Endoreduplication also occurs in root hair cells that grow from the main root where plants absorb nutrients from the soil.

Although the phenomenon of endoreduplication has been known for a long time, the mechanism was not understood. The Plant Functional Genomics Research Team at RIKEN succeeded in discovering the gene that controls endoreduplication, and produced results that have elucidated part of the mechanism.

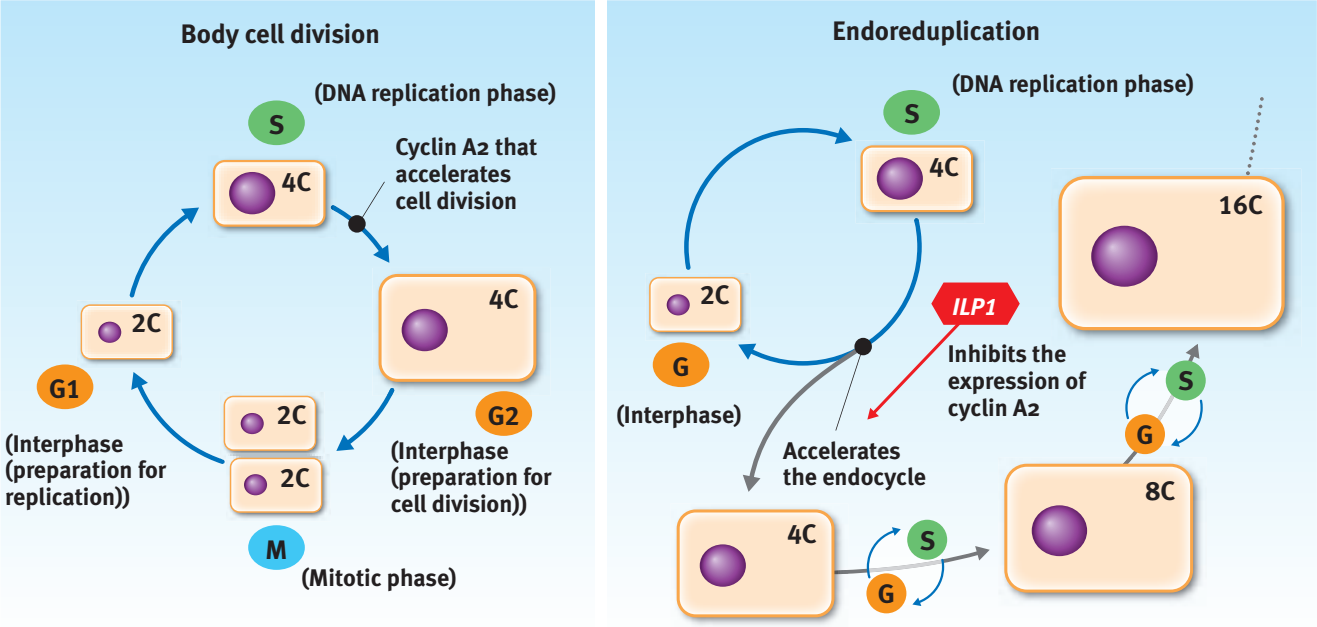
Growing large tomatoes

The Plant Functional Genomics Research Team used the cruciferous plant Arabidopsis thaliana in their studies of endoreduplication. Arabidopsis thaliana is widely used in research because it is only 40 cm tall, easy to grow indoors, has a short generation time of only two months, and the smallest genome size among complex plants. The total genome sequence was decoded in 2000.

Matsui and his team members investigated in detail the difference in the amount of DNA between a wild-type strain and a mutant strain, finding an overexpression of the gene *ILP1* in the mutant strain. The ILP1 protein contained in the nucleus of a cell can inhibit the expression of genes. Further investigation revealed that the expression of the *Cyclin A2* gene was reduced in the mutant strain. "Cyclin is a protein that controls the cell cycle," explains Matsui. "We know several kinds of Cyclins." He adds and continues to describe how Cyclin A2 acts as an accelerator for cell division after the DNA in a cell has been duplicated, thus acting as an inhibitor of endoreduplication. "To sum up, overexpressed ILP1 proteins inhibit the expression of *Cyclin A2* genes, which results in the acceleration of the endoreduplication cell cycle, the so-called endocycle."

Figure 3 is a graph that compares the amount of DNA in the cotyledons of a wild-type strain with that in a mutant strain in which *ILP1* genes are overexpressed. The cotyledon area of the mutant strain is larger than that of the wild-type strain by about 30%. The graph shows that the mutant strain contains more 16-ploid cells than the wild-type strain; even 32-ploid cells are found. This implies that the overexpression of *ILP1* genes accelerated endoreduplication in the mutant strain, leading to a growth in cell size.

This finding may have far-reaching benefits. "We are conducting joint research with Shizuoka University, in which researchers use tomatoes," says Matsui. It is known that endoreduplication actively occurs in the fruit cells of tomatoes. Thus, the overexpression of genes such as



Fi ure 2 : Body cell division and endoreduplication.
A DNA-doubled 4C cell develops into two 2C cells because the M-phase (mitotic phase) follows the S-phase (DNA replication phase) during the cell cycle of the body cells. In contrast, the 4C state of the cell remains because the endocycle (endoreduplication cell cycle) skips the M-phase. Multiple endoreduplication can produce, for example, 8C, 16C, and 32C cells.

ILP1 and the resultant acceleration of endoreduplication would make it possible to produce larger tomatoes.

Matsui places greater expectations on the control technique of endoreduplication. "Plants produce a wide range of materials, a lot of which are very useful to us. For example, anthocyanin and flavonoid are said to be good for our health," explains Matsui. When the amount of DNA is doubled, owing to endoreduplication, the genes involved in material production are also doubled. In other words, the material producing capacity can be doubled. "I think we can use sugarcane to produce alcohol effectively, by accelerating its endoreduplication process, and thereby increasing the production of sugar," says Matsui. Plant-derived alcohol, or so-called biofuel, is drawing great attention as an alternative fuel to oil. He adds, "Creating larger cells can contribute to a variety of applications."

Exploring applications by adding more functions

"In plant studies, basic research and its applications are very closely related," says Matsui. "For applications, functionally enhanced crops are required for such purposes as increasing productivity, producing larger crops, accelerating growth and development, and enhancing drought resistance." Thus, the Plant Functional Genomics Research Team uses two different methods to create functionally enhanced variants. One is the activation tagging technique that introduces either an enhancer into the genome, or a base sequence that accelerates the expression of genes. We used this method to create more than 70,000 mutant strains, which have been disclosed in a database (http://amber.gsc.riken.jp/act/top.php).

"Since the database provides pictures and is classified into several categories, such as flowers, seeds, and leaves, a user can easily find interesting mutant strains and get to the genes that relate to specific characteristics. This function has never before been available."

The activation tagging technique was also used to create mutant strains that have a large amount of DNA leading to the analysis of the mechanism of endoreduplication. "But just assembling a database is not enough," says Matsui, explaining that the database will not be used unless it is recognized as a useful tool for studies and a means to produce valuable results. "We conducted the research for endoreduplication to verify the availability of databases. I am sure that the database of the mutant strains generated good publicity."

Although the current database is centered on visible mutant strains, invisible mutant strains such as metabolite amount will be added to the database in the future. The activation tagging technique, however, has a disadvantage. Overexpressing genes are sometimes located close to an introduced enhancer, and sometimes far away. Thus, it is difficult to find the correct one-to-one mapping between genes and their characteristics.

Matsui and his team members then developed another method, FOX Hunting (Full-length cDNA Over-Expressor Gene Hunting System). The acronym implies the aim: to hunt for useful genes. "FOX Hunting is a technique that adds functions by introducing full-length cDNA. Since

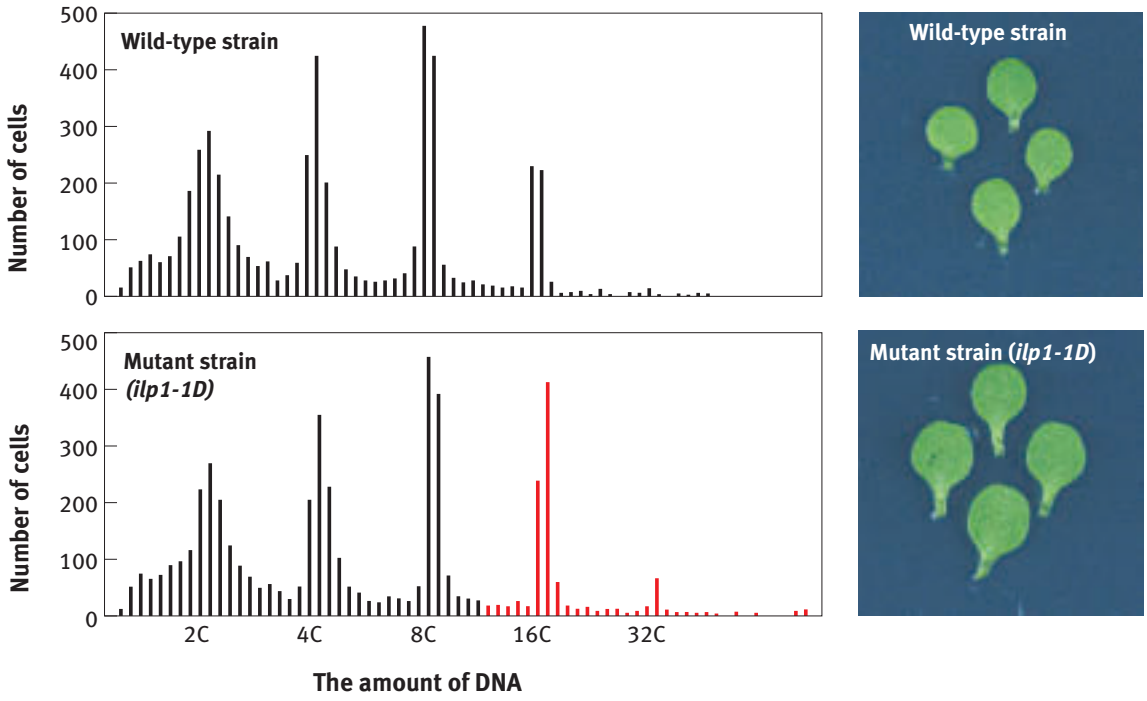


Figure 3 : Comparison of the difference in the amount of DNA between a wild-type strain and a mutant strain in which *ILP1* genes are overexpressed.
The cotyledon area of the mutant strain is larger than that of the wild-type strain by about 30%. The cell size of the mutant strain is also larger.

collecting full-length cDNA is a RIKEN-specific technique, our team is the only one in the world that is capable of using the technique."

DNA is transcribed into RNA, which is then spliced and reassembled into mRNA. Proteins are formed on the basis of this genetic information. Since full-length cDNA is generated by reverse transcription of mRNA, it provides all the genetic information. Thus, introducing the full-length cDNA of the gene of interest would enable the functions of the gene to be investigated. "The biggest benefit of introducing full-length cDNA is that the technique can be introduced to different species," says Matsui.

"For example, the rice plant has more than 40,000 genes," he continues. "You will have a hard time finding which genes contribute to an increase in crop yields." He points out that to make matters worse, there is a delay of half a year before the rice plant develops grains of rice from which the results can be determined. Arabidopsis thaliana, however, has a short generation time of only two months, providing more opportunities to test genes. Matsui adds that in addition, research will progress faster if genes that have already been proven to be useful are introduced into rice plants. "At present we are conducting joint research on rice genes with the National Institute of Agrobiological Sciences and the Research Institute for Biological Sciences Okayama," he says. "We think FOX hunting using Arabidopsis thaliana will be useful for plants with long generation time such as trees."

What is his research perspective for the future? He points out that the complete genomes of various organisms have been decoded one after another. Basic research tends to search for common terms among organisms. However, his team aims to focus, only on those that are specific to plants. "Research on growth based on endoreduplication is exactly what we want to work on in the future."

"Plant mechanisms are very interesting," says Matsui with excitement. He adds that plants have many sophisticated functions that are difficult for humans to deduce. They are capable of changing even the mechanism of their cell cycle in response to environmental changes so that they can adapt themselves to the external environment. "As a biologist, I want to clarify the mechanisms by which plants grow."

1. Press release, 24 October 2006) published by Ohm Inc., Tokyo (in Japanese). Japanese Patent, No. 2003-523656.

About the researcher

Minami Matsui was born in Tokyo, Japan, in 1958. He graduated from the Faculty of Sciences, Saitama University, in 1981, and obtained his PhD in 1986 from Kyoto University. After working in Nippon Medical School, he joined the RIKEN Frontier Research Program as deputy head in 1995. He worked on plant photomorphogenesis focusing on protein degradation. From 1999 he became a team leader of the Plant Functional Genomics Research Group at the Genomic Sciences Center, where he developed gain-of-function mutants of Arabidopsis. In 2006 he joined the Plant Science Center as a group director and is continuing research on plant functional genomics.

Joint Meeting on Activation and Regulation of the Immune System

The first Max Planck Society (MPG)–RIKEN joint workshop 'Activation and Regulation of the Immune System' was held April 16 and 17 in Berlin. Participating scientists were from the RIKEN Research Center for Allergy and Immunology (RCAI, Yokohama), the Max Planck Institute for Infection Biology (MPIIB), the Max Planck Institute for Immunobiology (MPI), and the German Rheumatism Research Center (DRFZ). The sessions were held in a modern research facility that houses both the MPIIB and DRFZ located on the historic Charité medical campus in the heart of Berlin. This venue was particularly fitting for a joint German/Japanese immunology meeting because it was here that German and Japanese bacteriologists Emil Behring and Kitasato Shibasabur collaborated in groundbreaking studies in the 1880s.

The meeting covered the spectrum of current immunologic research, providing an opportunity for scientists from both countries to learn the latest developments in their respective fields and to establish personal relationships that should foster future collaborations. The meeting featured talks by senior scientists including: Thomas Boehm (MPI), who described his efforts to trace the evolutionary origin of the MHC-peptide system used for antigen presentation in contemporary vertebrates; Sergei Nedospasov (DRFZ), who spoke about the ongoing efforts to create mice humanized for the cytokine tumor necrosis factor (TNF) and TNF receptors as a model to allow the study of the in vivo effects of TNF blockers; Michael Reth (MPIIB), who described new transgenic mice in which Cre recombinase is driven by mb-1 regulatory elements; Toshitada Takemori (RCAI), who issued a challenge to the textbook immunology view that memory B cells are generated during the germinal center reaction; and Masaru Taniguchi (RCAI) and colleagues, who reported that they have solved a long-standing puzzle of how BCG immunization suppresses IgE production and have identified the central role of the NK T cell. The clinical implications of the studies by Taniguchi and his co-workers in terms of allergy abatement are currently being tested by BCG immunization in humans.

The initial memorandum of understanding to establish formal collaborations between RIKEN and MPG was signed in 1984. The participants at this joint meeting all agreed that it was a successful first edition that would stimulate collaborations and scientific exchanges, and that there should be more such meetings in the future. ■

International Joint Graduate School Program

In autumn 2006, RIKEN set up an International Program Associate system. This is a system to accept outstanding foreign graduate students to work at RIKEN and to nurture them with the aim of developing an international network in the near future. Prior to making full-scale agreements under this system from 2008, RIKEN concluded an agreement for an international joint graduate school program with the University of Galati in Romania and Xi'an Jiaotong University in China, both in the field of VCAD research.

The VCAD (volume CAD) System Research Program provides software on the Internet (http://vcad-hpsv.riken.jp/en/release_software/) that is useful for manufacturing and biological research, such as modeling living cells. The organizers of the VCAD System Research Program hope that this software will become widely used in university education, industrial applications, and as a research tool. The University of Galati has collaborated with RIKEN for seven years in the simulation of manufacturing, which is related to VCAD. Xi'an Jiaotong University is one of China's leading universities, and has developed a strong emphasis on science and engineering.

From now on, RIKEN will accept graduate students from both universities, and these universities will appoint RIKEN researchers as visiting professors with the opportunity to teach their students. ■

RIKEN BSI – OLYMPUS Collaboration Center opened

On June 1, RIKEN BSI – OLYMPUS Collaboration Center (RIKEN BOCC) opened at the Brain Science Institute(BSI) in Wako. This center is the cooperative research hub between RIKEN and Olympus Corporation, one of the leading Japanese manufacturers of consumer digital cameras and medical and lifescience research instrument. It is the first organization in RIKEN set up under the name of a private company. RIKEN BOCC shares the research resources of RIKEN and Olympus, to contribute to progress in life sciences research over the next generation with a mid- to long-term outlook.

RIKEN BOCC will carry out developing bioscience imaging systems and the technology related to them. It will have the most advanced instruments in bio-imaging research, and two engineers are dispatched from Olympus to provide technical support for their use in sample preparation, instrument manipulation and observation. In addition to the research, the center will promote the popularization of bio-imaging technology by holding workshops and symposiums open to researchers outside RIKEN.

The most attractive instrument available at the center is the multi photon excitation laser scanning microscope. Multi photon excitation by a near-infrared laser enables imaging deep into an organism without damaging it. This microscope equips two lasers to study deep into the brain; one is used to observe the tissue at a depth of 500–800 micrometres and the other is used simultaneously to do optical stimulation the brain by laser. The center also owns an incubation imaging system that can study the cell function while it is cultured over few days. ■

Academic Outreach

Postgraduate courses affiliated with universities stem from RIKEN's determination to develop the full potential of its budding researchers

Since its establishment in 1917, education has always been a focal point of RIKEN researchers. They have been rewarded by young researchers obtaining expertise under their supervision and even moving to and flourishing at other academic or business organizations. RIKEN laboratories have also been willing to accept researchers from companies for training, and university students wanting to write theses on specialized topics.

Researchers at RIKEN didn't consider that formal lecturing was part of their job; they preferred to build a good relationship with students based on mutual trust.

In the early 1980s, RIKEN proposed establishing an in-house graduate school so that they could provide academic degrees independently to students. Japan's education ministry at that time showed a positive interest in the proposal, but this ambitious idea soon came to a standstill because of many regulations and resistance.

However, in 1985, an unexpected opportunity emerged: Saitama University unofficially invited RIKEN to jointly create a graduate school. At that time, the mid-sized public university near Tokyo operated a master's program, but had difficulty in establishing a doctoral program due to insufficient numbers of suitable faculty staffers.

Within RIKEN, some researchers wondered about the merit of tying up with Saitama University. Although RIKEN wanted to create its own postgraduate courses, presidents of both organizations agreed in 1987 the collaboration would benefit the future development of education. They believed the tie-up would extend the scope of education and research beyond the existing framework, promoting researcher exchange, and ultimately stimulating mutually beneficial collaborations.

In 1987, the education ministry approved the idea, and two years later Japan's first affiliated postgraduate program opened at Saitama University. The new program started by offering three major courses: materials science, manufacturing information science and biological environment. Sixteen RIKEN researchers joined the program as visiting professors and eight as assistant professors to teach students and assess their dissertations.

At that time, RIKEN's research disciplines were diversifying from cosmic radiation physics and organic materials to molecular cell biology and genetic information studies. Initially, 32 students joined the new program. RIKEN and Saitama University frequently held symposiums to strengthen their relations.

Figure 1: As part of its efforts to strengthen educational collaboration with other countries of Asia, RIKEN houses laboratories of Thailand's Kasetsart University in Wako.

As the educational affiliation went well, RIKEN decided to open similar affiliated postgraduate courses at other universities. By March 2005 the number of partner universities had grown to total 21 nationwide, and more than 1,100 students had enrolled in RIKEN's courses.

Meanwhile, in 2001 RIKEN also started to affiliate with Asian universities through an initiative of former RIKEN president Shun-ichi Kobayashi. Under the scheme, RIKEN nurtures top-class postgraduate students in the early stage of their career, and invites them to conduct research at its laboratories in Japan for terms of a few years.

So far, RIKEN has invited students from Hanoi University of Science, Vietnam, the National Chiao Tung University, Taiwan, Peking University, China, Kasetsart University, Thailand, University Sains Malaysia, Malaysia, and Pusan National University, Korea (Fig. 1). RIKEN's unique effort to integrate its knowledge with academia has stimulated other research institutes and universities to form similar affiliations. To meet the high expectations stemming from being a pioneer, RIKEN keeps improving its affiliated postgraduate courses by taking advantage of its rich research environment and invaluable human resources.

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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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