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Linking diet and prostate cancer

RIKEN

Medicine

Uncovering secrets of a childhood disease

In-depth genomic analysis helps researchers inch closer to an understanding of an enigmatic cardiovascular disorder that predominantly affects young East Asian children

For more than 40 years now, scientists have grappled with the mysteries of Kawasaki disease (KD). New genetic data from a large team of Japanese and American scientists may help fill in the gaps[1]. The condition (Fig. 1), which primarily affects children under the age of five, is often harmless. However, up to a quarter of all children with KD will succumb to potentially serious cardiovascular damage if left untreated, including dilation of the coronary artery and aneurysm. "Patients who have giant aneurysms have an increased risk of sudden death, require anticoagulant therapy and have exercise limitations," explains Yoshihiro Onouchi of the RIKEN Center for Genomic Medicine in Yokohama.

KD is the leading cause of heart disease among children in much of the developed world, but the problem is especially acute in East Asia, particularly Japan. Estimates suggest that in the US and UK, respectively, only 20.8 and 8.4 out of every 100,000 children aged four or younger will be diagnosed in a given year. In contrast, Japan and Korea respectively see 222.9 and 113.1 new cases of KD each year per 100,000 children in this age group.

Experts hypothesize that the condition may be triggered by infection, although the causative agent has not been identified. Nevertheless, there is striking evidence for a hereditary component and Onouchi has been in active pursuit of genetic risk factors for more than a decade. "I am also a pediatrician and started focusing on the genetic aspect of KD in 1997 as a postgraduate student," says Onouchi. Most recently, he and colleagues led an extensive population genetics study that validated some previously identified candidate risk factors while also spotlighting some promising new ones.

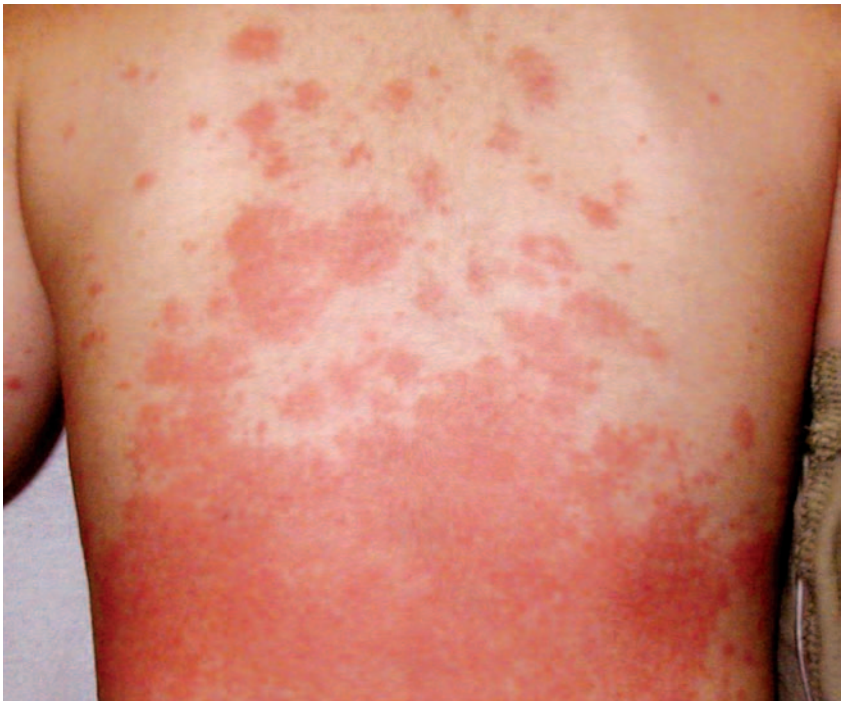


Figure 1. Kawasaki disease may be diagnosed based on inflammatory symptoms, such as the rash shown here.

Sifting through sequences

Genome-wide association studies (GWAS) are the weapon of choice for exploring the roots of hereditary disease risk. In a GWAS, researchers perform genomic analyses of large cohorts of patients afflicted with a given condition, analyzing small DNA sequence changes known as single-nucleotide polymorphisms (SNPs) scattered throughout the genome. By statistically determining which SNP variants are disproportionately present in the genomes of KD patients versus demographically similar cohorts of unaffected controls, it becomes possible to identify regions containing risk factors and, in some cases, actual disease-causing mutations.

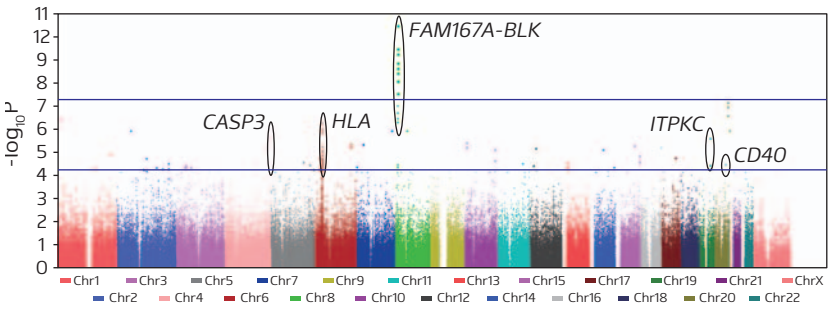


Figure 2: Statistical analysis of SNPs associated with KD revealed a number of 'hits' in various chromosomal regions (circled dots), but the strongest association was found for SNPs in the vicinity of the BLK gene.

In this study, Onouchi and colleagues analyzed SNPs from a population of Japanese children, including 447 KD patients and 3,397 controls. They subsequently validated the top candidates from this round by performing confirmatory analyses in other case-control pairs to ensure that the findings were statistically robust.

Among their new discoveries was a clear and strong disease association for several SNPs in the vicinity of the BLK gene (Fig. 2), which encodes a protein associated with signaling by antibody-producing B cells. The researchers also identified a strong association for a SNP in the vicinity of the CD40 gene. CD40 is expressed by a host of different immune cell types, and previous studies have linked variations in this gene with autoimmune and inflammatory disease. These specific variations can markedly alter the function of the encoded CD40 protein and Onouchi and colleagues found preliminary evidence that they may also contribute to the etiology of KD, although further confirmation will be necessary.

This study also spotlighted SNPs near FCGR2A, a gene that has been strongly linked to KD in analyses of both Asian and European cohorts, and which also highlights a potential immune basis for the disease. "The FCGR2A protein plays an important role in up-regulating the inflammatory response," says Onouchi. "However, there is also a cluster of receptor genes around FCGR2A, any one of which could be a true susceptibility gene." Two other previously identified KD-associated loci, ITPKC and CASP3, were among the top hits identified in this study.

Filling in the map

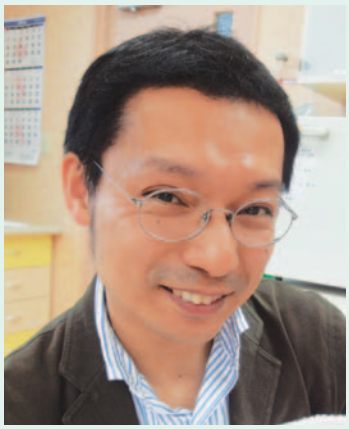
This study did not reveal statistically meaningful associations for several other loci identified in previous GWAS, and this current dataset also revealed possible links to genes whose association with KD has not been observed in other cohorts. However, Onouchi notes that this is simply an inherent challenge in performing GWAS—particularly for a condition with such a skewed geographic distribution. "Every GWAS for KD has the limitation of small sample size, such that susceptibility loci with moderate effect size might be missed," he says, "and it is important to recognize that relative contribution of each susceptibility locus can differ among ethnicities." He also notes that a recent independent analysis by Taiwanese researchers has validated many of the loci identified both in this study and in previous work.

Collectively, these results may not resolve the mystery of KD, but they provide tantalizing, and somewhat unexpected, insights that could steer future investigations. For example, multiple links between risk factors associated with KD and autoimmune conditions like lupus and rheumatoid arthritis could imply related disease pathways. "The data suggest the possibility that there is some overlap between pathophysiology of these diseases," says Onouchi. "I also think it is surprising that the same genetic factors play roles in the pathogenesis of different diseases—once in early childhood, and then later in adulthood."

In follow-up studies, he and his colleagues intend to delve deeper in their genomic analysis of high-risk East Asian populations, with the aim of charting out the disease's natural history, as well as potential indicators of disease severity that might help guide the treatment of KD patients at greatest risk from their disease.

1.Onouchi, Y., Ozaki, K., Burns, J.C., Shimizu, C., Terai, M., Hamada, H., Honda, T., Suzuki, H., Suenaga, T., Takeuchi, T. *et al*. A genome-wide association study identifies three new risk loci for Kawasaki disease. *Nature Genetics* **44,** 517–521 (2012).

ABOUT THE RESEARCHER



Yoshihiro Onouchi was born in Kyoto, Japan, in 1968. He graduated from the Osaka University School of Medicine in 1994. After a three-year internship at the pediatric department of the Osaka University School of Medicine and its affiliated hospital, he went on to graduate school at the same university and obtained his PhD in 2001. Onouchi came to RIKEN as a research scientist in 2002 after postdoctoral training at the Institute of Medical Science, the University of Tokyo. He was promoted to senior research scientist in 2005. In 2011, he moved to Chiba University as a lecturer and his title at RIKEN changed to visiting researcher. His research focuses on the genetic background of diseases in childhood, especially Kawasaki disease.

Getting to the heart of frustrated magnetism

A detailed mathematical model reveals the elusive origins of the unusual magnetic properties of thin films of solid-state helium

Thin films of helium atoms with nuclei of two protons and one neutron—helium-3—intrigue physicists because they have exhibited unusual and unexpected magnetic behavior in experimental investigations. To better understand how this behavior arises, a research team from Japan and France has developed a mathematical model that provides some important clues[1]. The model also predicts the appearance of a new quantum state in solid helium-3 films. "The search for a novel quantum state of matter is one of the most exciting aims of condensed-matter physics," says team leader Tsutomu Momoi from the RIKEN Advanced Science Institute in Wako.

Momoi and his colleagues focused on solid-state helium-3 because it enabled them to study a phenomenon known as frustration. Helium-3 thin films are 'frustrated' by interactions between localized areas of magnetism known as spins. The atoms in these films are organized into a triangular lattice, so the interaction between nearest-neighbor-pairs requires that spins act in the same direction—a mechanism known as ferromagnetism. At the same time however, exchange interactions between multiple spins are antiferromagnetic; that is, alternating spins act in opposite directions.

This contradiction leads to the frustration that gives helium-3 its unusual magnetic properties. In general, helium thin films are an ideal system for studying frustration because the ratio of the competing spin interactions can be tuned by varying the density of helium atoms. Momoi and his colleagues provided a more complete understanding of this material by mathematically modeling its lowest energy arrangement, or ground state.

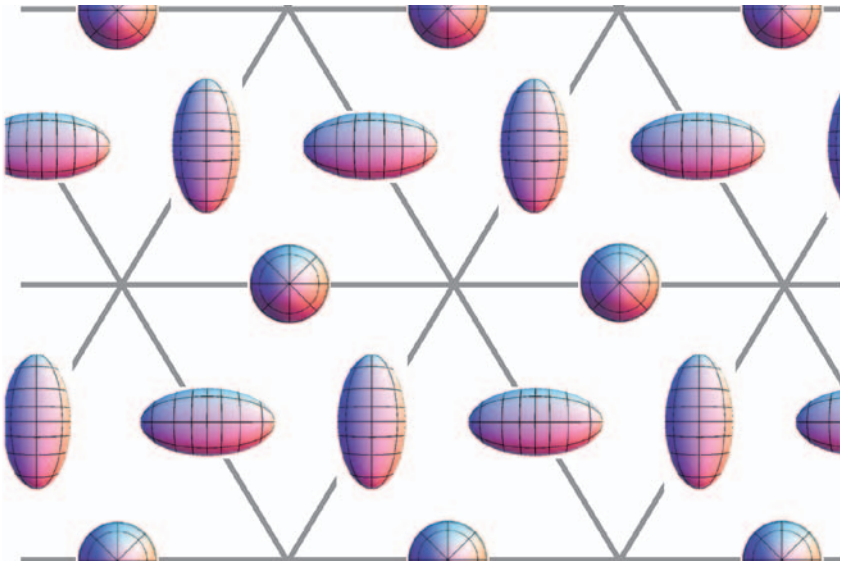


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Figure 1: The spin configuration of the quantum ground state of thin films of solid-state helium-3, as revealed by a sophisticated mathematical model.

Previous theoretical models included two-spin and four-spin interactions that provided information about the system's ground state when in an external magnetic field. "But these models were too simple to describe the delicate balance of competing interactions," explains Momoi. Indeed, experimental studies have indicated that five-spin and six-spin interactions also play a role, particularly in the absence of an external field. Thus, the researchers extended the multiple-spin exchange model to include five-spin and six-spin interactions.

They found a previously unknown ground state that has a so-called octahedral spin nematic order; that is, the spins are arranged such that they point along a particular direction, and these 'directors' are orthogonal to each other (Fig. 1). Momoi and colleagues believe that it is this unusual arrangement that causes the anomalous magnetic behavior of two-dimensional solid helium-3. "Because this is a completely new state, we next need to develop the theory for clarifying characteristics that will be helpful for observing this phase directly in experiments." ■

1. Momoi, T., Sindzingre, P. & Kubo, K. Spin nematic order in multiple-spin exchange models on the triangular lattice. *Physical Review Letters* **108**, 057206 (2012).

Shining lights of chemistry

Molecules with rare and valuable light-absorbing abilities finally give up their structural secrets

Potential applications for organic molecules that can interact with light in unusual ways range from next-generation solar cells to light-activated anticancer drugs. One of the oldest families of these brightly colored molecules is the phthalocyanines that were discovered in 1907 and subsequently prized by industry as dyes. A research team from Japan and Russia recently discovered a new branch of this family[1]. The unusual light-capturing properties of these so-called 'expanded phthalocyanines' look set to springboard the phthalocyanines into a range of high-tech applications.

Nagao Kobayashi at Tohoku University in Japan, and Evgeny Luk'yanets at the Organic Intermediates and Dyes Institute in Russia, led the research. "They were discovered accidentally," says Atsuya Muranaka, a team member based at Japan's RIKEN Advanced Science Institute in Wako. "It took about 10 years for us to determine their exact molecular structure."

Phthalocyanines are flat, disc-like molecules with a hole at the center, within which usually sits a single atom of copper or a similar metal, forming brightly colored compounds ranging from blue to green. But the researchers discovered that when they made phthalocyanines from certain salts of the metals molybdenum or tungsten, a small amount of brown-colored material could also form. Muranaka and his colleagues have now run the full gamut of analytical tests, including x-ray analysis, to reveal the compounds responsible. They are indeed expanded phthalocyanines, containing not one but two metal atoms within their central cavity (Fig. 1).

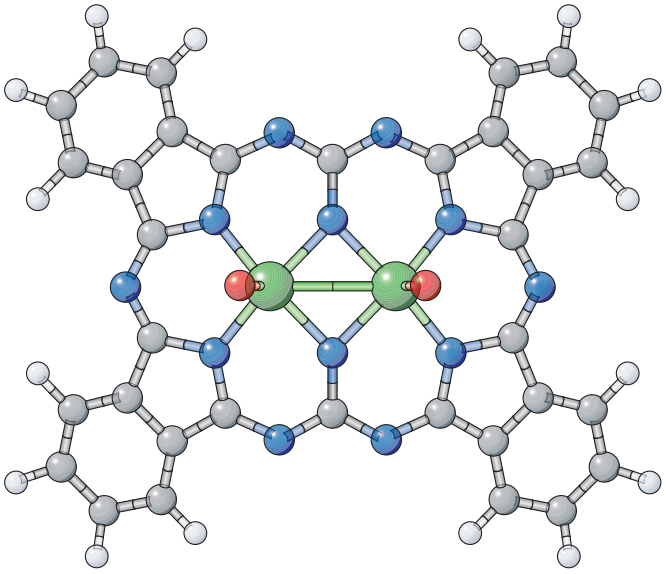

Figure 1: An image of an expanded phthalocyanine reveals the origin of its unusual optical properties: two central metal atoms (green) rather than the usual one (white, hydrogen; grey, carbon; blue, nitrogen; red, oxygen; green, molybdenum).

Thanks to their stretched structure, the expanded phthalocyanines have different properties—especially their optical properties—compared with regular phthalocyanines, Muranaka says. "The expanded phthalocyanines absorb near-infrared light, in a range where few other organic molecules [can]," he says.

One potential application for the unusual light-capturing ability of the expanded phthalocyanines could be within tandem solar cells, in which two active compounds are used to gather more solar rays than a single compound can capture. It could also be useful in a cancer treatment called photodynamic therapy, in which light-absorbing compounds are used to generate a form of oxygen known as 'singlet oxygen' that kills cancer cells. By carefully aiming light at the tumor, only the cancerous cells are killed by these molecules. As infrared light passes particularly well through human tissue, photodynamic compounds that absorb this light are particularly sought after.

Before they consider such applications, however, Muranaka and his colleagues first plan to find better ways to make expanded phthalocyanines. "At present, they are obtained in quite low yield," he says. ■

1. Matsushita, O., Derkacheva, V.M., Muranaka, A., Shimizu, S., Uchiyama, M., Luk'yanets, E.A. & Kobayashi, N. *Journal of the American Chemical Society* **134**, 3411–3418 (2012).

Unraveling a new twist in DNA folding

Findings from a quantitative analysis of chromatin structure challenge the classical model of static regularity

The DNA in the human genome is organized into irregularly folded fibers during cell division, according to a recent study by a team of researchers led by Kazuhiro Maeshima of the RIKEN SPring-8 Center and RIKEN Advanced Science Institute in Japan[1].

DNA is wrapped around proteins called histones to form nucleosome fibers, which are tightly compressed into the chromosomes by large protein complexes called condensins (Fig. 1). Many previous studies suggest that nucleosomes are organized into regular fiber structures that are 30 nanometers in diameter, which led to the classical model of overall chromosome structure. However, other studies suggest that these regular fiber structures exist only in specialized cell types.

To resolve these conflicting results, Maeshima and his colleagues investigated chromosome structure in mitotic, or dividing, HeLa cells using three different techniques: cryo-electron microscopy, which allows for observation of frozen, hydrated biological structures at high resolution without producing the artifacts seen in conventional EM; small-angle x-ray scattering (SAXS), which detects repeating structures in solutions of biological samples; and ultra-small x-ray scattering (USAXS), a newly developed type of SAXS that can resolve repeating structures across larger dimensions.

All three techniques produced consistent results. The researchers detected repeating structures at approximately every 11 nanometers, but not at longer distances, suggesting that chromatin is organized like beads on a string with an irregular folding pattern. The USAXS method further revealed that the chromosomes have a fractal nature: the same organization repeats itself at different scales, and that is the structures arrange into a rod shape.

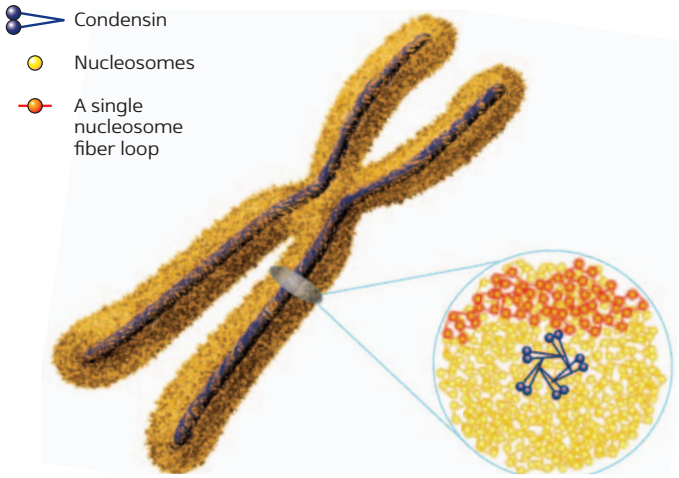

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Figure 1: Chromosomes (left) consist of tightly compacted and irregularly folded nucleosome fibers.

Maeshima and colleagues propose two possible explanations for the rod-shaped and large-scale organization of the chromosome. One is that the condensins bind to specific sites in the DNA, causing it to form self-assembling loops that interact with each other and produce repeating structures along the axis of the chromosome. Alternatively, the formation of regularly repeating looped structures alone might be sufficient to generate the observed rod shape because of repulsive forces between adjacent loops.

The researchers predict that irregular folding would make chromosomes more flexible: this type of organization has fewer physical constraints than a regular structure. They also suggest that irregular folding is common to chromatin in interphase, or non-dividing cells, as it makes DNA more accessible to the molecular machinery for RNA transcription and DNA replication.

"We focused on chromosomes in dividing cells," says Maeshima, "but we assume that a similar irregular folding also exists in interphase cells, and are now assessing that assumption." ■

1. Nishino, Y., Eltsov, M., Joti, Y., Ito, K., Takata, H., Takahashi, Y., Hihara, S., Frangakis, A.S., Imamoto, N., Ishikawa, T. & Maeshima, K. Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure. *The EMBO Journal* **31**, 1644–1653 (2012).

Seeking a splice for better rice

A survey of genomic contributions to metabolic activity in rice reveals a bounty of information that might steer future efforts at crop enhancement

Every organism produces a staggering variety of molecules, each with its own particular biological function. Complex interplay between genetic and environmental factors determines the production levels for each compound. By deciphering these factors, plant geneticists can use the information to derive organisms with useful properties, such as crops that are more resistant to pathogens.

A research team led by Kazuki Saito and Fumio Matsuda of the RIKEN Plant Science Center in Yokohama has begun to unravel how genetic variations affect metabolite production in rice, a core dietary staple around the world[1] (Fig 1). Saito's group, which has acquired considerable expertise in characterizing the chemical contents of plants, partnered with agricultural specialists at Japan's National Institute of Agrobiological Science (NIAS) in Tsukuba. "NIAS is the leading research center for rice breeding in Japan and has developed useful experimental lines for genetic analysis of rice qualitative traits," says Saito.

Saito, Matsuda and their colleagues examined 85 different lines generated via crosses between cultivars representing two different types of rice, known as Indica and Japonica. "Indica rice is fluffy and used for curry dishes, while Japonica short-grain rice is the moist, sticky, bright white rice used in sushi or risotto," explains Matsuda. From these various lines, the researchers collected data describing 759 different metabolites. Then they determined the extent to which genetic variations associated with Indica or Japonica genomes correlate with differences in the levels of these compounds.

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Figure 1: Rice is a core component of the diet of billions of people. Identification of the genetic factors that make the plant more robust, or nutritionally beneficial, may further boost the usefulness of this crop.

Although many metabolites appeared to be primarily modulated by non-genetic factors, Saito, Matsuda and their colleagues identified several molecules whose production is strongly affected by differences at particular genomic loci. The researchers even identified a 'hotspot'—a cluster of loci on chromosome 3 that coordinates production of a number of different amino acids and fatty acids.

Rice plants produce molecules known as flavone glycosides, which protect them from consumption by herbivores but also exhibit antioxidants and anti-inflammatory properties in humans. These could therefore represent promising targets for crop engineering. The research team found clear evidence for heritable factors that affect production, including an Indica-specific variant that is associated with greatly elevated levels of one particular flavone glycoside.

Finer mapping will be needed to pinpoint pertinent genetic alterations, as a possible prelude to targeted modification and optimization. However, this work also reinforces the value of 'old-fashioned' genetic engineering techniques. "Our study demonstrates that the metabolic composition in rice grains can be improved by traditional breeding programs, using natural genetic variations in the rice population," says Saito. ■

1. Matsuda, F., Okazaki, Y., Oikawa, A., Kusano, M., Nakabayashi, R., Kikuchi, J., Yonemaru, J., Ebana, K., Yano, M. & Saito, K. Dissection of genotype-phenotype associations in rice grains using metabolome quantitative trait loci analysis. *The Plant Journal* **70**, 624–636 (2012).

Finding genes that expand waistlines

Two large-scale genetic analyses reveal previously unknown genetic variants associated with obesity in east Asians

Some common genetic variants associated with obesity in east Asians have been identified by two international research teams involving RIKEN researchers[1,2]. Yukinori Okada and Toshihiro Tanaka at the RIKEN Center for Genomic Medicine in Yokohama, Japan, co-led the first study, and Tanaka co-led the second in an international collaborative consortium, the Asian Genetic Epidemiology Network (AGEN).

By analyzing data from over 62,000 east Asian individuals, the researchers found two of the variants, or single nucleotide polymorphisms (SNPs), in the *KLF9* and *MSTN* genes. *KLF9* encodes a transcription factor that activates expression of genes involved in numerous physiological processes, including one that is involved in fat cell differentiation and implicated in obesity. Mutations in *MSTN* are known to cause decreases in body fat (Fig. 1). Further analysis revealed that the newly identified SNPs interact with each other, suggesting that *MSTN* regulates the effects of *KLF9* on body mass index (BMI), a common measure of obesity.

In the second study, the researchers performed a meta-analysis on data from eight genome-wide association studies, including approximately 2.4 million SNPs in the genomes of nearly 28,000 east Asians. This revealed SNPs in three well-known genes, *FTO*, *SEC16B* and *MC4R*, which geneticists have previously found to be associated with BMI.

In an analysis of data from approximately 55,000 additional east Asian individuals, Okada, Tanaka and colleagues focused on 798 SNPs that they found were associated with BMI, and 50 additional SNPs linked to BMI in earlier studies. By combining these data sets, they confirmed that SNPs in six genes previously associated with obesity in Europeans are significantly associated with BMI in east Asians.

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Figure 1: Success or failure in battling weight gain can partly depend on the ancestry of your genes.

The researchers also identified three previously unknown variations that are significantly associated with BMI, located in or near the *CDKAL1*, *PCSK1* and *GP2* genes, all of which encode enzymes involved in metabolism.

Of all the variants identified, the *FTO* variant was most strongly associated with BMI, accounting for nearly 0.2% of the variation in the study population. This and nine other genes strongly associated with BMI account for nearly 0.9% of the individual variation between them, while all 22 of the genes identified together explained 1.18% of the individual variation in east Asians.

Large-scale studies such as these provide valuable information about the genetic bases of obesity. As well as identifying genetic variants that are important in east Asians, they allow for comparisons with European individuals. "We will continue [to clarify the function] of these SNPs, which might lead to a better understanding of metabolic syndromes," says Tanaka. ■

1. Okada, Y., Kubo, M., Ohmiya, H., Takahashi, A., Kumasaka, N., Hosono, N., Maeda, S., Wen, W., Dorajoo, R., Go, M.J. *et al.* Common variants at *CDKAL1* and *KLF9* are associated with body mass index in east Asian populations. *Nature Genetics* **44**, 302–306 (2012).
2. Wen, W., Cho, Y.-S., Zheng, W., Dorajoo, R., Kato, N., Qi, L., Chen, C.-H., Delahanty, R.J., Okada, Y., Tabara, Y., *et al.* Meta-analysis identifies common variants associated with body mass index in east Asians. *Nature Genetics* **44**, 307–311 (2012).

Tracking down prostate cancer risks

Diversifying the populations of Japanese men used in a study plotting their risk to prostate cancer reveals a potential dietary link

Three previously unknown loci, or regions of the genome, are associated with an increased risk of Japanese men developing prostate cancer, according to RIKEN-led research on single nucleotide polymorphisms (SNPs) associated with this disease[1]. An SNP associated with prostate cancer at a fourth locus—related to one found earlier in European men—appears to be associated with diet, the first hard evidence of such an environmental link.

These findings are the result of a genome-wide association study (GWAS) by Hidewaki Nakagawa of the RIKEN Center for Genomic Medicine, Yokohama, and colleagues at universities and institutes in Japan and the USA. Repeating earlier work in Europeans, they compared the genetic profiles of Japanese groups of prostate cancer sufferers with non-sufferers.

"We are aiming to construct a genetic risk model for the development of prostate cancer that we hope will [allow personalization of] prostate cancer diagnosis in the Japanese population," Nakagawa says.

The researchers broadened the scope of an earlier study by adding three, more diverse, populations of Japanese men. One population included a cohort of men from Hawaii and California with Japanese ancestry. They tested nine loci that their previous study had indicated were nominally associated with the development of prostate cancer, but were not significant statistically.

The latest study not only allowed for testing of a single ethnic group likely to be affected by different environmental factors, but also provided a larger population size when combined with those surveyed in the previous work for a meta-analysis. The joint numbers included 7,141 prostate cancer sufferers and 11,804 non-sufferers.

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Figure 1: A traditional Japanese breakfast of natto (fermented soy beans), sprinkled with compressed seaweed, is rich in vitamin K, a compound traditionally thought to fight cancer.

The most recently identified loci are on chromosomes 11, 10, 3 and 2. Those on chromosomes 10 and 3 are not related to any known genes or proteins, but the researchers suggest they may be regions linked with gene regulation. The locus on chromosome 11 is associated with a gene whose function is unknown, but the locus on chromosome 2 is linked with GGCX, a vitamin K-dependent enzyme that regulates blood clotting, bone formation and cancer biology. Japanese foods such as *natto* and seaweeds are rich in vitamin K, which is thought to protect against cancer (Fig. 1). Interestingly, the association of this SNP with prostate cancer was significant in all populations except for the Japanese in the USA, indicating that environmental factors, such as diet, are involved.

"These findings provide support for conducting GWAS for prostate cancer in diverse populations to identify risk loci for this genetically heterogeneous cancer," the researchers note. ■

1. Akamatsu, S., Takata, R., Haiman, C.A., Takahashi, A., Inoue, T., Kubo, M., Furihata, M., Kamatani, N., Inazawa, J., Chen, G.K., *et al.* Common variants at 11q12, 10q26 and 3p11.2 are associated with prostate cancer susceptibility in Japanese. *Nature Genetics* **44**, 426–429 (2012).

Following an immune cell's career path

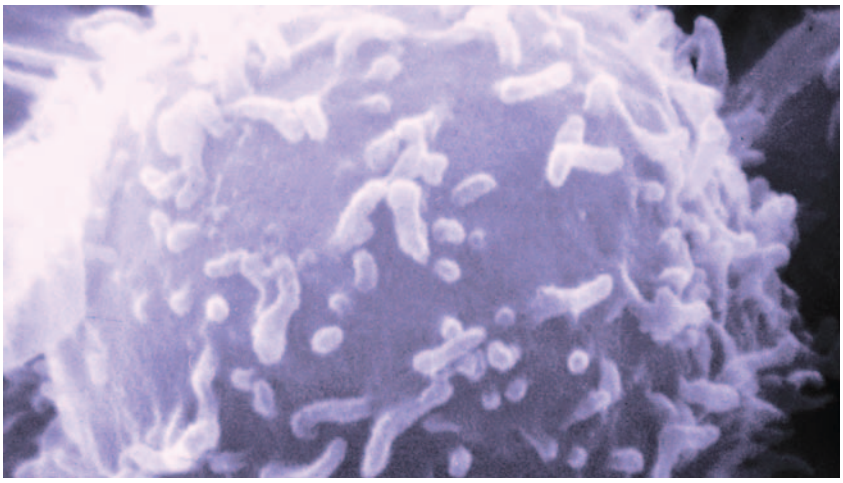
A protein 'fingerprint' used to identify certain immune cells is expressed more broadly than first thought, raising new questions about how these cells develop

The immune system produces diverse varieties of T cells (Fig. 1), such as pathogen-destroying cytotoxic T cells and immune response-boosting helper T cells. Regulatory T (T_{reg}) cells restrain these other cells and prevent the body from overreacting to threats or generating a dangerous autoimmune response.

T_{reg} cells are usually identified by expression of the transcriptional regulator protein Foxp3, but new work from a team led by Shohei Hori of the RIKEN Research Center for Allergy and Immunology in Yokohama has demonstrated that this is not a reliable signature[1].

Several groups have obtained data suggesting that T_{reg} cells can essentially 'change careers', losing their Foxp3 expression and transforming into other T cell types. However, findings from Hori and colleagues led them to propose an alternative 'heterogeneity model'[2]. "Our observations suggested that these phenomena can be fully explained by a minor 'uncommitted' population of Foxp3$^+$ T cells without assuming reprogramming," he says. His group has now provided compelling evidence for this hypothesis by using a labeling technique that allowed them to distinguish cells currently expressing Foxp3 from those that are not, but which have expressed this protein in the past.

The researchers identified two groups of Foxp3-expressing cells that responded differently to an immune stimulus. Most expressed this protein stably and at high levels, and exhibited the functional characteristics of T_{reg} cells. A minority fraction displayed transient bursts of Foxp3 expression, but ultimately developed into other T cell types. These 'exFoxp3' cells did not appear to represent reprogrammed T_{reg} cells, but rather a separate pool of T cells that only produce this protein sporadically.



Source: Dr Triche, National Cancer Institute, USA

Figure 1: The various classes of T cells are capable of responding to pathogenic threats and restraining the resulting immune response to avoid inflicting damage on host tissues.

Interestingly, his team also learned that some T_{reg} cells do enter a state where they stop expressing Foxp3, although they retain 'memory' of their identity as T_{reg} cells. This is achieved via chemical modifications to the DNA within the gene encoding Foxp3, and immune stimulation promptly leads to robust re-expression of this protein. "This should force people to reconsider the popular but oversimplified view of Foxp3 as the master regulator of T_{reg} cells," says Hori.

The events that determine this expression profile are therefore likely to prove more important in establishing cellular identity than the presence or absence of Foxp3. "It has been speculated that the T_{reg} lineage is determined by a higher-order regulatory pathway upstream of Foxp3, but the nature of this system is unknown," says Hori. In future work, he plans to partner with colleagues at other RIKEN laboratories to investigate this question more closely. ■

1. Miyao, T., Floess, S., Setoguchi, R., Luche, H., Fehling, H.J., Waldmann, H., Huehn, J. & Hori, S. Plasticity of Foxp3$^+$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. *Immunity* **36**, 262–275 (2012).
2. Komatsu, N., Mariotti-Ferrandiz, M.E., Wang, Y., Malissen, B., Waldmann, H. & Hori, S. Heterogeneity of natural Foxp3$^+$ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. *Proceedings of the National Academy of Sciences USA* **106**, 1903–1908 (2009).

Building a faster snapshot of cell function

Automation of a protocol for rapidly analyzing gene expression on a large scale will yield faster results at less cost

To generate an overall view of cell function, molecular biologists build simultaneous expression, or activity, profiles of thousands of genes. Gene expression begins with a process called transcription, during which the DNA sequence encoding a gene is copied into RNA. The information contained in the transcript is then translated into a chain of amino acids that folds up to form a functional protein molecule.

Building on a technique called cap analysis of gene expression (CAGE) to identify and analyze transcription start sites and their expression levels, a research team in Japan, led by Masayoshi Itoh of the RIKEN Omics Science Center in Yokohama, has developed a large-scale technique for analyzing gene expression that increases productivity eight-fold compared to previous methods[1].

The original CAGE protocol involves 17 steps including a process that 'traps' the ends of the original transcripts, numerous extractions using organic solvents, and centrifugation to create complementary DNA (cDNA) libraries[2]. Itoh and his colleagues simplified and eliminated the PCR amplification step to reflect the original expression profile[3]. The prepared libraries consisted of millions of DNA molecules, each corresponding to the transcription start site. The researchers then determined the sequence of these cDNA molecules, and the amount of each one, using a single molecule sequencer called the HeliScope Genetic Analysis System.

Since each step in the original protocol was performed manually, the technique was labor intensive and it took approximately six weeks to prepare 96 cDNA libraries. Itoh and colleagues currently have two of the new systems in place that allow them to generate 192 cDNA libraries every eight days. The automation cuts sequencing costs because it involves less manual work, and the system can be used with the three most common DNA sequencing platforms.

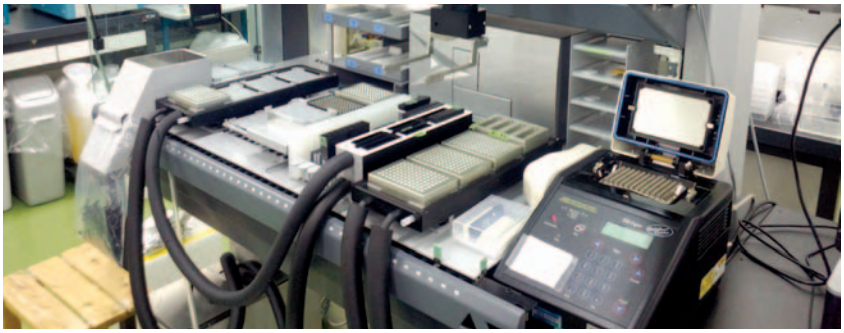


Figure 1: The automated CAGE cDNA preparation system for rapid genome-wide gene expression analysis.

Itoh and his colleagues achieved this improvement by adapting CAGE so that all the steps are completed by an automated system consisting of a robotic manipulator arm equipped with an 8-channel liquid-handling device (Fig. 1). They simplified the protocol further by using magnetic beads to purify the cDNA. The automated method is significantly faster than the original protocol and reliably produces highly accurate sequences. Since constant supervision is not required, two or more systems can be easily operated simultaneously.

"We are currently developing the automation system for the next generation Illumina HiSeq2000 sequencing platform," says Itoh. "This involves improving the steps that produce and trap the transcription start site cDNAs, and we aim to establish the new library preparation system in the first half of 2012." ■

1. Itoh, M., Kojima, M., Nagao-Sato, S., Saijo, E., Lassmann, T., Kanamori-Katayama, M., Kaiho, A., Lizio, M., Kawaji, H., Carninci, P., *et al*. Automated workflow for preparation of cDNA for cap analysis of gene expression on a single molecule sequencer. *PLoS ONE* **7**, e30809 (2012).
2. Shiraki T., Kondo S., Katayama S., Waki K., Kasukawa T., Kawaji H., Kodzius R., Watahiki A., Nakamura M., Arakawa T., Fukuda, S., Sasaki, D., Podhajska, A., Harbers, M., Kawai, J., Carninci, P., Hayashizaki, Y. Cap analysis gene expression for high-throughput analysis of transcriptional starting point and identification of promoter usage. *Proceedings of the National Academy of Sciences* **100**, 15776–15781 (2003).
3. Kanamori-Katayama M., Itoh M., Kawaji H., Lassmann T., Katayama S., Kojima, M., Bertin, N., Kaiho, A., Ninomiya, N., Daub, C. O., Carninci, P., Forrest, A. R. R., Hayashizaki, Y. Unamplified cap analysis of gene expression on a single molecule sequencer. *Genome Research* **21**, 1150–1159 (2011).

Making sense of molecular fragments

A new computer program overcomes difficulties in rebuilding the shape of full-length RNA molecules from high-throughput sequence data

Data from high-throughput next generation sequencers (NGS) and genome tiling arrays have greatly enhanced scientists' ability to recreate RNA molecular structures, which is vital to disease and biotechnology research. However, high levels of noise and bias in some processes lead to uneven gene-expression values for segments belonging to the same molecule. Reconstructing the complete, or 'full-length', information of molecules as they occur in cells is therefore difficult.

To improve accuracy by reducing noise and bias, Tetsuro Toyoda, Shuji Kawaguchi, and Kei Iida at the RIKEN Bioinformatics And Systems Engineering division (BASE) in Yokohama, together with scientists from the RIKEN Plant Science Center, have developed a statistical algorithm for reconstructing full-length information of RNA molecules using output from tiling arrays and NGS[1]. They implemented this algorithm in a computer program called 'ARabidopsis Tiling-Array-based Detection of Exons' (ARTADE).

The genome encoded in an organism's DNA holds the blueprint for building and maintaining cells. For this building and maintenance to work, the DNA blueprint is copied, or 'transcribed', by molecules of RNA 'transcripts'. RNA molecules use this code to create proteins or act themselves as functional molecules and regulate cell activities. Transcriptome is the name given to all the transcripts present at any one time in a cell. Transcriptomes hold vital information about living organisms, including how different protein genes are switched on and off in response to different environmental stresses.

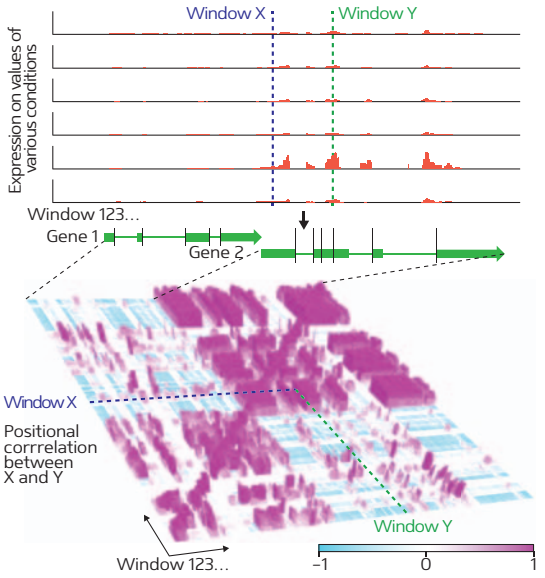


Figure 1: From next generation sequencer expression data for two genes (green), expressed in different plant tissues and conditions, the ARTADE2 computational algorithm produces more accurate representations of RNA molecule reconstructions (bottom) than the pre-processed transcriptional expression level indications shown in red (top). Unclear representations of the genes (top left), for example, are visible after applying ARTADE2 (bottom left).

Toyoda and his team further developed ARTADE, ARTADE2, so they could rebuild a virtual representation of the transcriptomes comprising RNA molecules. "Understanding transcriptomes is essential for research on molecular mechanisms of diseases and development of biotechnology with plant species," Toyoda explains. "Both genome tiling arrays and NGS have output problems with uneven expression values from fragmentation and noise and bias from machinery. This makes it difficult to form a perfect reconstruction."

ARTADE2 uses a new 'positional correlation analysis' developed by Kawaguchi and Iida so that it can analyze any species and be used for NGS output (Fig. 1). This process identifies areas where the transcriptional activities among multiple cellular conditions—such as differences in tissues, developmental stage, or environment—are highly correlated. Positional correlation removes output problems, providing a better representation of the original molecules.

The team has now begun developing a database using information from the rebuilt transcriptomes. This new technology and database will lead to deeper understanding of molecular structures and their alteration according to environmental stresses and disease, furthering understanding of the relationship between genome sequences and cell activities. ■

1. Kawaguchi, S., Iida, K., Harada, E., Hanada, K., Matsui, A., Okamoto, M., Shinozaki, K., Seki, M. & Toyoda, T. Positional correlation analysis improves reconstruction of full-length transcripts and alternative isoforms from noisy array signals or short reads. *Bioinformatics* **28**, 929–937 (2012).

Making live cell microscopy affordable

An inexpensive and non-destructive microscopy method for illuminating living cells brings advanced biology within reach of under-resourced laboratories

Researchers in Japan have developed a low-intensity light source that allows cell biologists to visualize and handle live cells without destroying them during prolonged exposure[1]. In addition to laying the foundation for new cell manipulations, the development will make advanced biology requiring fluorescence microscopy accessible to underfunded laboratories. Led by Teruhiko Wakayama from the RIKEN Center for Developmental Biology, Kobe, the researchers developed an adapter, equipped with a halogen lamp, for a conventional microscope.

Typically, conventional microscopes used in live cell studies rely on powerful ultraviolet (UV) lamps or lasers to illuminate cells labeled with a fluorescent dye molecule. However, extended imaging times or continuous exposure to these high-intensity light sources results in harmful effects to cells. Although the problem seemed complex, Wakayama explains that simply lowering the strength of the light source via a halogen lamp solved the problem of phototoxicity.

The adapter developed by the researchers consisted of a small excitation filter and a diaphragm that allowed some light to leak around its periphery. They placed it on top of the microscope's condenser lens, which concentrates light from the lamp onto the samples. By closing the diaphragm, they channeled all the light through the filter, yielding only fluorescence. Opening the diaphragm produced a bright field image that merged with the fluorescent signal. The researchers found that they could tune the relative intensities of both images by varying this opening.

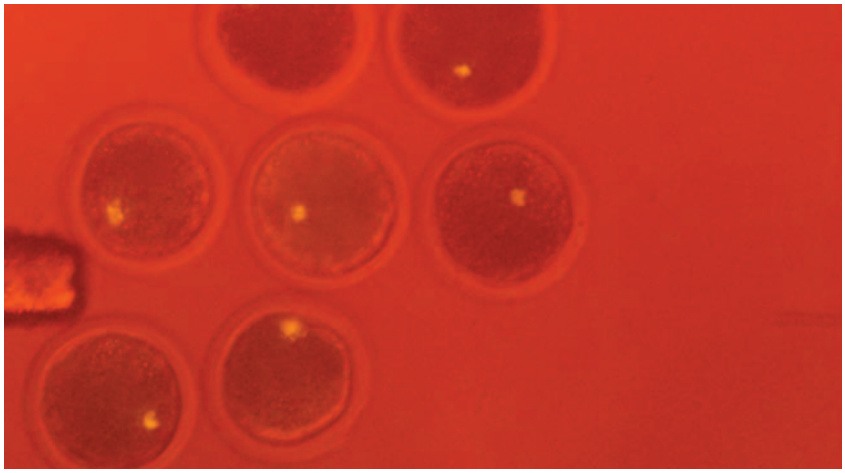


Figure 1: An image of fluorescently labeled unfertilized cow oocytes using the halogen lamp filter adapter. The nuclei are clearly observed at the center of the cells.

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Wakayama and his colleagues tested the performance of their device for monitoring the enucleation, or the removal of metaphase chromosomes, from female reproductive cells called oocytes. They discovered that, unlike traditional enucleation approaches, the new method allowed them to successfully remove the chromosomes in quantitative yields. This made it unnecessary to use additional analytical techniques to confirm the absence of chromosomes (Fig. 1). Overall, compared to conventional approaches, the halogen lamp method significantly simplified fluorescent observation of the enucleation procedure and reduced processing times without affecting cell viability.

The team is currently planning to use their fluorescence imaging system to rapidly detect the fertilization of human oocytes.

"Previously, we could not observe these oocytes using fluorescence microscopy [owing] to UV lamp-induced cell damage, but now we can thanks to our adapter," says Wakayama. Moreover, the researchers are proposing to introduce their device to high schools, which use simple microscopes that lack fluorescence imaging capabilities. "Our system will make it possible for all students to have access to advanced fluorescence microscopy without excessive costs," he adds. ■

1. Yamagata, K., Iwamoto, D., Terashita, Y., Li, C., Wakayama, S., Hayashi-Takanaka, Y., Kimura, H., Saeki, K. & Wakayama, T. Fluorescence cell imaging and manipulation using conventional halogen lamp microscopy. *PLoS ONE* **7**, e31638 (2012).

KUNIYA ABE

Team Leader
Technology and Development Team for
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RIKEN BioResource Center

New techniques to rediscover the value of bioresources

Research in the modern life sciences cannot evolve without using biological genetic resources, or 'bioresources', including mice, plants, cells, microorganisms and genes. Kuniya Abe, leader of the Technology and Development Team for Mammalian Cellular Dynamics at the RIKEN BioResource Center (BRC), is working to utilize such research resources in more effective ways to help promote research activity in the life sciences as a whole. "My motto is: Learn from the past," says Abe. "Bioresources have been produced by many researchers over the long history of scientific studies. If we examine them from different viewpoints using novel techniques, we may be able to realize their hidden potential. The wide variety of bioresources that has been collected to date at the BRC truly represents a treasure trove that will never lose value."

Exploring bioresources from three perspectives

"We are working on creating useful bioresources, and developing new techniques to analyze their characteristics, thereby increasing their value," says Abe. "We are also mindful of carrying out unique investigations that can only be conducted using these bioresources. In characterizing bioresources, we focus on three main categories: genotype, epigenotype and phenotype."

It is well known that DNA, which constitutes the genome or total genetic information of an organism, is encoded by its base sequence, or the arrangement of the four bases: adenine (A), thymine (T), guanine (G) and cytosine (C). This genetic makeup, found in all organisms, is known as the genotype.

"Our personalities and abilities are not solely dependent on the genome we inherit from our parents—instead, they can be varied by differences in gene expression. It is the process, which causes some genes to function and others to be inactivated. The DNA winds around the histone protein, forming a structure called chromatin, which is housed in the cell nucleus (Fig. 1). Gene expression is regulated as the chromatin is loosened and condensed to change its structure. The chromatin structure is altered by genome modifications such as DNA methylation, in which a methyl group attaches to the DNA, and histone modifications, in which histone is methylated or acetylated by the attachment of an acetyl group or a methyl group."

This pattern of acquired genome modifications—whereby gene expression is regulated without changing the base sequence of the DNA—is known as the epigenotype.

Phenotypes represent the observable characteristics of an organism, such as facial shape, hair color and disease susceptibility, which arise due to the expression and functioning of genes. Although researchers have long been engaged in extensive investigations to determine how genotypes are reflected in phenotypes, they have only recently begun to focus on epigenotypes, which occupy an intermediate position between genotypes and phenotypes.

Why use wild mice?

In the category of genotype, comparative genomic analyses of laboratory mouse strains and wild-derived strains are described here.

Currently, the most widely used, standard mouse strain is C57BL/6 (B6). The origins of this standard laboratory strain

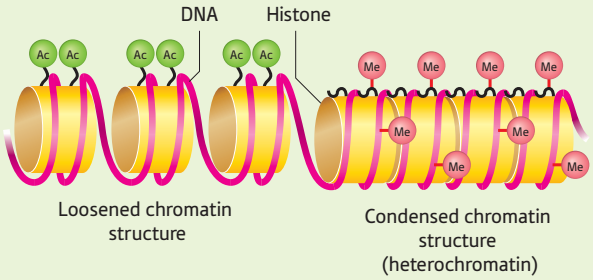
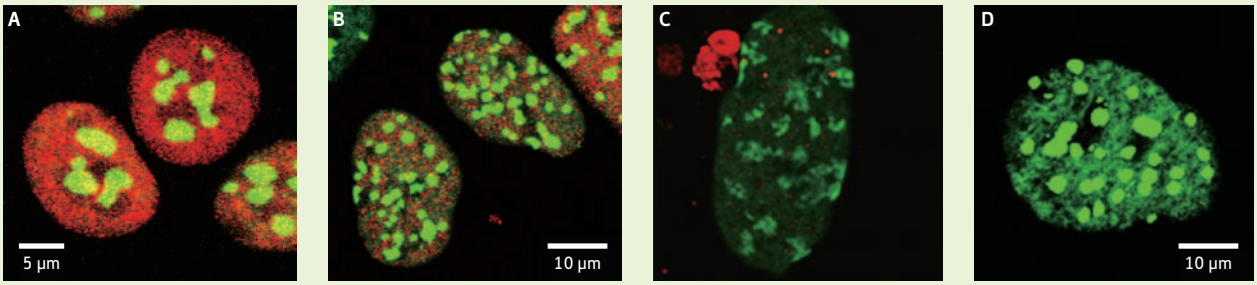


Figure 1: Chromatin structure and visualization of DNA methylation in ES cell differentiation

The DNA winds around the histone protein to form a structure known as chromatin. When an acetyl group (Ac) attaches to a particular site of histone, the chromatin structure is loosened to allow the gene to be switched on (left). Meanwhile, when a methyl group (Me) attaches directly to the DNA, and another methyl group to another site of the histone, the chromatin is condensed to form heterochromatin, and the gene is switched off (right). Gene expression is regulated as the chromatin structure is loosened or condensed by such genome modifications.



A: Undifferentiated state

Methylated DNA is shown in green. Several green gigantic chromocenters are seen. A chromocenter is an assembly of bundles of many chromosomes formed by mutually associating heterochromatins in centromeres. A marker for undifferentiated cell (OCT3/4 protein) is shown in red, indicating that the cell has not differentiated.

B and C: States in the midst of differentiation

Once differentiation has begun, chromocenters are separated and redistributed, whereas the undifferentiation marker decreases (B). As the differentiation proceeds, the heterochromatin is loosened, and the DNA methylation level lowers; the undifferentiation marker is no longer present (C). The red portion in the upper left of panel C is an artifact.

D: State approaching terminal differentiation: the DNA methylation level rises again.

had been unclear. Many of the laboratory mouse strains widely available today, including B6, are thought to be offspring of pet mice in Western Europe, which were transferred to the USA in the early 20th century. The mice were subjected to inbreeding by geneticists to establish new strains with the same genetic background. Inbreeding can be completed after 20 generations or more of sib-mating (Fig. 2).

"An interesting thing to note is that the phenotypes of laboratory mice are highly diverse," comments Abe. "If they had originated from a few mice that were offspring of a mouse subspecies group (the hierarchical class under the species in biological taxonomy) from Western Europe, this remarkable diversity would not have emerged. A likely cause is that evolutionarily isolated genomes mingled together. In fact, a past study had provided evidence for the mingling of an Asian mouse genome in the B6 strain. However, the true story as a whole had been unclear. This was because the B6 strain was the only mouse strain whose genomic information, in the form of base sequences, was then known in its entirety, whereas the base sequences of the Asian mouse genome were largely unknown. Therefore, we proceeded to conduct a genomic analysis on the Asian mouse and compare its genome sequence with that of B6."

In 2004, Abe and his colleagues constructed a BAC library of an inbred strain from a Japanese wild mouse referred to as MSM/Ms, or simply MSM. A BAC clone is prepared by inserting a fragment of genomic DNA into a bacterial artificial chromosome (BAC) vector; several hundreds of thousands of BAC clones are gathered into an assembly comprehensively carrying the base sequences of the entire genome. These assemblies, known as BAC libraries, are now commonly used as an essential resource for analyzing genomes, as a broad range of analyses can be conducted by using BAC clones without directly handling the whole genome.

"The base sequences of B6 and MSM were found to differ by nearly one percent. Human beings and chimpanzees are considered to have evolved into different species some five million years ago, and the base sequence difference between these primates is 1.2%. With this in mind, we can say that B6 and MSM are considerably far from each other in terms of evolution despite their belonging to the same species. Our analytical results support the previous estimation that the differentiation into the two subspecies groups occurred about one million years ago."

Abe and his colleagues then proceeded to map the positions of single-nucleotide polymorphisms (SNPs), which represent genomic differences by one base, as compared with the base sequences of the two strains B6 and MSM (Fig. 3). They found that there are some regions representing extremely low frequency of SNPs, hence regions with high sequence homology, in their genomes. "This finding demonstrates that the genome sequence of the B6 strain is for the most part derived from the Western European subspecies groups but partially from the Asian subspecies, and that artificial hybridization of the two subspecies resulted in mingling of their genomes in a mosaic state."

Furthermore, using an SNP marker for the combination of MSM and B6, Abe and his team examined other laboratory mouse strains to determine the type of SNPs in their base sequences. All strains examined were found to have SNPs of the MSM type, as does B6, with the SNP distribution pattern differing little by little among the strains. It is therefore evident that laboratory mouse strains resulted from shuffling the genomes of the Asian and Western European mouse subspecies, which occurred in different modes among the different strains.

"The two subspecies are chronologically apart from each other by about one million years on the evolutionary timescale, and their genomes differ widely," says Abe. "The phenotype diversity found in

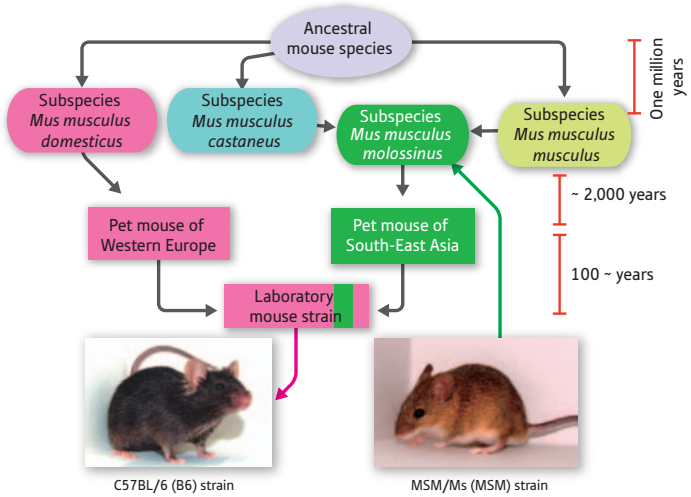


Figure 2: Mouse dendrogram

Mice are believed to have differentiated into several subspecies groups about one million years ago. The various subspecies groups exhibit their own respective phenotypes. The laboratory mouse strain exhibits diverse phenotypes although they were supposedly derived from a small number of pet mice.

the various laboratory mouse strains may be due to different combinations of genomes coming from the two subspecies." Further investigations will be necessary to obtain experimental data for testing this hypothesis. Abe and his colleagues posit that the interaction of two different genomes may trigger some change in the expression of the genes. In fact, within certain pairs of genes, it has been discovered that one of the pair is always subject to repression. Abe comments, "An organism is a system in which multiple genes interact with each other to exhibit their functions. However, the functioning of the system may be altered as two different genomes encounter each other, and this may produce varied phenotypes. If so, this issue would lead not only to a better understanding of the genetics and evolution of the mouse, but also to the much wider elucidation of gene interactions and networks."

When Abe began analyzing the genome of the Japanese wild mouse, one question he was often asked by overseas researchers was: "Why do you use wild mice?" However, it was owing to the Japanese wild mouse—a unique bioresource, viewed from a novel perspective using new techniques—that made Abe's research breakthroughs possible. "Currently, American and European researchers are paying attention to Asian wild mice. This is because the genome information of Asian mice may also lead to a deeper understanding of laboratory mouse strains. While the life sciences evolved by concentrating on a few model organisms, we have entered an era in which both the uniqueness and universality of living systems can be revealed by examining and comparing diverse subjects using a wide variety of advanced analytical techniques."

Real-time visualization of DNA methylation in embryonic stem cells

Another significant area of research at Abe's team is the study of the epigenotype. "In higher animals such as mammals, a genome modification such as DNA methylation causes chromatin to be condensed and form a structure called heterochromatin (Fig. 1). The relevant gene is then switched to repress expression. Epigenotypes such as DNA methylation are considered to change dynamically in the processes of cell differentiation and development of individuals. However, no method had been available for analyzing these processes in a single living cell."

In 2007, Abe developed a technique to visualize DNA methylation in living cells, and applying this method, he succeeded in observing how the DNA methylation pattern changes for embryonic stem cells (ES cells) as they differentiate, as well as the morphological changes in highly methylated heterochromatin. In Figure 1, panel A shows the cells in an undifferentiated state, panels B and C show intermediate states of differentiation, and panel D shows a state approaching terminal differentiation. Methylated DNA is shown in green and OCT3/4 protein (a marker for the undifferentiated state) in red, demonstrating that the cell has not differentiated. In panel A, the larger green portions are chromocenters, each one of which is an assembly of bundles of many chromosomes formed by association of centromeric heterochromatins. Once differentiation has begun, the chromocenters are separated and redistributed (panel B). As differentiation proceeds, the DNA methylation level lowers, which in turn loosens the heterochromatins, resulting in the reduction of the green signals for methylation (panel C). As terminal differentiation is approached, the DNA methylation level rises again, forming an epigenotype that is different to the original (panel D).

"I was astonished to find that DNA methylation changes so widely in the process of cell differentiation," says Abe. "Another notable finding was that the intranuclear structure changes concurrently.

Figure 3: Mosaic genome structure in the B6 strain

The positions of single-nucleotide polymorphisms (SNPs) representing genomic differences by one base between the two strains, B6 and MSM. Regions with an extremely low incidence of SNPs between the two (with very similar sequences) are shown in green. Regions with SNPs frequently detected between the two strains (with different sequences) are shown in pink. This finding demonstrates that the genome sequence of the B6 strain is mostly derived from the Western European subspecies and partially derived from the Asian subspecies, and that artificial hybridization of the two subspecies resulted in the mingling of the genomes of the two subspecies in a mosaic state. Chr1 to Chr19 and ChrX represent the 19 chromosome pairs and X chromosome in the mouse.

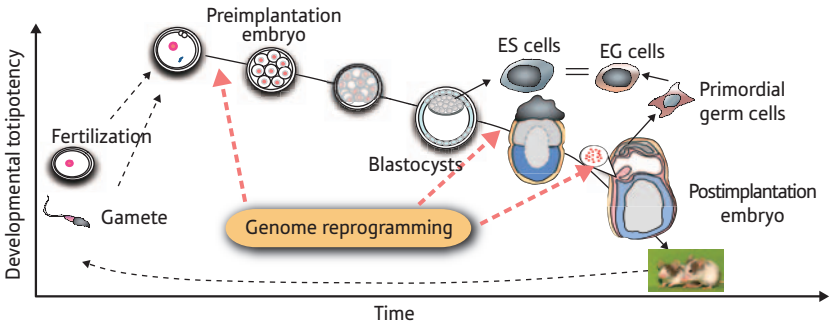


Figure 4: Germ cell lineage and genome reprogramming

A mouse germ cell lineage is shown along the temporal axis of development. The Y axis represents developmental potential. Fertilized eggs are totipotent. In the processes of mouse early embryogenesis and primordial germ cell formation, genome reprogramming occurs in which epigenetic modifications are reset on the genomic DNA, resulting in dramatic changes in the epigenotype. Accordingly, the cell nucleus significantly changes its structure. Embryonic stem (ES) cells originate from blastocysts and primordial germ cells are the origins of germ cells. Because the epigenetic modifications accumulated during the development is reprogrammed, mouse germ cell lineages can be the most suitable subject of research into genome reprogramming. Abe is working to generate epigenome maps of germ cell lineages.

While in an undifferentiated state, the chromocenter is largely located in the nucleus, although it is partially in contact with the nuclear lamina, a basket-like layer of fibers inside the nuclear membrane. However, upon differentiation, the heterochromatin changes its position so that it becomes embedded in the nuclear lamina. Hence, the shape of heterochromatin and the intranuclear positions of chromosomes were found to change dynamically in synchronization with epigenetic changes. Additionally, when we took a series of images in living cells at constant time intervals, we found that nuclear division appeared to trigger conversion of the undifferentiated state into differentiated states. We think this represents the very instance of ES cell differentiation. The intranuclear structural change should also influence gene expression."

Although research into the relationship between cell nuclear structures and development and differentiation has only just begun, the observations made by Abe and his team have caused a stir in the field. Although the visualization of DNA methylation in ES cells has been achieved *in vitro*, Abe is currently working to visualize DNA methylation in living organisms in real time. He is also developing a technique to detect methylation of a particular base sequence, as well as studying the analysis of DNA methylation in the entire genome using just a small number (of between tens to one hundred) of cells.

The goal: To generate epigenomic maps for germ cell lineages

With regard to future research goals, Abe comments, "Life begins at the very moment an egg is fertilized by sperm. The fertilized egg repeatedly undergoes cell cleavage to become an embryo, in which cells differentiate into a wide variety of tissue and organs, eventually resulting in the birth of an individual. In this developmental process, some cells of the embryo differentiate into primordial germ cells, which serve as sources of ova and spermatozoa. The primordial germ cells become ova and spermatozoa, and again acquire the capacity to undergo fertilization."

"I was interested originally in early embryogenesis and the formation of germ cells, such as spermatozoa, ova, and primordial germ cells," Abe continues. "These processes involve many biologically critical phenomena, including the mechanisms for genome protection, for revitalization of genome through genome reprogramming—by which genome modifications are reset—and the mechanisms for creation of genomic diversity through chromosome crossover. My research goal is to generate epigenomic maps, made up of a comprehensive combination of gene expression, genome modifications, structural changes in cell nuclei and other phenomena in the cell lineage, from early embryos to germ cells (Fig. 4), and to understand the meaning of these epigenetic changes. However, there are only a very small number of cells of this lineage in the embryo, and so collecting and examining them involves painstaking work. For this reason, people tend to use ES cells, which have been artificially induced to differentiate *in vitro*. However, the real answer should reside in the development of living organisms. I believe epigenomic maps will deepen our understanding of the relationship between gene expression and changes in epigenetic status or intranuclear structures, and the nature of genome reprogramming as well as abnormalities associated with this process."

Currently, Abe and his colleagues at the BRC are conducting joint research into abnormalities in cloned mice. "Anomalies of clones arise partially due to incomplete genome reprogramming. In this sense, the clones can be described as epigenetic mutants. I think that epigenetic mutations also accumulate in the process of iPS cell generation, which has been a hot topic in recent years. Epigenomic maps may enable us to distinguish between the normal and the abnormal. Once clues are obtained, there should be a way of dealing with the abnormalities. This will also lead to the creation of new bioresources."

"The RIKEN BRC possesses a wealth of knowledge in the form of bioresources. The seeds of new ideas are awaiting discovery. I aim to share unique research achievements that would not be realized without using the BRC's resources."

ABOUT THE RESEARCHER

Kuniya Abe was born in Fukuoka, Japan, in 1955. He graduated from the College of Natural Sciences, the University of Tsukuba, and obtained his PhD in 1983 from the Institute of Biological Sciences, the University of Tsukuba. He worked at the Memorial Sloan-Kettering Cancer Institute in New York from 1983 to 1986, where he undertook research in mammalian developmental genetics. From 1987 to 1989, he continued studies in mouse genetics at the University of Texas at Austin. In 1989, he joined the ERATO MorphoGene Project and started research in primordial germ cell development. From 1991 to 2001, he worked at the Institute of Molecular Embryology and Genetics at Kumamoto University, and then moved to RIKEN as a team leader of Mammalian Cellular Dynamics at the BioResource Center in 2002. His research focus is basically in two areas: mouse functional genomics and epigenome dynamics during early embryogenesis and germline development.

KEN SHIMIZU

Evaluation Section, Policy Planning Division
Office for the Promotion of an Administrative Information System, General Affairs Division
System Adviser, Personnel Division
RIKEN Headquarters

Developing more effective ICT solutions

Why did you join RIKEN?

Through my involvement with systems development and consulting for a wide range of different-sized businesses and indeed my experience of business start-ups, I became acutely aware of the way in which the question of how to effectively manage information with regard to such factors as marketing and management throughout the whole business so often underpins the kind of root-and-branch reform of organizations that leads to innovation. I am always brainstorming and looking for more workable information and communication technology (ICT) solutions. An ongoing challenge at RIKEN is how to more effectively organize research-related information. I joined RIKEN because I would like to help overcome this challenge by applying to the role my ideas about systems development and my experience in the field.

What do you do at RIKEN?

Although I have several roles with different responsibilities, the common goal of all these roles is to enhance the work flow and systems development at RIKEN. I am also project managing four projects related to the human resources department. The goals of these projects are not simply to just build a system, but also to promote business process re-engineering and IT system restructuring in an integrated manner. These elements play a key role in effective ICT solutions. By aiming to achieve these goals, my team and I work together in striving to develop better ICT solutions for improving work processes at RIKEN.

What have been the highlights of your time at RIKEN so far?

At the outset of these projects, it soon became clear that many of the project members had little understanding of what went on outside of their own areas of responsibility and in some cases were unaware that their sections were connected to other sections in RIKEN. However, they worked assiduously on top of their existing day-to-day tasks to reform and improve work processes and the quality of information available with the common goal of developing the kind of services that people working at RIKEN have a right to expect. As a result, we achieved our team objective and created an enduring legacy which continued after the conclusion of the project. It was extremely gratifying to receive positive feedback and it emphasized to me that effective ICT solutions are all about the individual and the team.

How was the transition to life at RIKEN?

I think that in any type of work, not only within RIKEN but beyond, things may not run smoothly all of the time. However, by identifying targets and ways of tackling problems, I think the chance of success is high. Instead of just thinking about the IT system itself, it may be important to consider how to make use of the information in terms of how it flows, how we share, and how information is connected to and impacts our actions. Of course, the best solution varies from situation to situation; there are no absolute solutions. Therefore, I keep on thinking about what are the best solutions. I have been fortunate to be surrounded by many supportive colleagues at RIKEN.

What is the best thing about working at RIKEN?

If you wish, you can gain many opportunities to think about the true essence of things through various experiences.

CONTACT INFORMATION

For details about working at RIKEN, please contact the RIKEN Global Relations Office:
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E-mail: gro-pr@riken.jp

Celebrating 30 years of friendship

Over the last three decades, RIKEN and the Chinese Academy of Sciences (CAS) have conducted a wide variety of joint research programmes, with a total of 1,000 researchers taking part in exchanges that have continued to deepen ties between the two organizations. To commemorate the thirtieth anniversary of the official launch of research cooperation between the two organizations, a series of special events was held in Japan on 25 May 2012.

RIKEN President Ryoji Noyori (left) and the CAS President Chunli Bai (right) celebrate the signing of a joint communiqué pledging further research cooperation in the future

A delegation of 24 guests from the CAS, led by President Chunli Bai, visited RIKEN's Wako campus where, following a welcoming address from RIKEN President Ryoji Noyori, delegates had the opportunity to discuss current and future research collaborations. The delegates participated in a tree planting ceremony to celebrate the longstanding partnership.

A series of commemorative lectures was presented at one of Tokyo's most innovative venues, Roppongi Academyhills. More than 200 people attended the talks given by outstanding scientists from the two institutes. Representing the CAS, lectures were presented by Institute of Modern Physics Director Guoqing Xiao, Institute of Chemistry Director Lijun Wan, Institute of Metal Research Deputy-director Huiming Cheng, and Institute of Biochemistry and Cell Biology Deputy-director Naihe Jing. From RIKEN, the speakers included Masatoshi Takeichi, director of the Center for Developmental Biology and Kobe Institute, Yoshinori Tokura, director of the Advanced Science Institute Emergent Materials Department, Hiroyoshi Sakurai, chief scientist at the Nishina Center for Accelerator-Based Science, and Hou Zhaomin, chief scientist of the Advanced Science Institute Organometallic Chemistry Laboratory.

A series of commemorative lectures drew a large audience in downtown Tokyo

Following the lectures, a ceremony was conducted to recognize the achievements of seven individuals who had made significant contributions to strengthen the partnership between RIKEN and the CAS over the years. A joint communiqué was then signed to pledge further development of the partnership in the future. ■

RIKEN President visits Beijing

A delegation led by RIKEN President Ryoji Noyori visited Beijing, China from 10-12 June 2012 to meet with leading figures from the Chinese research community as part of RIKEN's ongoing program of strengthening global research ties. On the first stop of the visit Noyori joined six other newly-elected foreign members of the Chinese Academy of Sciences (CAS) in receiving their official certificates of CAS membership from the CAS President Chunli Bai at a presentation ceremony on 10 June. Noyori, elected to the CAS in 2012, is one of only 64 distinguished scientists to hold foreign membership of the CAS.

The presentation ceremony took place during the Consultative Meeting of Foreign Members of the CAS, attended by a total of 13 delegates, who offered suggestions for the future development of the Academy. Noyori spoke of the importance of further strengthening the relationship between RIKEN and the CAS and hoped that through debate and cooperation they could together "advance the cause of global science and technology".

RIKEN delegates visit the Institute of Microbiology, the CAS (IMCAS)

On 12 June the RIKEN delegation visited the Institute of Microbiology, the CAS (IMCAS) where they were welcomed by the institute director Li Huang and senior colleagues. The two teams reviewed the recent history of mutual cooperation between the IMCAS and the RIKEN BioResource Center, which jointly hosted the Third Meeting of the Asian Network for Research Resources Centers in 2011, in areas such as exchange visits of research personnel and the sharing of biological resources. The RIKEN delegation also heard a report of the work carried out by the CAS Sino-Japan Joint Research Center during its first five-year term. Noyori expressed his hope that both parties would strengthen their cooperation in research innovation, the training of young researchers and scientific exchange — sentiments which were echoed by Huang and the Chinese delegation. The RIKEN team subsequently visited the joint research center as well as the bacterial strain storage facility and the information center at the IMCAS. ■

Forging stronger ties with China

With 2012 marking the fortieth anniversary of the normalization of diplomatic relations between Japan and China, a host of events and activities throughout the year have commemorated and further consolidated the foundation of Japan-China friendship. RIKEN has continued to build on its strong ties with partner organizations in China by actively engaging in a diverse range of events designed to enhance person-to-person exchanges and strengthen collaborative research linkages.

Among some of the highlights of the commemorative year have been the opening ceremony of the RIKEN Beijing Representative Office in June 2011, at which a Memorandum of Understanding was signed between RIKEN and the Department of International Cooperation of the Chinese Ministry of Science and Technology (MOST), and a ceremony held at Xi'an Jiaotong University to celebrate the opening of the RIKEN-XJTU Joint Research Center in February 2012, attended by RIKEN President Ryoji Noyori and RIKEN Advanced Science Institute Director Kohei Tamao, as well as distinguished guests from MOST, the Chinese Ministry of Education, and Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Both the RIKEN Beijing Representative Office and the RIKEN-XJTU Joint Research Center are seen as prominent hubs for research exchange between Japan and China, and are testament to the skills, knowledge and talent of scientists of both nations.

Since the normalization of diplomatic relations between Japan and China in 1972, the bilateral relationship has grown significantly in all areas, not least in science and technology research. In recognition of the increasing importance of collaborative research efforts, President Noyori addressed audiences in China and emphasized "the need for Japan and China to work together to solve the global issues of energy, food, health and the environment that confront humanity today." ■

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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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